J. Braz. Chem. Soc., Vol. 12, No. 4, 499-506, 2001.
Printed in Brazil

0103 - 5053 $6.00+0.00

Article

Macroalgae as Lead Trapping Agents in Industrial Effluents - A Factorial Design Analysis

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Um planejamento fatorial de dois níveis foi empregado para analisar a influência da agitação, do tempo de contato, da quantidade de alga e do tipo de pré-tratamento sobre a remoção de metais pesados por algas A*rribadas*, num sistema em batelada contendo uma solução sintética simulando um efluente típico de fabricação de baterias. Amostras de 4 g de algas secas, moídas e peneiradas removeram 99% do chumbo de amostras de 100 mL do efluente sintético. Os percentuais máximos de remoção de zinco e ferro foram 37% e 80%, respectivamente. Como as algas arribadas são baratas, abundantes e de ocorrência natural, a remoção de chumbo através deste método pode ser vantajosa para aplicação industrial em larga escala.

A two-level factorial design was employed to analyze the influence of agitation, contact time, amount of algae and type of pretreatment on heavy metal removal by *Arribadas* algae, in a batch system consisting of a synthetic solution simulating a typical effluent from battery manufacturing processes. Dried, ground and sieved 4 g algae samples were able to remove 99 % lead from 100 mL samples of synthetic effluent. Maximum removals for zinc and iron were 37 % and 80 %, respectively. Lead removal using this method is potentially useful for large-scale industrial applications, because *Arribadas* algae are cheap, abundant, naturally occurring waste materials.

Keywords: factorial design, algae, lead, zinc, iron

Introduction

One of the undesirable consequences of increasing industrial activity is the increase in metal concentrations in natural water sources, caused by the large output of industrial effluents contaminated with heavy metals[1]. Removing from solution metallic species dispersed in natural environments is thus a matter of great practical interest, either because these species are highly toxic (mercury, lead, cadmium, zinc, nickel and chromium, for example) or because they have high aggregate value (gold, silver, and platinum).

Lead is a heavy metal occurring in effluents from battery manufacturing processes. It is an element with no known biological function. It is also highly toxic to living beings, even at low concentrations, because, like other heavy metals, it inhibits many enzyme-catalyzed biochemical reactions. Therefore, industrial plants that use lead as a raw material need efficient methods to reduce to a safe level the concentration of this metal in their effluents.

The traditional methods commonly employed to remove heavy metals from effluents, such as chemical precipitation, oxidation/reduction, filtration, electrochemical processes, adsorption by activated carbon or ion-exchange resins, are not always convenient. In some cases either they are not effective enough, or their cost is prohibitive, especially when the metals are present in low concentrations (1-100 mg L^{-1}) in large bodies of water [2].

In the last few years, increasingly stronger pressures from society at large and from environmental protection agencies

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have pushed forward the development of alternative methods of pollution fighting. This in turn has led to a growing interest in the use of biological organisms for trapping heavy metals. These technologies are less aggressive to the environment and also economically promising but, in spite of much research effort, few biosorption processes have reached commercial application. One possible reason is that, in order to be accepted by the engineering community, a new metal ion biosorption process must be economically competitive[3].

A number of researchers have investigated the feasibility of using cheaply available marine or fresh water algae for heavy metal removal[3-8]. However, these studies employed algae of a single isolated species, and to extend their results to large-scale treatments of industrial effluents would certainly require that some scheme for growing or collecting that particular species be established.

In the present work we investigate the possibility of using *Arribadas* algae to remove lead from the typical effluent of battery manufacturing plants. *Arribadas* algae consist of several species uprooted from their natural habitats and carried ashore by the action of winds and tides. On the Northeastern coast of Brazil, the algae washed ashore in certain areas are very easy to collect and handle, and occur in amounts large enough to allow consideration of their use as biomass in the treatment of industrial effluents. They also present high capacity for replenishing the stock between tides, probably due to reproduction through spores, thus constituting a low-cost renewable source of adsorption material. Further, since *Arribadas* algae are naturally occurring waste materials, reaping does not present any ecological problems and also helps to promote tourism-related activities.

