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Use of Response Surface Methodology in the Optimization of Coagulation-Flocculation of Wastewaters Employing Biopolymers

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ABSTRACT: The aim of this work was to optimize the coagulation-flocculation (CF) process applied to municipal wastewaters (WW). Optimization of CF was performed to minimize chemical oxygen demand (COD), turbidity, sludge produced, and some metals in sludge. A Box-Behnken design was used to evaluate the effects and interactions of three factors such as type of biopolymer (guar, mesquite seed gum and *Opuntia mucilage*), dose of biopolymer (25, 50 and 75 mg/L) and initial organic load of wastewaters WW (725, 1,425 and 1,325 mg COD/L). Regarding the statistical analysis, Results were assessed with various descriptive statistics such as p value, lack of fit (F-test), coefficient of R^2 determination and adequate precision (AP) values. p values <0.05 demonstrates that the model for those response variables were significant at the 95% confidence level. The PLOF values > 0.05 show that the F-statistics was insignificant implying significant model correlation between the variables and process responses. Regarding the fit of the model, the obtained R^2 values were up to 0.98 for sludge produced, 0.94 for COD removal, 0.91 for Cd, 0.90 for turbidity removal, and 0.75 for sludge density. It is noteworthy that response surface methodology (RSM) also allowed optimizing de CF process. Employing this methodology it is feasible to determine COD, turbidity, and salts removals, as well as the amount and quality of the produced sludges under hypothetical conditions within predetermined parameter ranges, without the need of carrying out experimental runs.

Key words: Wastewater, Coagulation, Flocculation, Guar gum, Mesquite Gum, Opuntia indica mucilage

INTRODUCTION

The process of coagulation-flocculation (CF) is one of the most efficient and widely applied methods to treat wastewater (WW) in an efficient and simple way (Khannous *et al.*, 2011). Coagulation is the destabilization of a colloid from neutralization of the electric charge and aggregation of fine particles in suspension. Flocculation involves an increase in the contact among fine particles to form flocs that settle easily. These processes are carried out by means of chemical compounds that are added to the WW (Weeber, 2003; Sincero and Sincero, 2003). In general Fe or Al salts and synthetic polymers (such as polyacrylamide) are used, which generate a poorly biodegradable sludge that may still contain traces of metals and polymers in the treated water. The use of biopolymers in WW treatment has been investigated because they offer certain advantages over the use of Fe and Al salts and synthetic polymers (Bolto and Gregory, 2007). Guar seed (*Cyamopsis tetragonologus*) and mesquite seed (*Prosopis laevigata*) gums are composed mainly of galactomannans (Gupta and Ako, 2005; Lopez-Franco *et al.,* 2006). Cactus mucilage (*Opuntis ficus-indica*) is composed of neutral sugars plus polygalacturonic acid (Sepulveda et al. 2007). These biopolymers have been used as coagulant-flocculant aid in CF instead of using Fe or Al or other polymers (Lopez-Franco *et al.*, 2006; Dakia et al, 2008; Miller *et al.*, 2008). Mijaylova et al. (1996) employed locust bean gum (LBG) and guar gum as an aid in CF of WW in Mexico City obtaining good turbidity removal efficiencies. Torres et al. (2012a) demonstrated that mesquite seed gum and *Opuntia* mucilage can be used to treat municipal WW with an initial organic charge of 837 mg/L as COD by CF process. Mesquite seed gum removed up to 90% of COD (pH 10, dose of 75 mg/L) and 60% (pH 7, dose of 50 and 150 mg/L). *Opuntia* mucilage removed 65% of COD (pH 10, dose of 50 mg/L). They highlighted the importance of

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employing these environmental-friendly products in WW treatment. Torres et al. (2012b) reported for the first time the use of the polysaccharides contained in seeds of two fruits very much appreciated for their nutritional and even pharmaceutical properties. They stated that seeds of *Annona diversifolia* and *A. muricata* are capable of reducing the COD, turbidity, and electrical conductivity in WW. *A. diversifolia*, seeds gum yielded COD removals of 37% (dose of 50 mg/L), producing 2 mL of sludge/L. *A. muricata* seeds gum removed 35% of the COD present (dose 100 mg/L) and produced 5 mL sludge/L.

