



# MS2 coliphage inactivation by Al/Fe PILC-activated Catalytic Wet Peroxide Oxidation: multiresponse statistical optimization



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## ABSTRACT

The optimization of the Catalytic Wet Peroxide Oxidation (CWPO) assisted by an Al/Fe-pillared clay (Al/Fe-PILC) was assessed in the inactivation of the MS2 coliphage in the presence of a synthetic surrogate of natural organic matter (NOM). The simultaneous effect of two experimental factors (i) H<sub>2</sub>O<sub>2</sub> dose - (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> (3.00–25.50 % of the H<sub>2</sub>O<sub>2</sub> theoretically required for full mineralization) and (ii) catalyst concentration (0.33–2.60 g/L), and four non-controllable variables (covariates) (a) circumneutral pH (6.00–9.00), (b) temperature (5.00–25.0 °C), (c) synthetic NOM concentration (2.0–20.0 mg C/L) and (d) MS2 titer (10<sup>4</sup>, 10<sup>5</sup> and 10<sup>6</sup> PFU/mL) was investigated by Response Surface Methodology (RSM). Every response was modeled and maximized: (1) MS2 inactivation, (2) fraction of reacted H<sub>2</sub>O<sub>2</sub>, (3) decolorization and (4) NOM mineralization. Multi-response optimization via desirability function based on responses (1) to (3) achieved excellent fitting (0.94 out of 1.0) and following set of optimal experimental conditions: 0.33 g Al/Fe-PILC/L, 3.36 % (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> (Fe<sub>active</sub>/H<sub>2</sub>O<sub>2</sub>) = 0.46, giving rise to 92.9 % of MS2 inactivation and 100 % of reacted H<sub>2</sub>O<sub>2</sub> at pH 7.07, 25.0 ± 0.1 °C, 16.06 mg C/L as starting NOM concentration, and MS2 titer of 10<sup>6</sup> PFU/mL after just 70 min of reaction.

## 1. Introduction

Contamination of water effluents is nowadays increasingly concerning worldwide. Over 1.2 billion people around the world do not have access to drinking water supply services, usually because of poverty (Huesca-Espitia et al., 2017). Consumption of poor-quality drinking water is associated with several public health problems because of the presence of chemical pollutants and pathogens (Tsydenova et al., 2015). It is well-known that many cases of gastrointestinal diseases as well as nervous system diseases in the world are caused by waterborne viruses (Giannakis et al., 2017b); in 2012 around to 1.5 million human deaths were caused by diarrhea, which usually results from the ingestion of contaminated water (Tsydenova et al., 2015).

Waterborne diseases can be caused by enteric viruses present in raw

or insufficiently treated waters (Nieto-Juarez et al., 2010). These microorganisms are more difficult to analyze in laboratory conditions than other pathogens (Cheng et al., 2014); therefore, MS2 coliphage, a single-stranded RNA virus that infects *Escherichia coli* has been frequently used as a model for viral studies due to the fact that its size and structural complexity are similar to those of enteric viruses. Besides, it is not pathogenic for humans and its culture is cost-effective and easy to perform in laboratory conditions to reach the high titers required for inactivation analyses (Giannakis et al., 2017a).

In the other hand, chlorination is one of the most applied disinfection treatments. However, its main disadvantage is the production of hazardous disinfection by-products (DBPs) (Cheng et al., 2014). Advanced Oxidation Processes (AOPs) are considered promising alternatives inactivating microorganisms (Galeano et al., 2017); these are a set of

