



SIMULTANEOUS REMOVAL OF DISSOLVED ORGANIC MATTER, *Microcystis aeruginosa*, AND MICROCYSTIN-LR BY PRE-OXIDATION AND COAGULATION-FLOCCULATION PROCESSES

REMOCIÓN SIMULTÁNEA DE MATERIA ORGÁNICA DISUELTA, *Microcystis aeruginosa* Y MICROCISTINA-LR POR LOS PROCESOS DE PREOXIDACIÓN Y COAGULACIÓN-FLOCULACIÓN

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Received: August 22, 2018; Accepted: October 25, 2018

Abstract

The aim of this work is to assess the effect of the presence of *Microcystis aeruginosa* (MA) and microcystin-LR (MC-LR) on the removal of dissolved organic matter (DOM) by coagulation-flocculation operated at optimal conditions. Also, the effect of pre-oxidation with NaOCl on the simultaneous removal of MA, MC-LR, and DOM was studied, using the same processes and conditions. The water used in the experimental tests was prepared with distilled water, strains of MA, microcystin-LR standards, and model compounds of DOM. All trials were carried out in a jar test equipment applying two designs: a fractional factorial design to identify those variables with significant influence, and a response surface to determine the optimal operational conditions for each of the evaluated processes. Results show that the simultaneous presence of MA and MC-LR reduce until 32.25% the DOM removal, because they were competitors for the coagulant. The main effect was associated to MA due to its physicochemical characteristics and its major concentration. The pre-oxidant dose did not have a significant influence on the removal of MA, MC-LR and DOM since the contact time used in the tests was very short.

Keywords: cyanobacteria, cyanotoxins, clarification, sodium hypochlorite, dissolved organic carbon, drinking water .

Resumen

En este estudio se evaluó el impacto de la presencia de *Microcystis aeruginosa* (MA) y de la microcistina-LR (MC-LR) sobre la remoción de la materia orgánica disuelta (MOD) por coagulación-floculación en condiciones óptimas. Adicionalmente, se determinó el efecto de la preoxidación con NaOCl en la remoción simultánea de MA, MC-LR y MOD, empleando esos mismos procesos y condiciones. En las pruebas se utilizó agua destilada dopada con una cepa de MA, con estándares de MC-LR y con compuestos modelo de la MOD. Los experimentos se realizaron en un equipo de prueba de jarras, utilizando un diseño factorial fraccionado para determinar los factores de influencia significativa, y uno de superficie de respuesta para estimar las condiciones óptimas de operación de los procesos evaluados. Los resultados mostraron que la presencia de MA y MC-LR disminuye 32.25% la remoción de la MOD por un efecto de competencia por el coagulante, asociado principalmente a la MA, ya que presentó una mayor concentración y características fisicoquímicas que la hacen más susceptible de removerse por coagulación. La dosis de preoxidante no tuvo influencia significativa en la remoción de los tres contaminantes, debido a que el tiempo de contacto utilizado en las pruebas fue bajo.

Palabras clave: cianobacterias, cianotoxinas, clarificación, hipoclorito de sodio, carbono orgánico disuelto, agua potable.

1 Introduction

Dissolved organic matter (DOM) includes all organic compounds with hydrophilic (HPI) and hydrophobic (HPO) characteristics that pass through a 0.45 μm pore diameter membrane. These compounds

are generated through natural biological, chemical and photochemical reactions issued from the decomposition of plants and animals in aqueous medium, and they are present in all types of water bodies (Hendricks, 2006; MWH, 2012). Removal of DOM is important because it affects the water organoleptic quality (color, odor, and taste), is a precursor of disinfection by-products, and it is the

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<https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Sandoval>
issn-e: 2395-8472

most abundant fraction of natural organic matter in water (up to 90% measured as dissolved organic carbon (DOC)) (Hendricks, 2006; Matilainen *et al.*, 2010; MWH, 2012). However, this fraction is the most difficult to remove by conventional potabilization processes due to its physicochemical characteristics: highly hydrophilicity or solubility, with negative electrical charge, a molecular weight between 500 and 3000 Da, and a composition integrated by 45-60% carbon, 4-5% hydrogen, 35-40% oxygen, and 1% nitrogen (Hendricks, 2006; Sillampaa, 2015). Furthermore, the removal of the DOM can be reduced by seasonal growths or blooms of cyanobacteria and its metabolites (AWWA, 2010; Zamyadi *et al.*, 2012).