In another work, Torres et al. (2012c) demonstrated that *Opuntia* mucilage is useful in treating high-load industrial WW. They presented four methods for producing mucilage and found that the production method can affect the CF´s efficiency of the mucilage. Heated Dry powder removed 41% of COD and the dry whole cladode removed 24%. Turbidity removals of 88% (dry whole cladode) and 41% (heated mucilage) were obtained. The efficiency of the CF can be affected by several factors such as type and dose of coagulant-flocculant, pH, contact time and stirring speed, and temperature (Ghafari et al, 2009; Khannous *et al.*, 2011). The Response Surface Methodology (RSM) is a set of mathematical techniques used in the treatment of problems in which a response of interest is influenced by several variables. The objective of RSM is to design an experiment to provide reasonable values of the response and then determine the mathematical model that best fits the data. With RSM the values of the factors that optimize the value of the response variable can be set (Montgomery, 1991). Application of RSM has already been reported in CF experiments. Rigas et al. (2000) used a central composite design (CCD) of the second order to investigate the effect of the dose of coagulant and flocculant and pH on turbidity, suspended solids and oil content in WW and found that optimal regions were within the intervals of the experimental design. Until now, there are not reports for the optimization of parameters using RSM based on the Box-Behnken design in COD and turbidity removals of municipal WW by CF. Our work group has published the use of Box-Behnken design with RSM to optimize CF of a high-load cosmetic industry WW (Carpinteyro-Urban et al. 2012). The parameter that strongly affected the calculation of COD, turbidity, oil and greases (O&G), and the amount of sludge including its metals content was the polymer´s dose. RSM graphs of COD and turbidity removals showed that the higher the initial organic load and biopolymer concentration, the greater clearance is obtained, with a maximum removal around 40% for COD, 70% for turbidity and over 60% of O&G removal. The Box-Behnken design is a free, spherical and rotable quadratic design, that consists of a central point and the midpoints of the edges of the cube restricted on the area (Ferreira et al. 2007; Kayan and Gözmen, 2012).

MATERIALS & METHODS

Municipal WW was collected at the Sewage Treatment Plant San Juan Ixhuatepec located in Estado de Mexico, Mexico.

WW was preserved at 4°C until employed. Physico-chemical parameters were analyzed like COD, BOD, TS, pH, conductivity, hardness, MBAS, oil and greases, as well as four metals, according to Standard Methods (APHA AWWA WPCF, 1989). The results of preliminary experiments at different doses and pH allowed us to establish ranges and parameters for a Box-Behnken experimental design (Montgomery, 1991). Three parameters (with three levels each one): type of biopolymer, dose of biopolymer and organic load of WW were selected as independent variables (Table 1). COD, turbidity removal, sludge produced and metal content as the dependent response variables. Jar test experimental conditions, *Opuntia* mucilage and mesquite seed gum were produced as in Torres et al (2012 a). Guar gum and $FeCl_3$ were of analytic grade. The experimental results were analyzed using Design Expert 8.1 and a regression model was proposed. The response variables were fitted by a second order model in the form of a quadratic polynominal equation:

$$y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} b_{ij} X_i X_j + \varepsilon \quad (1)$$

Table 1. Experimental design parameters and ranges

Ranges	Biopolymer type	Biopolymer concentration (mg/L)	Initial organic load (mg COD/L)
-1	Guar gum	25	700
0	Mesquite seed gum	50	1,300
1	*Opuntia* mucilage	75	1,400

Where y is the response variable to be modeled, i is the linear coefficient, j is the quadratic coefficient, b is the regression coefficient, k is the number of factors studied and optimized in the experiment and ε is the random error. Analysis of variance (ANOVA) was used for graphical analyses of the data to obtain interactions between the independent and dependent variables. The quality of the fit polynomial model was expressed by the coefficient of determination (R^2) and its statistical significance was checked by the Fisher F-test. Model terms were selected or rejected based on the p value (probability) with 95% confidence level. Three dimensional plots were obtained based on the effects of the levels of two factors. For the three biopolymers and $FeCl_3$ metal content (Al, Ca, Fe, K, Mg, Ns, Zn, Cu, Pb and Ni) was determined. Also, in produced sludges, some metal contents (As, Cd, Cr, Hg, Zn, Cu, Pb and Ni) were determined by the atomic absorption spectrophotometric method (NMX, 1981).