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technologies based on the production of strongly oxidizing radicals capable of mineralize almost any kind of organic matter (Huesca-Espitia et al., 2017). Particularly, Fenton and Fenton-like processes have been successfully proven for the inactivation of highly resistant microorganisms (Galeano et al., 2017; Huesca-Espitia et al., 2017). The Fenton reaction occurs when  $H_2O_2$  reacts with ferrous/ferric ions producing powerful oxidizing species such as the hydroxyl radical (HO.) (Giannakis et al., 2017a; Kim et al., 2010). Nevertheless, homogeneous Fenton reactions could be limited by the generation of high amounts of iron sludge together with restricted operational range of pH (Mirzaei et al., 2017; Nieto-Juarez et al., 2010), whereas the heterogeneous variation, so-called Catalytic Wet Peroxidation Oxidation (CWPO), has been considered more feasible because it widens the operational range of pH by using a solid catalyst more easily separable from the treated effluents (Mirzaei et al., 2017). CWPO activated by transition metals such as Fe and Cu has shown to be very efficient depleting several organic contaminants on different types of supports (Munoz et al., 2017b; Ribeiro et al., 2013); moreover, several authors have reported an excellent performance of Al/Fe-Pillared Interlayered Clays (Al/Fe-PILCs) catalyzing activation of the oxidizing agent  $H_2O_2$  (Galeano et al., 2014; Sanabria et al., 2009). Clays in general are interesting catalyzing AOPs because of their abundance and low cost, high resistance and stability (Hadjiltaief et al., 2015). Considering its high performance in the CWPO degradation of different types of pollutants, in our research an Al/Fe-pillared clay-catalyst was used (Munoz et al., 2017a). Although the efficiency of virus inactivation using classic Fenton and its modifications has been studied (Kim et al., 2010), as long as we know no attempts have been reported to determine the activity of the Al/Fe-PILC-activated CWPO technology against viruses. Since in the heterogeneous Fenton reaction many experimental factors may affect the catalytic response (Mirzaei et al., 2017), alongside the stringent quality parameters featuring drinking water, it is mandatory to optimize reaction conditions to obtain most significant MS2 inactivation through the lowest possible remaining fraction of hydrogen peroxide in output stream.

This study aimed at the simultaneous optimization of main operational parameters of the CWPO process in the MS2 coliphage inactivation using the RSM statistical tool. A set of experimental assays was carried out under a range of either Al/Fe-PILC clay-catalyst concentration and peroxide dose ( $H_2O_2$ )<sub>d</sub>, recording MS2 inactivation under variable either temperature (5.0–25.0 °C), pH (6.00–9.00), concentration of a synthetic surrogate of natural organic matter (NOM) given as initial Dissolved Organic Carbon DOC (2.0–20.0 mg C/L) and viral titer ( $10^4$ – $10^6$  PFU/mL) as non-controllable, experimental factors (covariates). Four responses were recorded as a function of the reaction time: MS2 inactivation, fraction of reacted  $H_2O_2$ , contaminant's mineralization (Dissolved Organic Carbon – DOC removal) and decolourization.

## 2. Materials and methods

$H_2O_2$  was purchased from Panreac (50 % solution). Polygalacturonic acid, polyacrylic acid, humic acid and polystyrene sulfonate standards in different average molecular weights (PSS1 = 1,000 kDa; PSS2 = 200 kDa) were all purchased from Sigma–Aldrich and used as received in preparation of the synthetic NOM in UHP type I water.

### 2.1. Strains and culture conditions

MS2 coliphage was the model organism for this study and *Salmonella typhimurium* WG49 was used as its host cell. Both strains were kindly provided by the Laboratory of Microbiology of Water Related to Health from University of Barcelona, Spain. A working of *S. typhimurium* ( $9.7 \times 10^9$  CFU/mL) and a stock of MS2 coliphage ( $1.4 \times 10^{10}$  PFU/mL) were prepared according to the International Organization for Standardization (ISO, 1995). Composition of growth broth was tryptone 10 g/L; yeast extract 1.0 g/L; NaCl 0.8 g/L and glucose 0.1 g/L. Incubation conditions were 37 °C for 18–22 h.