Cyanobacteria are prokaryotic, autotrophic, photosynthetic organisms which are classified as Gram-negative bacteria. A bloom or exponential growth of cyanobacteria occurs when the conditions in a water body are adequate for its development (temperature, nutrients, and pH). Nevertheless, cyanobacterial blooms have increased their frequency in number and intensity due to climate change and human activities (AWWA, 2010; May-Cua *et al.*, 2019; Navarro-Peraza *et al.*, 2017; Quiblier *et al.*, 2013). The main problem of blooms is the excessive production of toxic metabolites, known as cyanotoxins, which are part of the DOM and represent a health risk, even in trace concentrations. Drinking water treatment plants that use coagulation-flocculation do not have the capacity to efficiently remove these contaminants during blooms. As a result, the effluent does not comply the required organoleptic and toxicological water quality, the filtration runs of sand filters decrease, the demand of coagulants, flocculants, adsorbents and oxidants increases, as well as the operation and maintenance costs of water treatment plants (AWWA, 2010).

MC-LR is a cyanotoxin produced by most of the cyanobacterial species, which is very stable in water, one of the most toxic and it has been related with the highest number of intoxication reports in human beings (Stewart *et al.*, 2006; Westrick *et al.*, 2010; Pantelic *et al.*, 2013; Merel *et al.*, 2013). In Mexico, there are some reports of the presence of extracellular MC-LR (dissolved in water) with values above the limit ($1 \mu\text{g}\cdot\text{L}^{-1}$) recommended by the World Health Organization (WHO) (Alillo *et al.*, 2014; Vasconcelos *et al.*, 2010). As consequence, the MC-LR maximum concentration is the most regulated in international standards for drinking water. Mexico recently began to establish a standard (COFEMER, 2017). Due to its high abundance and physicochemical

properties (hydrophobicity, negative charge, and solubility), MC-LR competes with the rest of the DOM compounds to be removed through conventional potabilization methods. For these reasons, MC-LR and cyanobacterium MA, one of the main producer of this cyanotoxin, were selected to be evaluated in this work (Šejnohová, 2008).

Coagulation-flocculation (CF) is one of the most commonly conventional process used in water treatment facilities due to its low cost and because it is easy to operate. Only in Mexico, 24% of the installed drinking water treatment plants utilize it, thus this is equivalent to 69% of the drinking water production (SEMARNAT, 2016). Coagulation-flocculation has individual removal efficiencies of cyanobacteria ranging from 70 to 90%, cyanotoxins from 0 to 39%, and DOM (quantified as DOC) from 10 to 60% (Lambert *et al.*, 1996; Hendricks, 2006; Merel *et al.*, 2013b). However, the simultaneous removal of these three pollutants, and the effect of cyanobacteria and their cyanotoxins on the DOM removal by coagulation-flocculation have been scarcely studied. Thus, the main objective is to improve the CF efficiency to remove DOM once the three compounds are present. In particular it is priority to increase the removal of cyanobacteria because they cause operational problems to water treatment plants and they have a high sanitary importance (Sillampaa, 2015). For this purpose, one suggests the combination of CF with other processes such as pre-oxidation, which has been reported as a coagulant aid, easy to apply and has a low costs (Matilainen *et al.* 2010; Sillampaa, 2015).

In this work, NaOCl was applied as a coagulant aid in the simultaneous removal of these pollutants using the optimal operating conditions for a potabilization train, which is composed of the pre-oxidation-coagulation-flocculation-sedimentation (PCFS) processes.

2 Methodology

The methodology was divided into three main stages: the first one consists in the preparation of the water samples to be treated; the second one involves the elaboration of two experimental designs (fractional factorial to determine the factors of significant influence and response surface to obtain the optimal conditions for the maximum simultaneous removal of DOM, MA and MC-LR); and the last stage

includes laboratory tests performed to determine the effect of the presence of both MA and MC-LR on the removal of DOM and the application of pre-oxidation with NaOCl to evaluate its impact on the simultaneous removal of the three pollutants under optimal operating conditions for the treatment train proposed in this study.