RESULTS & DISCUSSION

The initial characterization of raw WW is presented in Table 2. As noted, the pH value is close to neutral (6.91), with a conductivity value of 2,003 μS and turbidity of 537 NTU. COD and BOD_5 values are of 1,141.3 and 391.1 mg/L, respectively, giving a BOD/COD ratio of 0.34 (moderately biodegradable). Total solids had a level of 1,591 mg/L, MBAS and oil and greases (O&G) were 117 and 76 mg/L. Four metals were measured, Al (2 mg/L), Cr (0.034 mg/L), Fe (2 mg/L) and Pb (0.310 mg/L). The results of 15 experimental runs of a Box-Behnken experimental design are shown in Table 3. Note that three more runs were done with $FeCl_3$ for comparison purposes. Final pH was around neutral value (from 7.1 to 7.65). The initial pH value of raw WW was 6.91 units, so biopolymers and $FeCl_3$ produced an increase in the pH value, which is good for the quality of the produced water. Salinity removals were as follows: guar gum removed between 3.25 to 3.75 %, mesquite seed gum removed 1.8 to 9.91%. *Opuntia* mucilage only removed 0.1 to 0.5%. $FeCl_3$ removed 0.2 to 1.1% at a dose of 25 and 50 mg/L. Although CF is not designed for removing salts from water, frequently a co-precipitation process has been observed, which removes certain amount of salts present in WW.

Guar gum removed 74.5 and 72.7 % of turbidity (run 3 and 1), followed by mesquite seed gum with 73.09 % (run 10). *Opuntia* mucilage removed 71.08 % (run 5). $FeCl_3$ removed 71, 73.5 and 75.5 % (for the different doses assayed).

COD removals as high as 56.1 and 52.81% were observed when mesquite seed gum was employed (run 13 and 10). The second best value corresponds to guar gum 51.4% (run 1); followed by *Opuntia* mucilage between 44.2 and 44.4% (run 7 and 5). For the assessments with $FeCl_3$, the maximum COD removal was as high as 65.4 (for a dose of 25 mg/L).

The net amount of COD removed is found at the 9th column of Table 3. Maximum COD removals (800 mg COD/L) were found when using mesquite seed gum (run 13). Another important value was 733 mg COD/L when employing guar and mesquite seed gum (run 1 and 11). With *Opuntia* mucilage, 633 mg COD/L were removed (run 5). $FeCl_3$ removed between 866 mg COD/L (run 17) and 933 mg COD/L (run 16). From other perspective when the total COD removal is divided by the biopolymer or salt dose (column 10, Table 3), values up to 29 mg COD/mg biopolymer, when using guar gum (run 1), were obtained. 28 mg COD/mg biopolymer (run 10) were found when using mesquite seed gum. *Opuntia* mucilage is closely placed with 25 mg COD/mg biopolymer (run 5). The best value calculated for $FeCl_3$ was of 37 mg COD/mg salt (run 16).

Table 2. Wastewater initial characteristics

Parameter	Value	Parameter	Value
pH	6.91 units	Hardness ($CaCO_3$)	379.57 mg/L
Conductivity	2003μS	MBAS	117.25 mg/L
Color Pt/Co	2010 units	Oil and greases	76.66 mg/L
Turbidity	537 NTU	Al	2.007 mg/L
COD	1141.3 mg/L	Cr	0.034 mg/L
BOD_5	391.1 mg/L	Fe	2.099 mg/L
TS	1591 mg/L	Pb	0.310 mg/L