Emphasis is given here to an empirical evaluation of the lead-removing capacity of these algae, based on a relatively simple experimental design. No attempt is made, at this stage, to develop a mechanistic model for lead biosorption. Four factors were identified as the most likely to influence the efficiency of the heavy metal adsorption process: intensity of agitation, time of contact, amount of algae and type of pretreatment. The effect of these factors on lead adsorption was studied with factorial designs, to determine the experimental conditions under which lead removal was most effective. Zinc and iron removal efficiencies were also monitored.

A 2^3 factorial design in the first three variables was carried out for each of three pretreatments, the response being heavy metal removal. In a complete two-level design, the value of each controlling variable (or factor) is kept fixed at one of two possible levels and the experiments are done at every possible combination of all levels. With 3 factors, this leads to a minimum of 2^3=8 experiments, hence the name of this specific design.

Traditional methods of optimization, which allow variation of only one factor at a time, all other factors being kept fixed, are adequate when the factors are independent of one another. In the complex systems normally associated with environmental questions, where synergic or antagonistic interactions are common, univariate optimization might yield misleading results[9]. Factorial designs are based on the alternative multivariate approach, in which all factors are considered simultaneously, and on an equal basis. These designs have the considerable advantage of furnishing information concerning not only the individual effect of each factor on the response of interest, but also about the possible interactions between all factors, which often prove very significant[10].

Experimental

Biomass preparation

Arribadas algae were collected at Itamaracá beach (State of Pernambuco, Brazil), washed and dried at 32 ± 1 ^oC for 4 days. They were then divided in three kinds of samples: dried only (D), dried and ground in a knife mill (DG), and dried, ground and sieved in 35-mesh sieves (DGS).

Synthetic effluent preparation and analysis

The solutions for the adsorption experiments were prepared from the $Pb(NO_3)_2$, $Zn(NO_3)_2.6H_2O$ and $Fe(NO_3)_3.9H_2O$ salts in 0.1 mol L^{-1} nitric acid, with concentrations of 2 mg L^{-1} for Zn and 30 mg L^{-1} for both Pb and Fe. These values simulate the typical effluent from battery plants as to pH (equal to 1) and the presence of interfering ions (zinc and iron), and exceed by 50% the corresponding average concentrations observed in the raw effluent of an actual manufacturing plant located in Northeastern Brazil. Lead, zinc and iron contents were quantified by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) before and after the experiments were carried out. Removal efficiencies were calculated from the concentration differences.

Determination of solid residue, ash and water contents in algae samples

1g duplicate algae samples were dried at 32 ± 1 ^oC and weighed. Percent weight loss was recorded as the water content at 32 ± 1 ^oC. The samples were then heated to constant weight at 105 ± 1 ^oC. Accumulated percent weight loss relative to the natural material was recorded as the water content at 105 ± 1 ºC. To determine solid residue and ash content the dried samples were calcinated at 600 ± 1 ºC and then incinerated at 900 ± 1 ºC.

The 2^3 factorial design

The experimental conditions for the removal experiments are given in Table 1. All experiments were made in duplicate, to obtain an estimate of experimental error, and another experiment was carried out in triplicate at intermediate conditions. In all, therefore, 22 experiments were done, in 100 mL batches of the synthetic effluent. The ranges initially chosen for factors **W** and **T** (weight of the algae sample and time of contact) were 0.2-0.6 g and 1-5 h, respectively. Since lead removal under these conditions remained below 30%, the factor ranges were extended until removal in the 90% vicinity was reached, resulting in the design of Table 1. Following the usual convention, the two extreme levels are denoted by minus one (lower level) and plus one (higher level). This, as we shall see, leads to a convenient algorithm to analyze the experimental results.