2.1 Materials and reagents

Polluted samples, prepared in a type II water (resistivity 15 M Ω .cm), were used in all experimental tests carried out in this work. The preparation of these samples consisted in adding different amounts of one MA strain, MC-LR standards, and seven representative model compounds of the different DOM fractions present in surface waters (Table 1). DOM was quantified as Dissolved Organic Carbon or DOC (mg/L) in a TOC-LCSH Shimadzu analyzer. The strain of *Microcystis aeruginosa* was obtained from the bacteriology laboratory of the FES-Iztacala, UNAM; its culture was carried out under dynamic conditions at a temperature of 28 ± 0.5 °C in a 14h photoperiod and it was quantified in a Neubauer chamber (cell mL⁻¹). MC-LR standard (Cayman Chemical) was used applied and its concentration was quantified by HPLC-PDA with a previous concentration from solid phase extraction using STRATA-X cartridges. To provide alkalinity and turbidity to the water samples, sodium bicarbonate (NaHCO₃) (J.T. Baker) and bentonite (SIGMA-ALDRICH) were used, respectively. Aluminum sulfate (Al₂ (SO₄)₃·18H₂O) (J.T. Baker) was used as coagulant and sodium hypochlorite (NaOCl) as a pre-oxidant. The pH was adjusted using sulfuric acid (H₂SO₄) and sodium hydroxide (NaOH), both of reactive grade.

2.2 Stage 1: Preparation of water samples for treatment

In each experimental laboratory test, 2 L of synthetic water sample were used; the samples were prepared in distilled water by adding: 1) 1.5 g of sodium bicarbonate to obtain an alkalinity of 750 mg L⁻¹ as CaCO₃; 2) DOM compounds as presented in Table 1, in amounts according to the experimental design, in all cases the DOM initial concentration was 5 mg DOC L⁻¹; 3) the MA strain; 4) the MC-LR standard; 5) bentonite to obtain an initial turbidity of 5 UTN, and 6) sulfuric acid or sodium hydroxide to adjust the initial pH. The DOC concentration used in the tests was the average value reported for most of the drinking water supply surface sources. The turbidity was the average value reported for most of water surface supplies during the dry season. The alkalinity value was selected to avoid pH variations due to the coagulation-flocculation process (Hendricks, 2006; MWH, 2012).

2.3 Stage 2: Experimental designs

After preparing the water samples, trials of pre-oxidation-coagulation-flocculation-sedimentation were performed in a jar test equipment (JARTESTER PB 700 PHIPPS and BIRD), based on two experimental designs, which were established using the STATGRAPHICS software and selecting as response variables the removal efficiency of DOM, MA, and MC-LR. The duration of each process was established based on the times applied in "Los Berros" (Ortíz, 2014) and "Madín" (Félix, 2010) water treatment plants, and also on the results reported by Cerón *et al.* (2016) (Table 2).

Table 1. Typical composition of DOM fractions as DOC and representative model compounds.

Fraction	% DOC	Model compound
Hydrophobic acid (HPOA)	47	Fulvic and humic acids
Hydrophobic basic (HPOB)	2	Aniline
Hydrophobic neutral (HPON)	3.7	Gallic acid
Hydrophilic acid (HPIA)	16.3	Citric acid
Hydrophilic basic (HPIB)	3	Glutathione
Hydrophilic neutral (HPIN)	28	Polysucrose

Source: Imai *et al.*, (2001); Kennedy *et al.*, (2005); Zularisam *et al.*, (2006/2007)

Table 2. Experimental conditions of P-C-F-S processes.

Parameter	Time	Gradient
Pre-oxidation	10 s	600 s ⁻¹ (273 rpm)
Coagulation	1 s	600 s ⁻¹ (273 rpm)
Flocculation	5 min	100 s ⁻¹ (83 rpm)
	5 min	42 s ⁻¹ (45 rpm)
	10 min	25 s ⁻¹ (30 rpm)
Sedimentation	30 min	-

The first experimental design used in this work was a fractional factorial type to evaluate the effect of six factors and to determine those of significant influence on the three response variables (removal of DOC, MA, and MC-RL). The formula to calculate the number of experiments is $2^{k-p} + n_c$, where: 2 is a constant that indicates the levels of the factors (-1 and +1 in coded values), k is the number of factors, p indicates the experimental design fraction to be selected for performing the tests, and n_c is the number of replications of the central point experiment. A quarter of experiments of the original design was selected with three replications of the central point, thus a total of 19 experimental tests were performed with the pre-oxidant. With a quarter of this design a resolution IV is obtained, in other words the main effects of the factors can be estimated and, as a consequence, the determination of those parameters of significant influence can be made. In addition, the replicates in the central point allowed to evaluate the existence

of curvature, which indicates the need of a higher order model (Gutiérrez and de la Vara, 2008). The factors evaluated in this experimental design were: initial pH, coagulant dose, pre-oxidant dose, initial concentration of MA, initial concentration of MC-RL, and the percentage ratio between hydrophobic and hydrophilic water characteristics (HPO:HPI).