Table 3. Results for the jar test experiments using biopolymers or $FeCl_3$

Run	Biopolymer type	Biopolymer Dose (mg/L)	COD *	Final pH	Removal			COD removed (mg/L)	COD removed/ dose	Sludge produced (mL/L)	Sludge density (g/L)
					Salinity	Turbidity	COD				
1	Guar gum	25	3	7.23	0	**72.7**	**51.44**	**733.34**	**29.33**	10	0.026
2		50	3	7.21	**3.759**	71.56	44.42	633.34	12.66	13	0.028
3		50	2	7.33	3.467	**74.57**	37.72	500	10	**15**	0.028
4		75	1	7.66	3.25	65.78	32.16	233.34	3.11	7	0.025
5	*Opuntia* mucilage	25	3	7.14	0.506	**71.08**	**44.42**	**633.34**	**25.33**	13	0.026
6		50	1	7.36	0	45.78	22.97	166.67	3.33	4	0.034
7		50	2	7.39	0.138	68.64	**45.26**	600	12	**20**	0.025
8		75	3	7.1	0	**72.53**	42.09	600	8	13	0.027
9	Mesquite gum	25	1	7.56	4.015	60.52	45.94	333.34	13.33	3	0.053
10		25	2	7.6	**5.894**	**73.09**	**52.81**	**700**	**28**	**18**	0.018
11		50	3	7.24	1.807	71.08	51.44	**733.34**	14.66	10	0.028
12		50	3	7.23	4.049	66.74	46.76	666.7	13.33	10	0.036
13		50	3	7.37	0	68.19	**56.12**	**800**	16	9	0.034
14		75	1	7.65	0	59.47	36.75	266.67	3.55	4	0.042
15	$FeCl_3$	75	2	7.47	**9.916**	70.12	42.75	566.67	7.55	**16**	0.017
16		25	3	7.36	0.289	**71.08**	**65.47**	**933.34**	**37.33**	8	0.039
17		50	3	7.32	**1.156**	73.49	60.79	866.67	17.33	10	0.041
18		75	3	7.55	0	**75.42**	63.13	900	12	10	0.019

* 1= 725mg COD/L *2= 1,425 mg COD/L *3= 1,325 mg COD/

Regarding the amount of produced sludge, the maximum value corresponds to the mucilage at a dose of 50 mg/L for the highest COD load WW, with 20 mL/L. As can be noted, higher values of sludge produced (15-20 mL/L) were observed for the more concentrated WW, followed by the group where the COD load was intermediate (10-13 mL/L) and at the end for the low COD load WW (3-7 mL/L). When using $FeCl_3$, sludge volumes of 8-10 mL/L were achieved for the medium COD load WW. Sludge density did no vary significantly among all experimental runs, despite of different type and dose of biopolymers and initial COD load of WW. Sludge densities varied from 0.018 to 0.05 g/L.

The ANOVA results of the quadratic models are shown in Table 4. Results were assessed with various descriptive statistics, such as *p* value, the lack of fit F-test (PLOF), coefficient of determination R^2 and adequate precision (AP). *p* values < 0.05 (COD removal, turbidity removal, sludge produced and Cd) demonstrate that the model for those response variables were significant at the 95% confidence level. The PLOF values > 0.05 (for all response variables) show that the F-statistics was insignificant, which implies a significant model correlation between the variables and process responses. The fit of the model, yielded R^2 values up to 0.98 for sludge produced, 0.94 for COD removal, 0.91 for Cd, 0.90 for turbidity removal and 0.75 for sludge density. AP values higher than four (all response variables, except total metals) confirm that all predicted models can be used to navigate the design space defined by the Box-Behnken design. Quadratic equations are shown in Table 4. Using the quadratic equations it is possible to predict response variables (i.e., the COD or turbidity removal values) for some given biopolymer, dose of biopolymer at a given initial organic load of WW.

The most important parameters that affect the efficiency of response variables (COD, turbidity removals and sludge production) are biopolymer dose and initial organic load for COD and turbidity removals. In contrast, type of biopolymer and initial organic load affected more the sludge production. This was because the *p* value of those parameters was less than 0.05 and

Table 4. ANOVA and regression analysis of experimental design

Response	p	PLOF	R^2	AP (adequate precision)	Equation *
COD removal	0.0122	0.8805	0.9436	10.323	51.42 1.36 A -4.42 B +4.25 C +2.86 AB +5.86 AC -0.14 BC -7.96 A^2 +0.43 B^2 -7.28 C^2
Turbidity removal	0.0387	0.1968	0.9071	8.484	68.54 -3.26 A +0.34 B +7.63 C -0.96 AB +1.98 AC -0.47 BC +0.55 A^2 +4.39 B^2 -7.04 C^2
Sludge produced (mL/L)	0.0010	0.1336	0.9801	17.855	10.03 +0.71 A +0.092 B +6.57 C -0.43 AB +1.60 AC -0.68 BC +1.79 A^2 +0.39 B^2 -0.44 C^2
Sludge density (g/L)	0.2838	0.1792	0.7567	4.430	0.033 +2.381E-004 A -2.774E-003 B - 9.852E-003 C +2.867E-003 AB +3.987E-004 AC +3.255E-003 BC -4.778E-003 A^2 - 4.469E-003 B^2 +3.383E-003 C^2
Total metals (mg/Kg)	0.7572	0.1545	0.5223	3.438	795.99 +54.86 A +109.72 B +65.41 C -48.75 AB -6.59 AC + 86.49 BC -52.76 A^2 -42.46 B^2 +154.4 C^2
Cd (mg/Kg)	0.0288	0.3751	0.9183	7.544	5.26 -1.11 A -0. 67 B -4.67 C +1.03 AB +0.79 AC +1.29 BC +0.66 A^2 -0.40 B^2 +4.17 C^2