Table 1. Factor levels for the 2^3 factorial design. Level combinations were applied to samples submitted to each of three pretreatments: (a) dried only (D), (b) dried and ground (DG), and (c) dried, ground and sieved (DGS). Experiments at the lower and higher levels were carried out in duplicate and those at intermediate levels in triplicate. Runs 1 to 8 correspond to all possible combinations of the two extreme levels of the factors. The ninth and tenth are intermediate points. All experiments are run in 100 mL batch samples of the synthetic effluent.

		Levels		
Label	Factors	-1	Intermediate	+1
A	Agitation	Without	—	With
W	Algae weight (g)	2	3	4
T	Contact time (h)	3	6	12

	Factor levels		
Run	**A**	**W**	**T**
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	-1	0	-0.5
10	+1	0	-0.5

Results and Discussion

Solid residue, ash and water contents

The algae contain on average 89.6% water, leaving only 10.4% of dry matter at 105 ºC. The solid residue is 8.7% at 600 °C and 1.8% of ashes at 900 °C. Use of biomass dried at 32 ± 1 ºC is probably advantageous over the other conditions, because the energy requirements are low, and the dried algae keep well and are more easily stored. Also, a 1 g sample of natural algae is reduced to only 0.12 g of dry matter.

Removal of heavy metals from solution

Lead removal

The results obtained using the experimental design of Table 1 for lead removal are given in Table 2. Replicate runs were carried out, to yield an estimate of pure experimental error.

The analysis of a two-level factorial design begins with the calculation of the main effects of all factors and the interactions between them. All effects are calculated as differences between two averages, each average containing half of the experimental responses at the extreme levels of the design[10-11]. With 3 factors, as in Table 2, each average

Table 2. Percent lead removal values (R_i) for the experimental runs specified in Table 1, for algae pretreated in three different ways: just dried (D), dried and ground (DG), and dried, ground and sieved (DGS). Within each series, the experiments were performed in random order. The subscript (1), (2) or (3) identifies genuine replicates. The subscript (av) indicates average of replicate results, which are used to calculate main and interaction effects.

Run	D				DG				DGS			
	R_1	R_2	R_3	R_{av}	R_1	R_2	R_3	R_{av}	R_1	R_2	R_3	R_{av}
1	66.86	58.58	-	62.72	54.84	72.99	-	63.92	60.51	67.89	-	64.20
2	65.84	49.24	-	57.54	60.43	64.31	-	62.37	60.96	63.57	-	62.27
3	89.95	84.79	-	87.37	87.45	99.11	-	93.28	98.19	98.63	-	98.41
4	86.09	80.07	-	83.08	95.01	98.97	-	96.99	99.09	98.82	-	98.96
5	58.28	53.27	-	55.78	54.27	65.82	-	60.05	73.81	64.81	-	69.31
6	57.41	73.86	-	65.64	54.76	66.22	-	60.49	62.32	68.33	-	65.33
7	86.47	89.97	-	88.22	95.99	99.11	-	97.55	99.36	99.25	-	99.31
8	93.84	98.71	-	96.28	95.31	98.80	-	97.06	99.32	99.16	-	99.24
9	77.79	74.19	75.94	75.97	89.02	95.14	83.49	89.22	96.42	95.43	96.98	96.28
10	86.58	89.20	85.08	86.95	86.39	95.38	86.79	89.52	98.29	97.51	98.79	98.20

contains four responses. Each of these responses is an average of duplicate values. The three main effects are simply the differences between the average response at the higher level of the factor in question and the corresponding average at the lower level. In a simple factorial design, the intermediate runs are ignored at this stage, and used only to investigate possible curvatures in the experimental response surface. To obtain the main effects one thus applies to the responses in Table 2 the signs of the corresponding columns in Table 1, performs the algebraic sum, and divides the result by four.

For example, using the R_{av} values, the main effect of factor **A** (agitation) on lead removal by the dried algae is given by

A = (1/4) [- 62.72 + 57.54 - 87.37 + 83.08 - 55.78 + 65.64 - 88.22 + 96.28 - 75.97 + 86.95] = 2.1%.