### 2.2. Preparation of synthetic NOM surrogate solution

In order to simulate a matrix of real surface water, a synthetic surrogate of NOM was prepared in order to find out the effect of the virus inactivation in the presence of natural compounds commonly found in real water supplies of drinking water treatment plants; thus, DOC in starting solutions was fixed between 2.0 and 20.0 mg C/L, a range corresponding to DOC values typical in real surface waters at south-western Colombia. Composition in terms of carbon contribution was 25.0 % of humic acid (standard solution 1,000 mg C/L), 30.0 % of polygalacturonic acid (standard solution 1,000 mg C/L), 20.0 % of polyacrylic acid (standard solution 1,000 mg C/L), 12.5 % of polystyrene sulfonate 1 PSS1 (standard solution 2,000 mg C/L) and 12.50 % of polystyrene sulfonate 2 PSS2 (standard solution 2,000 mg C/L) (Ramírez and Galeano, 2017).

### 2.3. Experimental setup of inactivation experiments

Catalytic experiments were adapted from a previously described procedure (Galeano et al., 2012). A 1 L synthetic NOM solution was spiked with MS2 at different final titers of  $10^3$ – $10^6$  PFU/mL in a 1.5 L glass, jacketed semi-batch reactor under continuous mechanical stirring (600 rpm) and constant air bubbling (around 2.0 L/min, STP). Temperature, pH, MS2 titer and initial DOC concentration were all fixed at starting values according to the statistical experimental design (Table 1); pH values were only adjusted at the beginning of each experiment (dropping NaOH 0.1 mol/L) but thereon just monitored. All covariates were randomized within mentioned ranges, in order to better mimic typical conditions in common real surface waters. The standard experiments (except dark one) were developed during May to July 2016 at Pasto (Nariño, Colombia) latitude +1.25, longitude -77.25. According to Data Access Service provided by NASA, all sky insolation incident on the horizontal surface of Pasto in that period was in average 3.964 kW-hr/m<sup>2</sup>/day within the range (2.490–5.940 kW-hr/m<sup>2</sup>/day). Al/Fe-PILC clay catalyst was used as active solid for the heterogeneous Fenton reaction with following physicochemical characteristics: Iron content (12.31 as Fe<sub>2</sub>O<sub>3</sub> w/w %; 0.62 as Fe w/w % incorporated by the pillaring procedure, which can be considered for practical purposes the Fenton-active iron - Fe<sub>active</sub>);  $d_{001} = 1.71$  Å;  $S_{BET} = 146$  m<sup>2</sup>/g;  $S_{pp} = 115$  m<sup>2</sup>/g. The detailed procedure employed in the preparation of this clay catalyst is already available elsewhere (Muñoz et al., 2018). First of all, the powdered catalyst (60 mesh) was added to the reactor containing the synthetic NOM surrogate solution spiked with the desired MS2 titer and stirred for 30 min. Subsequently, heterogeneous Fenton reaction was performed during 40 min;  $H_2O_2$  solution (84.0 mL) was started to be dosed at a constant flow rate of 2.1 mL/min to reach final concentrations between 1.65 and 27.43 mg/L in the reactor (assuming no consumption through the addition) (Galeano et al., 2012); such amounts were equivalent to 3.0–25.5 % of the theoretical amount of  $H_2O_2$  assumed to be required for full oxidation of the initial DOC loading in each experiment, calculated as follows: 1000 mg  $H_2O_2$ /L achieves removal of up to 470.6 mg/L of Chemical Oxygen Demand (COD) in iron-activated Fenton reactions (Deng and Englehardt, 2006); likewise, an experimental ratio (0.3 mg DOC/mg COD) was determined for the NOM standard solution used in this study. Therefore, the experimental ratio 7.07 mg  $H_2O_2$ /mg DOC in solution was used to calculate theoretical ( $H_2O_2$ )<sub>d</sub> required for full oxidation of the starting DOC concentration in each experiment. The addition of the catalyst into the reacting mixture was taken as zero time of every experiment, although  $H_2O_2$  addition only started 30 min later. Sampling in all assays was carried out as indicated in Table 2; the samples were filtered through a 0.45 μm syringe membrane and treated with 233 μL of sodium sulfite (1.92 mol/L) in order to scavenge any remaining  $H_2O_2$  that might latter interfere analytical measurements, except of course in the case of the sample used to determine  $H_2O_2$  concentration. In addition, four blank assays were ran in order to realize the individual role of different factors:  $H_2O_2$  blank (B<sub>H<sub>2</sub>O<sub>2</sub></sub>), containing  $H_2O_2$  but not catalyst; catalyst blank (B<sub>cat</sub>), where catalyst was added but not  $H_2O_2$ ; NOM