Table 3 shows the codified and natural values of the evaluated factors, as well as the references and the criteria applied for their selection.

For *M. aeruginosa* (MA), the cell density reports for algae consortiums detected in the Valle de Bravo reservoir reached maximums of 1×10^5 cell mL⁻¹ on October 2000 and April 2001 and for later on June 2010, it was 14×10^4 cell mL⁻¹ (Gaytan *et al.*, 2011; Alillo *et al.*, 2014). The value selected for this study was of 1×10^5 cell mL⁻¹.

The second experimental design was based on the response surface, using the Box-Behnken type ($2^k + 2k + n_c$), to evaluate the influence of four factors. For this design, three replications of the central point test were performed, thus 27 experimental tests were carried out. The experimental results were analyzed to obtain a statistical model that was used to calculate the optimal value of each factor to allow the maximum simultaneous removal of contaminants. This design was of second order, thus it is able to determine the optimum point within the limits of the experimental space established by high (1) and low (-1) levels of the factorial design (Gutiérrez and de la Vara, 2008).

Table 3. Real and coded values of the factors evaluated by fractional factorial design.

Factors	Unity	Level			Criterion	Reference
		-1	1	0		
[MC-RL] _o	μg/L	1.5	3.25	5	Maximum dissolved concentration reported in a body of water to supply the largest water treatment plant in Mexico	Alillo <i>et al.</i> (2014)
[Coagulant] _o	mg Al/L	3.6	8.55	13.5	Typical aluminum dose for initial DOC concentrations until 10 mg/L	Cerón <i>et al.</i> (2016)
pH	-	5	6.5	8	Typical pH in surface water	Cerón <i>et al.</i> (2016)
[MA] _o	cell/mL	20 000	60 000	100 000	Minimum and maximum concentrations found in a body of surface water	WHO (1999)
HPO:HPI	%:%	80:20	50:50	20:80	Typical variations of water composition	Cerón <i>et al.</i> (2016)
[NaOCl] _o	mg/L	0.25	1.125	2	Typical doses of NaOCl that do not cause damage to the cell wall of cyanobacteria	Zamyadi (2012)

2.4 Stage 3: Laboratory water treatment tests

Jar tests were performed in duplicate applying the calculated optimal conditions to determine the effect of pre-oxidation with NaOCl in the simultaneous removal of MA, MC-LR, and DOM and the influence of the presence of MA and MC-LR on the DOM removal.

3 Results and discussion

3.1 Factors of significant influence (factorial experimental design)

An analysis of variance (ANOVA) with a 95% confidence level of the results of the factorial design tests (a quarter of the experimental design) was performed. The number of data values of this design were sufficient to identify factors of significant influence on the response variables (removal of DOM, MA, and MC-LR) (Gutiérrez and de la Vara, 2008).

3.1.1 Factors of significant influence on the DOM removal

The Pareto chart of the DOM removal results (quantified as DOC) is presented in Figure 1, which shows that the factors of significant influence were: coagulant dose, HPO:HPI ratio, and pH. Due to the coagulant dose showed a positive effect on the process, thus a dose increase will produce a rise of the DOM removal efficiency. This factor was the one with the greatest significant influence in these tests, probably due to its capacity to destabilize the particles charge and ability to favor the formation of complexes with contaminants with their subsequent removal from water (Hendricks, 2006). The effect of the HPO:HPI ratio was negative, and thus the DOM removal efficiency will augment as the concentration of the hydrophobic organic matter increases; these results agree with data reported in literature (Zhang *et al.*, 2012; Sillampaa, 2015), which are explained on terms of the HPO organic matter composition. This DOM fraction is mainly composed of humic substances with high molecular weight, which consists of several phenolic structures, conjugated double bonds, and carboxyl groups that are efficiently removed using aluminum salts. The pH showed a negative significant influence, and thus a lowering

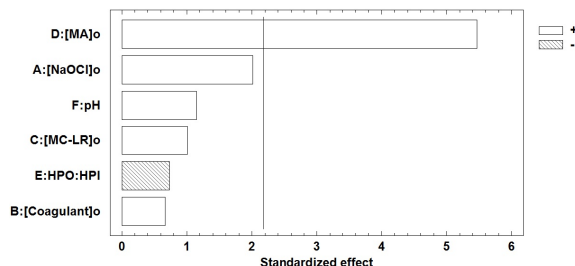


Fig. 2. Factors of significant influence in the removal of MA by P-C-F-S.