The interaction effects are linear combinations of the form

$$\frac{1}{4}\sum_{i}^{n} a_i y_i, \qquad (1)$$

where y_i is the average response in run *i* and the coefficient a_i is set equal to plus or minus one, depending on the sign of the product of the columns of the factors involved. For example, to calculate the three-factor interaction **AWT**, the sign of the response in run number 2 is given by (**A**)(**W**)(**T**) = (+1)(-1)(-1) = +1. In all, four interaction effects are determined, three of these being two-factor and one three-factor. All calculated effects are presented in Table 3.

Table 3. Main and interaction effects and their standard errors, calculated from the responses given in Table 3. D, DG and DGS refer to the pretreatments to which the algae samples were submitted. Units are percent lead removal. Statistically significant effects, at the 95% confidence level, are shown in boldface.

Effect	Pretreatment		
	D	DG	DGS
Average	74.6 ± 1.4	79.0 ± 1.6	82.1 ± 0.7
Main Effects			
A – Agitation (no/yes)	2.1 ± 2.8	0.5 ± 3.2	-1.3 ± 1.4
W – Algae Weight (g)	**28.3** ± 2.8	**34.5** ± 3.2	**33.7** ± 1.4
T – Retention time (h)	3.8 ± 2.8	-0.4 ± 3.2	2.3 ± 1.4
Two-factor interaction			
AW	-0.2 ± 2.8	1.1 ± 3.2	1.6 ± 1.4
AT	**6.8** ± 2.8	-0.6 ± 3.2	-0.7 ± 1.4
WT	3.2 ± 2.8	2.5 ± 3.2	-1.8 ± 1.4
Three-factor interaction			
AW	-0.7 ± 2.8	-1.5 ± 3.2	0.4 ± 1.4

Before trying to interpret the physical meaning of the numerical values calculated for the effects, it is necessary to obtain an estimate of the experimental uncertainty associated with them. The usual procedure is to pool the standard deviations of the replicate responses into a single overall estimate of experimental error, s_p. Since each effect is given by a linear combination of independent observations, the variances at each experimental setting can be combined into a single value representing the variance of an effect:

$$\hat{V}(\text{effect}) = \sum_i a_i^2 s_i^2 = \frac{s_p^2}{2}\sum_i a_i^2, \qquad (2)$$

where $a_i = \pm 1/4$ is the coefficient of the *i*th response and s_p^2 is an estimate of the pooled variance of that response[10]. The square root of $\hat{V}$ *(effect)* is the standard error of an effect. Substituting into this equation the responses in Table 2, standard errors of 2.8, 3.2 and 1.4% are obtained for the dried, dried and ground, and dried, ground and sieved algae, respectively. At the 95% confidence level, these values imply that only effects with absolute values exceeding 6.2% for dried, 7.1% for dried and ground and 3.0% for dried, ground and sieved algae can be considered statistically significant.

The only significant main effect is the amount of algae (**W**), irrespective of the pretreatment employed. In the preliminary experiments, when smaller contact times were used, time itself also presented a significant effect, which disappeared with the longer times of Table 1. This is an indication that with these longer times lead removal reaches saturation, that is, the "removal equilibrium" has been attained. Use of agitation, on the other hand, does not seem to have any effect on lead removal, indicating that resistance against ion diffusion in the outer layers of the biosorbant is low. No interaction effects are statistically significant. Although the **AT** interaction for dried algae is a borderline value at this level of significance, we prefer to consider it as a statistical artifact, since it is an isolated case, absent from the other pretreatments.

The overall conclusion of the factorial analysis, then, is that changing the amount of algae from 2 g to 4 g leads to an average increase in lead removal of 28.3%, 34.5% and 33.7%, respectively, for D, DG, and DGS algae samples. The absence of significant interactions means that these results are not affected by changes in time of contact (over the levels considered in the experiments) or use of agitation. Figure 1 is a traditional and convenient way of visualizing these results. Main effects are differences between average responses on opposing faces of the cube. The effect corresponding to increasing algae weight is perceived as a contrast between the higher lead removal values on the upper face of the cube and those on the lower face.