p = Probability of error. PLOF= probability of lack of fit. AP = adequate precision. *A: biopolymer type ,B: biopolymer concentration (mg/L), C: initial COD load of WW (mg/L).

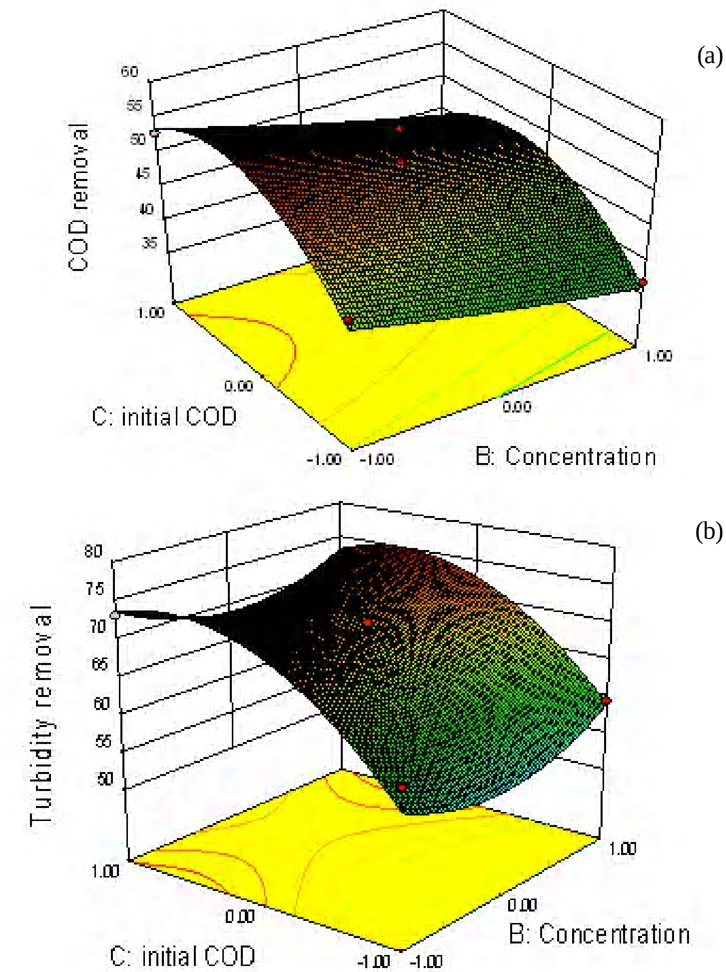

Fig. 1. Design expert plot: response surface plot for: a) COD removal, b) turbidity removal in WW treated after CF processes

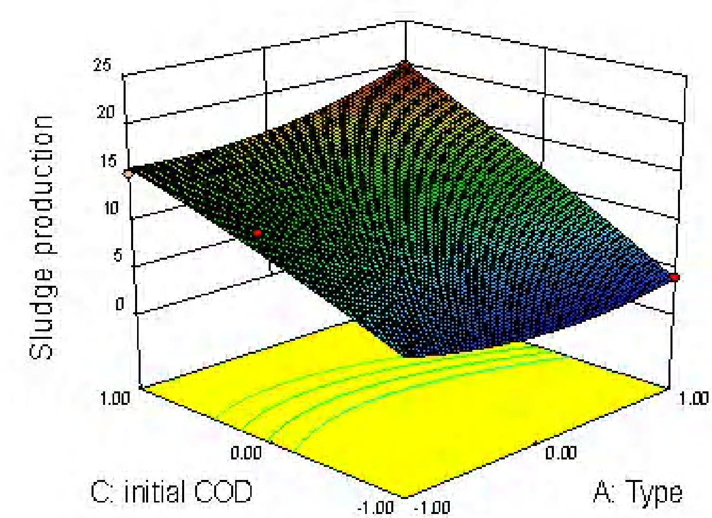

Fig. 2. Design expert plot: response surface plot for sludge production (mL/L) produced after CF processes