of the pH value represents a greater DOM removal. This effect is associated to pH determines the aluminum species, the coagulation mechanism, the speciation of NaOCl, and the ionization of the different functional groups of MON, MA, and MC-LR (Rivasseau *et al.*, 1998; Hendricks, 2006; Merel *et al.*, 2010; Zhang *et al.*, 2012); therefore, at acidic pH values, the removal can be favored due to the predominance of Al polymeric species, which have higher density of positive charge compared to the monomeric species formed at higher pH. Also, in acid conditions the hypochlorous acid (HOCl) concentration increases (which is the most reactive species of the NaOCl hydrolysis), the stability of MA decreases, and the dissociation of DOM and MC-LR compounds increments (Rivasseau *et al.*, 1998; Hadjoudja *et al.*, 2010; Meneses, 2011; Cerón *et al.*, 2016).

3.1.2 Factors of significant influence on the MA removal

Figure 2 shows the Pareto chart for the MA removal, which illustrates that the MA initial concentration was the only factor of significant and positive influence; consequently, the MA removal depends on its own initial concentration, and thus the efficiency will increase with an augmentation of this factor. As shown in Figure 2, the magnitude of the influence of this factor was significantly higher than the one of others. This means that the natural value of the upper level of the factor has been significantly low to observe a significant effect of the others (Gutiérrez and de la Vara, 2008), and therefore it is recommended to increase the value of this factor. However, it is difficult to detect concentrations of MA higher than the cell density proposed, considering the reports of Alillo *et al.* (2014), Gaytan *et al.* (2011), and WHO (1999); thus, it was chosen that the value of this factor remained constant for the optimization tests,

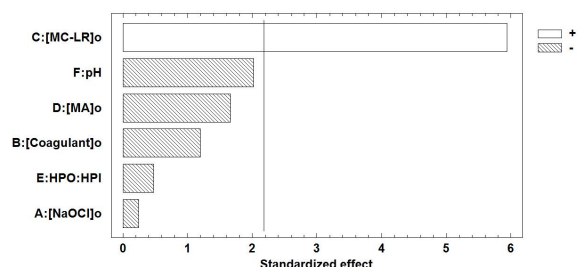


Fig. 3. Factors of significant influence in the removal of MC-LR by P-C-F-S.

which was the maximum concentration used in the first experimental design.

3.1.3 Factors of significant influence on the MC-LR removal

The statistical analysis (Pareto chart) of the MC-LR removal data is presented in Figure 3, which shows that the only significant and positive influence factor was the initial concentration of MC-LR. As occurred for the MA, this indicates that the initial value of the upper level used for this factor in the experimental design was excessively low and thus did not allow observing an effect of the others. However, it was selected to keep constant the initial MC-LR concentration at the highest value used in this experimental design, because it is the maximum dissolved concentration detected in a surface water used as supply source in the largest Mexican water treatment plant (Alillo *et al.*, 2014).

All factors of significant influence (coagulant dose, pH, and the HPO:HPI ratio) on the process were evaluated in the optimization tests. Also, even if the pre-oxidant dose in the studied interval did not show a significant influence on the MC-LR removal, this factor was evaluated since there is controversy about its effect as a coagulant aid (Meneses, 2011; MWH, 2012).

3.2 Optimization of the factors values of significant influence for the maximum simultaneous removal of pollutants (Box-Behnken design)

The value of coagulant dose, the HPO:HPI ratio, pH, and the pre-oxidant dose was optimized in this experimental stage. The test results were used to perform an analysis of variance with a confidence level of 95%, using the STATGRAPHICS software. This analysis fulfilled all the assumptions (normality,

constant variance, and independence) to validate the results obtained for each response variable (DOM, MA, and MC-LR); in addition, none of the residuals exceeded ± 2 sigmas to be classified as an atypical data since a value outside this range indicates a significant deviation from normality (Gutiérrez and de la Vara, 2008). The lack of fit was not statistically significant, indicating that the second order model obtained in this work describes adequately the behavior of the three response variables (p-value = 0.2548 for DOM, p-value = 0.4858 for MA, and p-value = 0.2712 for MC-LR).

The value of the determination coefficient (R^2) was appropriate to be used as a goodness-of-fit test, because the number of factors evaluated was less than five (Gutiérrez and de la Vara, 2008). The models and R^2 values are shown in Table 4; in all cases, the values of the determination coefficient were greater than 70%, and therefore the models are suitable for prediction purposes (Gutiérrez and de la Vara, 2008).