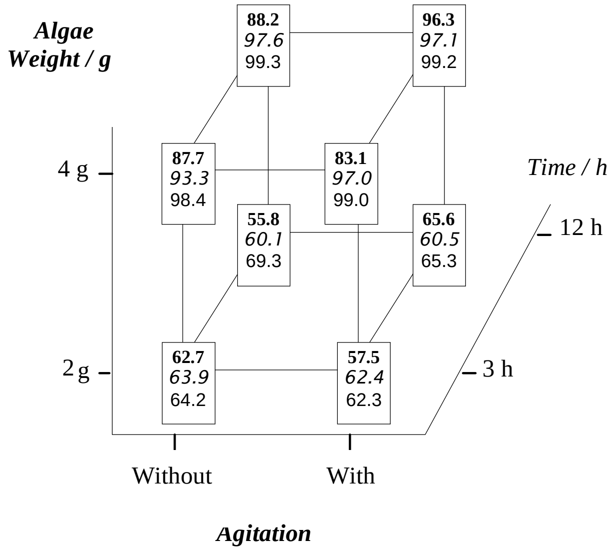

Figure 1. Geometrical representation of the results from the 2^3 design on lead removal. Values on top refer to results for dried samples, those in the middle to dried and ground samples, and those at the bottom to dried, ground and sieved samples. Units are percent lead removal. The only significant effect on lead removal – increasing algae weight – is perceived as a contrast of the values on the upper face of the cube with those on the lower face.

In the absence of significant interaction effects, linear models can adequately represent the responses for the three pretreatments:

$$\hat{y}_e = b_0 + b_1 x_A, \tag{3}$$

where b_0 and b_1 are estimates of the parameters of the model, given respectively by the overall average response and half the **W** main effect, $\chi_A = \pm 1$ is the weight of the algae sample in coded values, and $\hat{y}_e$ stands for predicted percent lead removal.

When the responses at the intermediate points are included, linear models exhibit lack of fit for all pretreatments. This is indicated by F-test results (at the 95% confidence level) and by the linear model residual plots (Figure 2).

Fitting a quadratic model, $\hat{y}_e = b_0 + b_1 x_A + b_2 x_A^2$, eliminates the systematic curvature observed in the residual plots for the linear models. Table 4 gives the parameter estimates and their standard errors for the quadratic models. All estimates are statistically significant at the 95% confidence level. The quadratic term implies that increasing the amount of algae in this range does not lead to a proportionate increase in lead removal, and this in turn suggests that removal equilibrium is being approached. Residual plots for the quadratic model fits are shown in Figure 3. The saturation effect is further confirmed by the decreasing spread of the residuals towards the right side of the second and third plots.