Table 5. Metal content in sludges produced after CF process and metal content in biopolymers (mg/kg)

Run	Biopolymer type	Biopolymer dose (mg/L)	COD (mg/L)	Metal in sludges (mg/kg)						Metals in biopolymers (mg/kg)			
				Zn	Cu	Pb	Ni	Cd	Total	Zn	Cu	Pb	Ni
1	Guar gum	25	3	71.43	206.61	61.59	28.93	9.8	485.12	27.15	2.71	25.81	141.25
2		50	3	219.36	94.96	21.00	10.38	4.49	350.28				
3		50	2	822.65	242.62	29.04	32.31	6.18	1141.38				
4		75	1	585.07	199.81	46.23	68.41	14.56	914.08				
5	*Opuntia* mucilage	25	3	491.14	155.39	20.25	20.26	3.76	693.84	156.63	37.19	39.01	37.05
6		50	1	479.74	181.12	45.86	59.90	13.42	780.03				
7		50	2	734.96	214.51	28.39	21.44	4.72	1010.51				
8		75	3	675.79	211.31	8.79	23.87	4.91	931.89				
9		25	1	570.48	185.42	44.93	89.44	15.45	905.71				
10	Mesquite seed gum	25	2	491.14	155.39	20.25	20.26	3.76	693.84	45.08	11.42	25.44	4.04
11		50	3	732.45	217.14	18.13	24.50	7.07	1001.03				
12		50	3	515.06	179.02	22.92	20.91	4.10	751.58				
13		50	3	640.96	159.94	25.47	28.18	6.58	861.29				
14		75	1	509.11	179.65	32.94	47.13	10.06	779.18				
15	$FeCl_3$	75	2	749.92	221.01	27.67	22.11	4.90	1027.05	267.79	5.04	11.26	22.29
16		25	3	671.68	178.67	29.48	31.33	7.34	921.30				
17		50	3	657.54	197.53	38.32	47.40	9.93	950.86				
18		75	3	335.27	115.78	26.23	56.99	7.99	542.26				

the coefficients were with positive sign (data no shown.): this means that they induced a significant effect on response variables.

In order to investigate the integrated effect of the biopolymer and the initial COD of WW, the RSM was used and 3D plots are the result. In WW treated (Fig. 1a), with an increase in the initial COD, the COD removal (%) increased with lower biopolymer dose (25 mg/L). Wang et al. (2005) have proposed ranges that are related with the dose of coagulant, that is, from one where the dose of coagulant is not enough to destabilize the colloids to another where there is an excess of coagulant concentration, causing the reversal of burden and stabilization of colloidal particles.

In Fig. 1b, the 3D plot indicated that turbidity removal increases with an increase of initial COD. There seems to be no significant difference among the three doses of biopolymer used (25, 50 and 75 mg/L). More than 70% is the maximum of turbidity removal for this model. This is quite enough if we consider that CF is a primary treatment and it is not the only method for treating WW.

The integrated effect of initial COD and type of biopolymer over sludge produced is shown in the 3D plot in Fig. 2. As expected, the higher the initial COD is, more sludge is produced (between 15-20 mL/L). *Opuntia* mucilage produced more sludge than guar and mesquite gums.

Of all metals analyzed in the biopolymers and $FeCl_3$ (Al, Ca, Cu, Fe, K, Mg, Na, Ni, Zn and Pb), *Opuntia* mucilage showed the highest values for all the metals (data no shown). We have the hypothesis that these metals arise from the water used for the production of the mucilage. This biopolymer acts as an adsorption column, trapping all the available metals. However, if we compare values of Cu, Ni, Zn, and Pb with the Mexican standard (NOM, 2002), the content of metals in sludges is below the permissible limits for heavy metals in biosolids. For example, experiments with *Opuntia* mucilage present values of Cd between 3.76-13.42 mg/kg and the Mexican standard accepts between 39-85 mg/kg. Cu in *Opuntia* mucilage was of 155-211; Pb, 8.79-45.86; and Zn, 479-734 mg/kg. In Mexican standard values are Cu, 1500-4300; Pb, 300-840 and Zn, 2800-7500 mg/kg. As expected, $FeCl_3$, had the highest concentration of Fe (188,201 mg/kg). In contrast, Ca, K, Na and Pb were present at lower amounts in $FeCl_3$ than in the biopolymers. It is important to remark that $FeCl_3$ coagulant employed in this work was of analytical grade, and it is assumed that the industrial-grade reactive might contain more metals at higher concentrations.