The desirability function was used to find a simultaneous removal optimum of the three pollutants. This function is applied if there is more than one response variable and allows to determine the point where all variables show simultaneously a satisfactory value (Gutiérrez and de la Vara, 2008). Desirability can have values between 0 (unacceptable) and 1 (the maximum desirable value). Table 5 shows the optimal value estimated by the desirability function for each of the evaluated factors to obtain the maximum simultaneous removal of the three pollutants; at these conditions the model calculated a desirability value of 0.8.

The experimental results obtained using the optimal conditions are shown in Table 6. When the removal values calculated by the model and the experimental results were compared, DOC showed the smallest difference. The experimental removal efficiencies obtained for MA and MC-LR were significantly lower than the values calculated by the model; this may be related to the significant experimental error reported for the analytical techniques used for the quantification of these pollutants, which could reach up to 30% (Bastidas, 2013).

These results, normalized by milligram applied of aluminum (Table 7), show that the depletion efficiencies of DOM and MA are within the ranges reported in studies conducted to remove separately both contaminants (Merel *et al.*, 2013b; Hendricks, 2006).

Table 4. Model of each response variable and its determination coefficient.

Model	R ² (%)
DOM = 2.91 + 0.09 * A + 0.78 * B - 0.19 * C + 0.07 * D - 0.45 * A ² - 0.05 * A * B + 0.03 * A * C - 0.24 * A * D - 0.60 * B ² + 0.58 * B * C + 0.46 * B * D - 0.02 * C ² + 0.41 * C * D - 0.47 * D ²	70
MA = 74444.3 - 15277.8 * A + 33888.9 * B - 5833.3 * C - 18333.3 * D - 2777.7 * A ² + 833.5 * A * B + 12500.0 * A * C - 4166.5 * A * D - 10694.3 * B ² - 2500.0 * B * C + 31666.7 * B * D - 25277.7 * C ² - 27500.0 * C * D + 21805.7 * D ²	84
MC-LR = 1.54 + 0.41 * A + 0.30 * B - 0.80 * C - 1.51 * D + 0.83 * A ² + 0.79 * A * B + 0.54 * A * C - 0.48 * A * D - 0.31 * B ² - 0.99 * B * C + 0.54 * B * D + 1.02 * C ² - 0.40 * C * D + 0.39 * D ²	76

A = pre-oxidant, B = coagulant, C = HPO:HPI relation and D = pH

Table 5. Optimal values estimated for each factor based on the function desirability (value = 0.8).

Factor	Estimated optimal value
Pre-oxidant dose (mg NaOCl/L)	0.44
Coagulant dose (mg Al/L)	12.23
HPO:HPI relation (%:%)	80:20
pH	7.35

Table 6. Simultaneous removal of pollutants by applying optimal operating conditions of the P-C-F-S processes.

Response variable	Model	Experimental
DOM (mg DOC/L)	2.56	2.59 ± 0.35
MA (cell/mL)	123397	90000 ± 5774
MC-LR (µg/L)	4.23	3.68 ± 0.02

However, the elimination of MC-LR was 35% higher than the average obtained in other studies (Lambert *et al.*, 1996). These results showed that in this study these three contaminants (DOM, MC-LR, and MA) could be efficiently removed simultaneously.

3.3 Effect of MA and MC-LR on the DOM removal by pre-oxidation and coagulation-flocculation

Figure 4 shows the amounts of DOM removed per mg of aluminum for three scenarios: with only the DOM present in water, with DOM and MC-LR simultaneously present, and finally when the three pollutants were simultaneously present.

The DOM removal at optimum operating conditions, using pre-oxidation, was 0.31 ± 0.002 mg DOC mg Al⁻¹ in the absence of MA and MC-LR, and 0.21 ± 0.01 mg DOC mg Al⁻¹, in the presence of both contaminants, which represents an efficiency depletion of 32.25%. The presence of MA produced a diminution of 0.08 mg DOC mg Al⁻¹, which is a decrease of 25.8% of the efficiency, and MC-LR only diminished 0.02 mg DOC mg Al⁻¹ (6.45%). This efficiency decrease can be associated to the competition of both cyanobacteria and their cyanotoxins with DOM for the consumption of NaOCl (pre-oxidant or coagulation aid) and aluminum sulfate (coagulant).

This competition is related to the initial concentration of each pollutant as well as to its physicochemical characteristics (size, solubility, surface charge, chemical composition), which can make them more susceptible to an attack with the pre-oxidant or to be removed by the coagulant.

Table 7. Simultaneous normalized and percentage removal of pollutants.