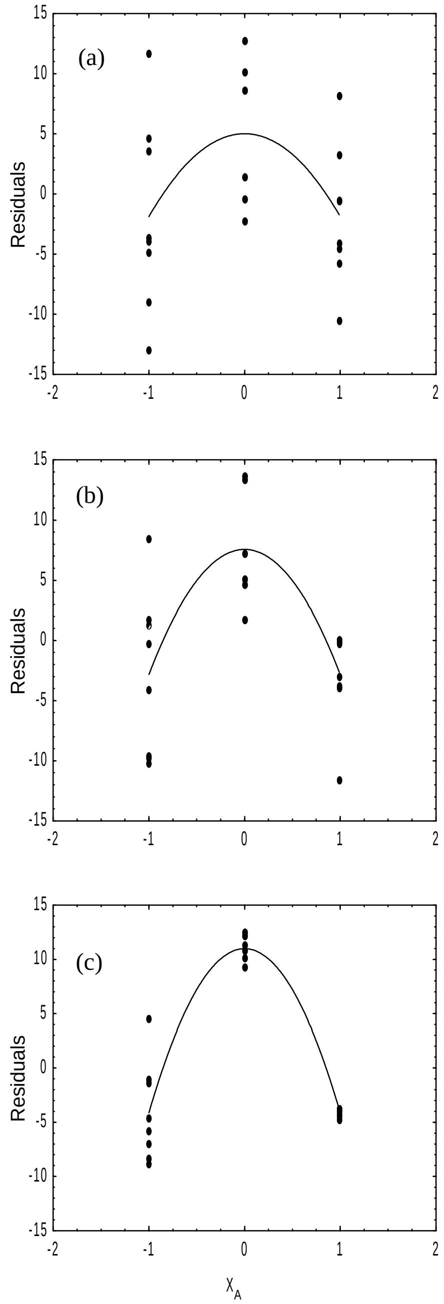

Figure 2. Residual plots for the fit of linear models. All plots present curvature, indicating lack of fit. (a) dried, (b) dried and ground, and (c) dried, ground and sieved.

Table 4. Parameter estimates and standard errors for the fitting of quadratic models to the responses in Table 2. Units are percent lead uptake. Errors are given in parentheses. Notation as in Table 3.

Pretreatment	b_o	b_1	b_2
D	81.4 (± 2.8)	14.1 (± 1.7)	-6.9 (± 3.2)
DG	89.4 (± 2.2)	17.2 (± 1.3)	-10.4 (± 2.6)
DGS	97.2 (± 1.1)	16.8 (± 0.7)	-15.0 (± 1.3)

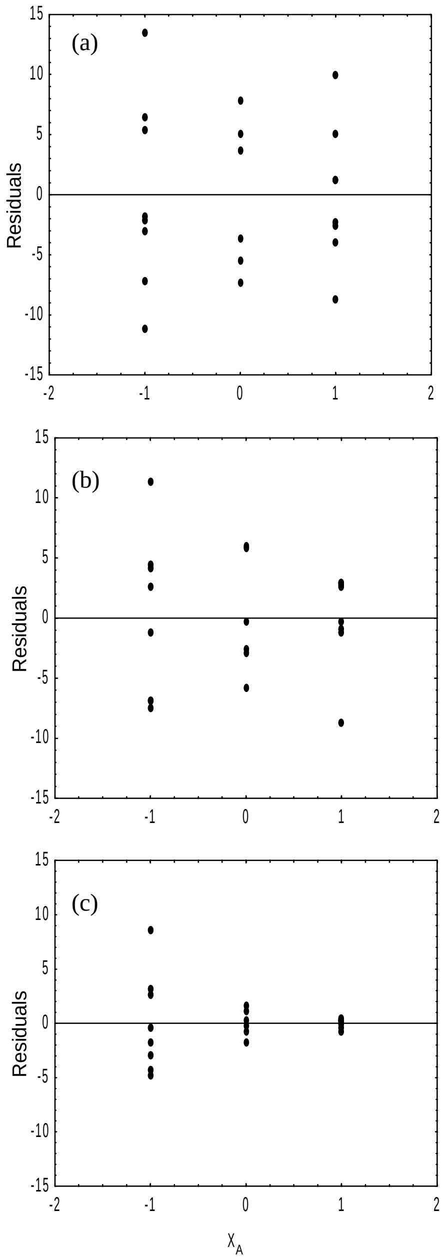

Figure 3. Residual plots for fitting of quadratic models. The second and third plots present smaller spreads at higher weight values, indicating saturation. (a) dried, (b) dried and ground, and (c) dried, ground and sieved.

Figure 1 shows that the *Arribadas* algae studied here, when dried, ground and sieved and used at the 4 g level, were able to remove an average of 99% of the lead from solution. When they are dried and ground but not sieved, the lead removal remains high, but slightly smaller – 96%, on average. Employing algae that were only dried results in an average removal of 89%. For large-scale industrial applications these slightly decreasing performances should be weighed against the increasing costs that grinding and sieving imply.

Zinc and iron removals

Zinc and iron removal results based on the same design used for lead are given in Table 5, and represented geometrically in Figures 4 and 5. Analysis is done in the same fashion.