**Table 1**  
Experimental conditions of the CWPO treatments and measured responses after 70 min of reaction.

Run	Factors		Covariates				Responses			
	Cat. (g/L)	(H <sub>2</sub> O <sub>2</sub> ) <sub>d</sub> (%)	pH <sup>a</sup>	T (°C)	DOC <sup>a</sup> (mg C/L)	Viral titer <sup>a</sup> (PFU/mL)	MS2 Inactivation (%)	H <sub>2</sub> O <sub>2</sub> reacted (%)	Decolourization (%)	NOM Mineralization (%)
1	0.33	14.2	6.26	13.3	3.47	2.23*10 <sup>3</sup>	94.3	86.9	8.0	2.42
2	1.46	14.2	7.30	16.3	7.77	3.35*10 <sup>3</sup>	82.5	30.8	15.7	21.49
3	1.46	14.2	7.32	15.2	14.3	3.12*10 <sup>5</sup>	77.7	50.3	25.8	13.91
4	0.66	22.2	7.47	18.5	6.28	1.63*10 <sup>5</sup>	97.4	99.1	16.0	24.24
5	2.27	6.30	6.85	16.7	3.27	1.70*10 <sup>3</sup>	81.5	100	47.5	15.18
6	0.66	6.30	5.85	17.3	5.28	1.74*10 <sup>5</sup>	95.8	100	24.0	17.37
7	2.27	22.2	5.45	16.2	10.5	4.75*10 <sup>4</sup>	88.8	60.3	23.5	9.37
8	1.46	3.00	6.29	11.8	10.3	2.75*10 <sup>4</sup>	71.1	100	36.1	14.25
9	1.46	25.5	7.09	15.5	14.0	6.20*10 <sup>5</sup>	96.5	68.0	27.9	14.38
10	2.60	14.2	7.01	17.4	19.8	8.90*10 <sup>5</sup>	91.0	70.6	32.8	31.75
11	1.46	14.2	7.20	14.5	12.4	9.35*10 <sup>4</sup>	69.6	79.1	20.7	17.51
B <sub>cat</sub>	2.27	0.00	6.98	25.0	14.8	9.40*10 <sup>5</sup>	82.6	0.00	29.5	0.68
B <sub>H2O2</sub>	0.00	3.36	6.90	19.4	16.2	1.52*10 <sup>5</sup>	25.3	39.5	5.90	5.19
B <sub>NOM</sub>	0.33	3.36	7.02	25.0	0.00	2.57*10 <sup>5</sup>	71.6	100	0.00	NA
B <sub>Dark</sub>	0.33	3.36	6.9	19.4	16.5	1.52*10 <sup>5</sup>	74.2	39.5	10.4	13.36
Opt.	0.33	3.36	7.07	25.0	16.1	3.00*10 <sup>6</sup>	88.4	100	20.2	14.20

<sup>a</sup> pH, DOC, and MS2 titer starting values were experimentally measured. NA: Not applicable.