Response variable	Removal		Individual removal efficiencies reported %
	Normalized	%	
DOM	7,359 ± 385 mg DOC/mg Al	89 ± 7	10-60
MA	0.21 ± 0.01 cell/mg Al	49.45 ± 4.9	70-90
MC-LR	0.30 ± 0.01 µg/mg Al	73.57 ± 0.42	0-39

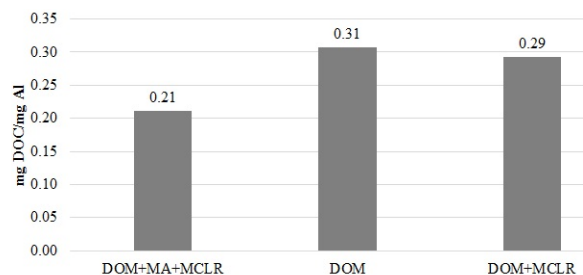


Fig. 4. Effect of the MA and the MC-LR on the removal of DOM (mg DOC/mg Al) in the clarified, applying the optimal conditions of operation of the processes of P-C-F-S.

On the one hand, the pre-oxidant can interact with the conjugated aromatic compounds of DOM, with some groups of MC-LR (3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-group-dienoic acid), and can also affect the electrostatic stability and growth of MA (Ho *et al.*, 2006; Henderson *et al.*, 2008; Shen *et al.*, 2011; Ma *et al.*, 2012). On the other hand, coagulation is more effective to remove colloids with sizes ranging from 0.001 to 10 μm due to their negative charge, high hydrophobicity and molecular weight, such as humic and fulvic acids, which are removed after destabilization or neutralization of their charge by the coagulant. In contrast, coagulation is less effective in removing particles with no charge, hydrophilic character and low molecular weight, such as alkyls and polysaccharides, which can only be removed after formation of complexes or by adsorption on flocs (Hendricks, 2006; Gernaout *et al.*, 2010). Considering this information and that MA is a colloidal particle (4-6 μm) with negative surface charge and present in higher concentration than the one of MC-LR, thus it can be more easily removed by coagulation. For this reason, cyanobacteria had a greater impact on the decrease of the DOM removal. On the contrary, due to the small molecular size (0.003 μm), low surface charge and concentration of the MC-LR, it is more difficult to be removed by coagulation, and consequently this pollutant impacted in a lesser degree the DOM removal (Hendricks, 2006; Aiken, 2014; Sillampaa, 2015).

3.4 Effect of the pre-oxidation process on the removal of MA, MC-LR, and DOM by coagulation-flocculation

In this stage a treatment test was carried out using the optimal operating conditions of the coagulation-flocculation process, without addition of the pre-

oxidant dose, to determine its effect on the DOM, MA,

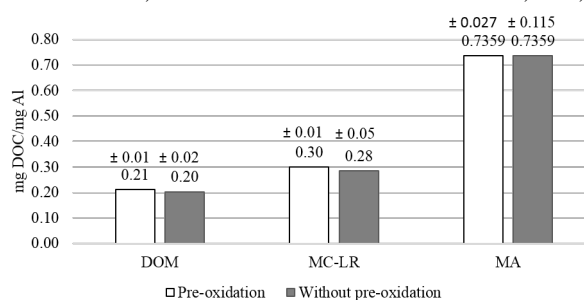


Fig. 5. Effect of the pre-oxidant on the removal of DOM (mg DOC/mg Al), MA ($\times 10^4$ cell/mg Al) and MC-LR ($\mu\text{g}/\text{mg Al}$).

and MC-LR removal. It was observed that there was no significant difference with or without application of NaOCl on the simultaneous removal efficiency of the three pollutants (Figure 5).

This result can be due to the low contact time (10 s) used in the test, which did not allow the development of none of the pre-oxidation mechanisms reported in literature (Henderson *et al.*, 2008; Ma *et al.*, 2012; MWH, 2012), such as transformation or alteration of the compounds, destabilization and inactivation of MA, and production of cellular exo-polymers, that serve as natural coagulants (Figure 6).