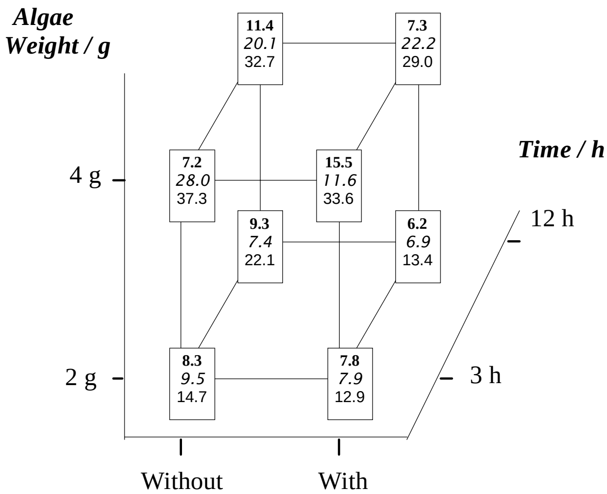

Figure 4. Geometrical representation of the results from the 2^3 design on zinc removal. Notation as in Figure 1.

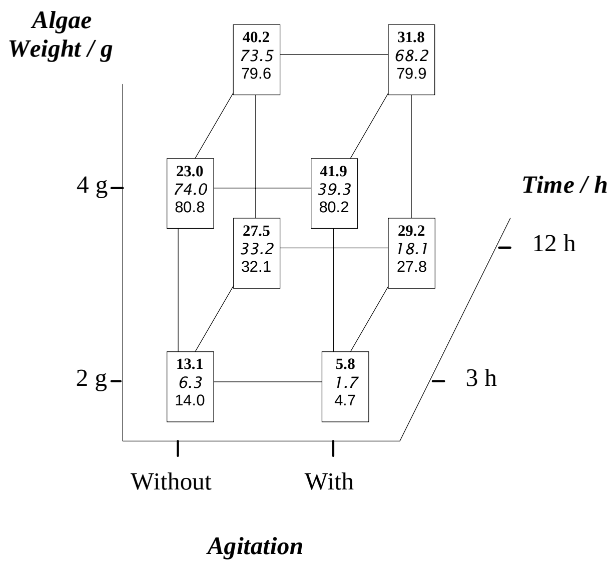

Figure 5. Geometrical representation of the results from the 2^3 design on iron removal. Notation as in Figure 1.

For zinc removal, the effect pattern is quite similar to the one observed for lead, but the responses are much smaller. Only the main effect corresponding to the size of the algae sample (**W**) is significant, and only when the sample is at least ground. For D samples, once again there is a marginally significant interaction value for contact time and agitation. The meaning of this (possible) interaction

Table 5. Main and interaction effects for zinc and iron removal. Notation as in Table 4. Statistically significant effects at 95% confidence level, are shown in boldface.

Effect	Pretreatment					
	D		DG		DGS	
	Zinc	Iron	Zinc	Iron	Zinc	Iron
Average	9.1 ± 0.7	26.6 ± 1.0	15.3 ± 1.4	39.3 ± 1.2	24.5 ± 1.6	49.9 ± 2.1
Main Effects						
A – Agitation (no/yes)	0.1 ± 1.2	1.2 ± 2.0	-6.4 ± 2.8	**-14.9** ± 2.5	-4.5 ± 3.2	-3.7 ± 4.2
W – Algae Weight (g)	2.5 ± 1.2	**15.3** ± 2.0	**14.8** ± 2.8	**48.9** ± 2.5	**17.4** ± 3.2	**60.5** ± 4.2
T – Retention time (h)	1.2 ± 1.2	**11.2** ± 2.0	-2.2 ± 2.8	**17.9** ± 2.5	0.4 ± 3.2	9.9 ± 4.2
Two-factor interaction						
AW	1.9 ± 1.2	4.0 ± 2.0	-5.3 ± 2.8	-5.0 ± 2.5	0.8 ± 3.2	3.3 ± 4.2
AT	**3.8** ± 1.2	4.5 ± 2.0	-2.6 ± 2.8	-4.7 ± 2.5	1.7 ± 3.2	-1.5 ± 4.2
WT	0.9 ± 1.2	**7.7** ± 2.0	-3.7 ± 2.8	3.7 ± 2.5	4.3 ± 3.2	10.7 ± 4.2
Three-factor interaction						
AWT	2.5 ± 1.2	**9.1** ± 2.0	-2.1 ± 2.8	**-9.9** ± 2.5	-1.7 ± 3.2	1.0 ± 4.2