Table 5, shows the contents of Zn, Cu, Pb, and Ni in biopolymers, $FeCl_3$, and in sludges produced after CF (in mg/kg). It is important to note that in the column named Total metals corresponds to the sum of all metals evaluated in the produced sludge. We attempted to find a correlation between the metals present in biopolymers and those in the produced sludges (data no shown). In the case of Zn, we observed a direct relationship between guar and *Opuntia* mucilage with correlation coefficients (R^2) of 0.94 and 0.65, respectively. This means that the higher doses of biopolymer used, the more Zn was present in the sludge. However, for mesquite seed gum and $FeCl_3$, the relationship was inverse (R^2= 0.05 and 0.78, respectively) i.e. the higher the dose, the lower was the content of Zn in the sludges. We have no explanations for this finding. Regarding *Opuntia* mucilage, Cu was directly correlated with the metal content in the produced sludge when employing *Opuntia* mucilage in CF (R^2= 0.99). $FeCl_3$ also presented positive correlation with Ni content in the produced sludge (R^2= 0.97) and an indirect correlation with Cu (R^2= 0.53). As far as we know, there are no previous reports regarding the natural coagulant-flocculant metal-contents; besides, there are very scarce reports regarding the characterization of produced sludges in terms of their densities and metal contents.

CONCLUSION

The studied WW presented COD and BOD_5 values are of 1,141.3 and 391.1 mg/L, respectively, giving a BOD/COD ratio of 0.34 (moderately biodegradable). Total solids had a level of 1,591 mg/L, MBAS and oil and greases 117 and 76 mg/L. Besides, four metals were measured. Upon the results of the jar tests it was found that guar gum removed 74.5 and 72.7 % of turbidity, followed by mesquite seed gum with 73.09 %. *Opuntia* mucilage removed 71.08 %. $FeCl_3$ removed 71, 73.5 and 75.5 % at the different doses used. COD removals as high as 56.1 and 52.81% were observed when mesquite seed gum was employed. The second best value corresponded to guar gum 51.4%; followed by *Opuntia* mucilage i.e., between 44.2 and 44.4%. For the assessments with $FeCl_3$, the maximum COD removal was as high as 65.4%. Regarding the statistical analysis, the PLOF values > 0.05 show that the F-statistics was insignificant, which implies a significant model correlation between the variables and process responses. Regarding the fit of the model, the R^2 values were up to 0.98 for sludge produced, 0.94 for COD removal, 0.91 for Cd, 0.90 for turbidity removal, and 0.75 for sludge density. Using the quadratic equations it is possible to predict response variables for some given biopolymer, dose of biopolymer at a given initial organic loads of WW. We attempted to find a correlation between metal present in biopolymers and in the produced sludges. In the case of Zn, we observed a direct relationship between guar and *Opuntia* mucilage with correlation coefficients of 0.94 and 0.65, respectively. This means that higher doses of biopolymer used, the more Zn was present in the sludge. However, for mesquite seed gum and $FeCl_3$, the relationship was inverse.

Regarding the RSM analysis, the most important parameters that affect the efficiency of response variables (COD, turbidity removals, and sludge production) are the biopolymer dose and initial organic load for COD and turbidity removals. In contrast, type of biopolymer an initial organic load affected more sludge production. As far as we know, there are no previous reports regarding the natural coagulant-flocculant metal-contents, besides, there are very scarce reports regarding the characterization of produced sludges in terms of their densities and metal contents. The RSM has shown to be useful for the design of experiments to investigate the effect of the three evaluated parameters (biopolymer type, dose, and COD load in WW load) on the response parameters (COD, turbidity and salts removals as well as on the amount and quality of the produced sludges). It is noteworthy that RSM also allowed optimizing the CF process. Employing this methodology it is feasible to determine COD, turbidity and salts removals, as well as the amount and quality of the produced sludges for hypothetical conditions within predetermined parameter ranges, without the need of carrying out experimental runs.

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