It is convenient to remember that this contact time is used in two of the main drinking water treatment plants installed in Mexico, "Los Berros" and "Madín" (Félix, 2010). Nevertheless, several laboratory studies only observed beneficial impacts of pre-oxidation using larger contact times (minutes). Lapsongpon *et al.* (2017) applied 3 mg L^{-1} of calcium hypochlorite in the pre-oxidation process and determined that it took 30 minutes to reach the maximum removal of MA by coagulation-flocculation. These authors mention that the removal was favored by the release of cellular exo-polymers. However, by applying longer contact times, a high generation of these compounds decreases the removal and increases the coagulant demand. They also observed that MA was inactivated with 2 mg L^{-1} and a contact time of 10 minutes, and this facilitated its removal and prevented its growth in subsequent processes. Ma *et al.* (2012) reported that after 20 minutes of pre-oxidation with a dose lower than 1 mg L^{-1} of NaOCl, the natural organic matter increased its molecular mass from 2524 to 2589; this improved 15% the DOC removal, and also the inactivation of MA was achieved. Nevertheless, it was found that with higher pre-oxidant doses, the release of cellular exo-polymers increases, and as

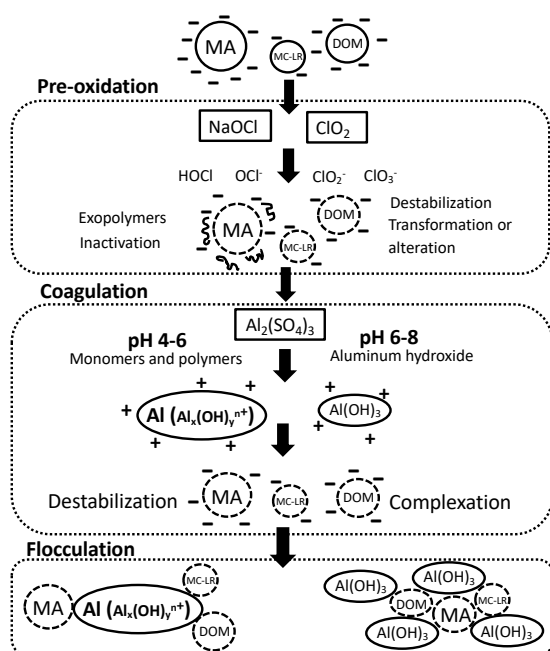


Fig. 6. The possible mechanisms of pre-oxidants and coagulant on simultaneous removal of DOM, MA, and MC-LR.

a result, the coagulant demand increased to obtain the same removal efficiency of pollutants.

Conclusions

In this study, *Microcystis aeruginosa* (MA), microcystin-LR (MC-LR) and dissolved organic matter (DOM) were simultaneously removed efficiently by applying a water treatment train with coagulation-flocculation (CF) as main process under optimal operating conditions. The removal efficiencies of the DOM and MA were within the ranges reported in literature, whereas the removal of MC-LR was 35% higher than the highest values reported.

DOM removal using CF decreased 32.25% in the presence of MA and MC-LR; the former caused the greatest depletion (25.8%) due to its colloidal characteristics and its higher concentration in raw water. In contrast, the MC-LR only decreased 6.45% the DOM removal efficiency, due to its significantly lower concentration ($5 \mu\text{g L}^{-1}$) compared with the DOM content (5 mg DOC L^{-1}). In addition, MC-LR could compete with humic substances ($\leq 0.001 \mu\text{m}$) for the coagulant dose due to its high surface charge and its low molecular size ($0.003 \mu\text{m}$).

The pre-oxidation process with NaOCl did not show a significant influence on the simultaneous removal of the three contaminants mainly for the low contact time used in the experiments. This short time did not permit to develop any of the reported mechanisms as enhancers of the CF process. This result is relevant because the contact time used in this work is similar to the values applied in drinking water treatment plants installed in Mexico. Therefore, it is recommended to use larger contact times than those used in such facilities, in order to have a significant beneficial effect in the simultaneous removal of MA, MC-LR, and DOM.

Acknowledgements

Juan Luis Sandoval Reyes is grateful to CONACYT and DGAPA (Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica, PAPIIT IV100616) for his PhD scholarships. The authors thank Dr. Pedro Ramírez García of the FES-Iztacala of the UNAM to provide the strain of *Microcystis aeruginosa*, to the QFB. Leticia García Montes de Oca of II-UNAM for the support provided in chromatography analyzes by HPLC, and to the Ing. José Luis Rodríguez Pérez of II-UNAM for the construction of the incubator for the cultivation of *Microcystis aeruginosa* strain.

Nomenclature

DOC	dissolved organic carbon
DOM	dissolved organic matter
HPI	hydrophilic
HPO	hydrophobic
MA	<i>Microcystis aeruginosa</i>
MC-LR	microcistina-LR
MOD	materia orgánica disuelta
NaOCl	sodium hydroxide
WHO	World Health Organization

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