can be seen in Figure 4. When time of contact increases from 3 h to 12 h, the average zinc removal under agitation decreases by 4.9%, suggesting that the adsorbed metal is starting to desorb. In the absence of agitation, the time effect (+1.6%) is indistinguishable from error. Overall, zinc removal is less efficient than lead removal, and the kind of pretreatment becomes more important. Even using 4 g of algae, average zinc removal is only 20% when the sample is dried and ground, and rises to 33% when it is also sieved. With D samples, average zinc removal falls to only 10%, and it does not appear to depend on the levels of any of the three factors. As these values themselves suggest, the **W** main effects are also smaller: +2.5% (D), +14.8% (DG) and +17.4% (DGS).

For iron removal the patterns are more complex, and the effects less clear-cut. For D samples, there are two significant main effects, **W** and **T**, and two significant interactions, **WT** and **AWT**. For DG samples, the significant effects are **A**, **W**, **T** and **AWT**. For DGS samples, the only significant effect is due to sample weight (**W**). These results are best interpreted by referring to the cube in Figure 5.

For DGS samples, increasing the amount of algae from 2 g to 4 g produces a dramatic rise in average iron removal (from 20% to 80%), and this does not depend significantly on the other two factors. The same weight effect is observed for the other two pretreatments, but is less pronounced and depends on the levels of agitation and contact time.

Longer times generally increase iron removal, but this effect is more pronounced with 2 g samples. With 4 g it is nonexistent for DGS samples (and also for DG samples without agitation), indicating that saturation has been reached.

The effect of agitation is the hardest to analyze. For 4 g samples of D algae, for example, introducing agitation increases removal with 3 h contact but the effect is reversed when the time is increased to 12 h. If 2 g samples are used, agitation reduces removal at 3 h, and shows no effect at 12 h. Similar variations are observed for the other treatments.

The type of pretreatment, as in zinc, also influences the extent of iron removal. The largest removal values (80%) occur with 4 g of DGS samples. For DG and D samples, maximum iron removals are 74% and 42%, respectively.

Conclusions

The results of the 2^3 design show that naturally occurring *Arribadas* algae are able remove up to 99% lead from a synthetic solution simulating a typical effluent from battery manufacturing processes, when 4 g of dried, ground and sieved samples are added to a 100 mL batch solution. The average lead removal for dried and ground samples is 96%, and for dried samples is 89%. Since no significant effects were observed for the time of contact or the use of agitation, it is less expensive to carry out the adsorption process at the shorter time (3h), without agitation. It is worth noticing that the experiments were done at a starting pH value of 1, which is unfavorable to adsorption, and in the presence of interfering ions. Under less stringent conditions, we would expect the process to perform better. It was observed, besides, that in the bio-interaction process Pb, Fe, Zn e H^+ ions in solution are exchanged with alkaline (Na e K) and alkaline-earth ions (Ca e Mg) present in the algae. As a consequence, the final pH value is about 5, the minimum value allowed by Brazilian legislation for effluents discharged on water bodies[12]. Adding a neutralizing agent before discharge would then be a minor concern.

Zinc and iron removal maximum values, 33% and 80%, respectively, were also obtained with 4 g of dried, ground and sieved algae. The algae based adsorption process suggested here is a promising alternative for the final treatment of lead-containing industrial effluents.

Acknowledgements

The authors gratefully acknowledge partial financial support from the government agencies BN, FACEPE and CNPq.

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Received: September 12, 2000
Published on the web: May 21, 2001