**Table 2**  
Sampling throughout every experiment of MS2 CWPO catalytic inactivation.

Sample	Assay time (min)	Reaction time (min)	Reaction conditions
t <sub>i</sub>	0	–	NOM standard surrogate spiked with MS2.
t <sub>0</sub>	30	0	NOM standard surrogate spiked with MS2 and catalyst Al/Fe PILC clay.
t <sub>1</sub>	40	10	25 % of H <sub>2</sub> O <sub>2</sub> added
t <sub>2</sub>	50	20	50 % of H <sub>2</sub> O <sub>2</sub> added
t <sub>3</sub>	60	30	75 % of H <sub>2</sub> O <sub>2</sub> added
t <sub>4</sub>	70	40	100 % of H <sub>2</sub> O <sub>2</sub> added
t <sub>f</sub>	100	70	NOM standard surrogate spiked with MS2 and Al/Fe-PILC clay-catalyst. No H <sub>2</sub> O <sub>2</sub> addition through final, recorded 30 min.

blank (B<sub>NOM</sub>), performed under the optimal catalyst and H<sub>2</sub>O<sub>2</sub> conditions but without the presence of synthetic NOM and finally a Dark assay carried out with under optimal experimental parameters as well, but dark conditions.

2.4. Analytical measurements

The viral titer was determined by the double-layer agar method (ISO, 1995). Remaining H<sub>2</sub>O<sub>2</sub> was determined in 0.5 mL samples by the iodometric method (Pupo-Nogueira et al., 2007). NOM decolourization was determined by measuring absorbance at 456 nm in 2.0 mL samples in a UV 1280 Shimadzu spectrophotometer using single wavelength 2120C Standard Method (APHA, 2005), and expressed in platinum-cobalt color units (CU). Finally, NOM mineralization (DOC removal) was measured in 15 mL samples in a Shimadzu TOC-L CPH Analyzer. In the case of inactivation, response was expressed as both Log (N/N<sub>0</sub>) and percentage.

2.5. Statistical design of experiments

A rotatable central composite (2<sup>2</sup>) statistical design of the inactivation experiments was defined to identify the effects of two experimental factors (Table 3) (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> (% of the theoretical amount of H<sub>2</sub>O<sub>2</sub> required

**Table 3**  
Levels of experimental factors in the rotatable central composite (2<sup>2</sup>) statistical design for RSM single-response optimization.

Experimental factors	Level				
(H <sub>2</sub> O <sub>2</sub> ) <sub>d</sub> (%)	3.00	6.30	14.25	22.21	25.50
Catalyst concentration (g/L)	0.33	0.66	1.46	2.27	2.60

for full oxidation of the initial DOC loading present in the synthetic NOM standard surrogate, related to the input DOC in solution as explained before), and catalyst concentration (g/L) (Table 1), in terms of four responses: MS2 inactivation, fraction of reacted H<sub>2</sub>O<sub>2</sub>, decolourization and NOM mineralization. All responses were analyzed at 40 min of H<sub>2</sub>O<sub>2</sub> addition. The experimental data was analyzed using the RSM approach in order to find out single-response optimums. It is noteworthy that these experimental factors were assessed within a range of values of other four non-controllable, so-called, covariates, let's say: initial MS2 titer (10<sup>4</sup>–10<sup>6</sup> PFU/mL), defined on the basis of literature reports (Niето-Juarez and Kohn, 2013; Niето-Juarez et al., 2010); pH (6.00–9.00), temperature (5.0–25.0 °C) and initial DOC loading (2.0–20.00 mg C/L) all defined based on experimental measurements performed on real samples of surface waters collected at south-western Colombia (at both Andean and Pacific-coast altitudes, not shown).

The full set of experiments consisted of eleven runs, including four axial points and three central point replicates (Table 1). Responses were estimated by means of a quadratic regression model expressed through the following general equation:

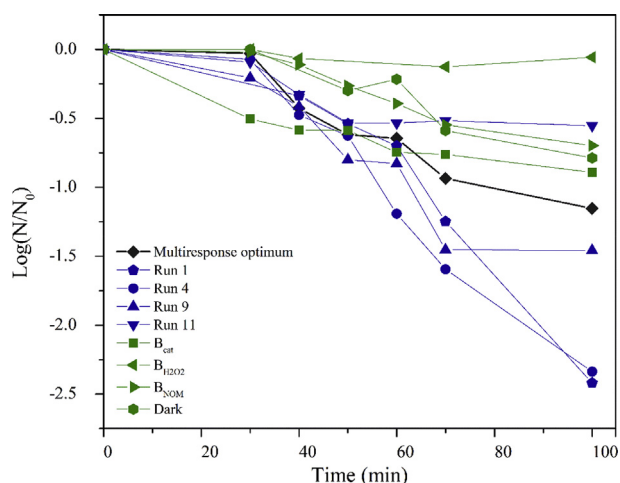
$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2$$

where  $\hat{Y}$  is the estimated value for each response, X<sub>1</sub> and X<sub>2</sub> are the factors evaluated and  $\beta_0$  to  $\beta_5$  correspond to estimated coefficients of the equation. The effect of the covariates on every response was estimated through multiple linear regression models. The results were analyzed by the least-squares method; individual linear, quadratic and interaction terms were determined by the variance analyses. The simultaneous optimization of the system in terms of the four measured responses (multi-response optimization) was achieved by raising a desirability function, giving same relative weight to every single response; the responses were expressed per mass unit of catalyst (%/g cat) in order to disregard the effect of adsorption. All statistical analyses and experimental design were carried out using Statgraphics Centurion® Software Package.

3. Results and discussion

3.1. CWPO inactivation of MS2 coliphage

Inactivation of the MS2 coliphage under different conditions of the CWPO treatment is plotted at Fig. 1. During the first 30 min of the assays, no CWPO reaction was performed since reactors did not contain any (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub>; however, inactivation was still observed at a maximum of



**Fig. 1.** CWPO inactivation of the MS2 coliphage under different experimental conditions. Multi-response optimum: 0.33 g cat/L  $(\text{H}_2\text{O}_2)_d = 3.36\%$ .  $B_{\text{cat}}$ : 2.27 g cat/L.  $B_{\text{H}_2\text{O}_2}$   $(\text{H}_2\text{O}_2)_d = 3.36\%$ .  $B_{\text{NOM}}$ : 0.33 g cat./L  $(\text{H}_2\text{O}_2)_d = 3.36\%$  without NOM. Dark: 0.33 g cat./L  $(\text{H}_2\text{O}_2)_d = 3.36\%$  without natural solar light irradiation.

0.33-log in run 9. Similar inactivation was observed in the catalyst blank ( $B_{\text{cat}}$ ), in spite that  $\text{H}_2\text{O}_2$  was not added at all along the full experiment. This experiment displayed the higher inactivation through the initial 30 min (0.50-log) and a total of 0.38-log inactivation after 100 min (Fig. 1), suggesting that adsorption effect took place on the catalyst surface. Furthermore, after washing of the spent solid catalyst with buffer solution composed of 1.00 % meat extract (10 g/L), 3.0 % Tween 80 (30 mL/L) and 2.92 g NaCl/100 mL at pH 9.00 followed by ultrasound for 3 min and filtration through 0.22  $\mu\text{m}$  membrane (Mendez et al., 2004), no coliphage count was observed, what probably suggests that inactivation observed in these cases obeyed to irreversible adsorption of the virus in the catalyst surface, phenomenon that could involve either electrostatic interactions, van der Waals forces and/or organic coating present on the catalyst surface that might favor virus-catalyst particle interactions (Nieto-Juarez and Kohn, 2013).

At 70 min of experiment, inactivation varied from 0.46-log (run 11) to 2.26-log (run 4) (Fig. 1). A previous report using magnetite in the heterogeneous Fenton-like treatment achieved a reduction of two magnitude orders in viral MS2 titer (Nieto-Juarez and Kohn, 2013), whereas it reached from 2-log to more than 3-log reductions in wastewater by heterogeneous photo-Fenton using maghemite and nano-sized maghemite under 900  $\text{W}\cdot\text{m}^{-2}$  solar simulator and temperature below 38 °C (Giannakis et al., 2017b).

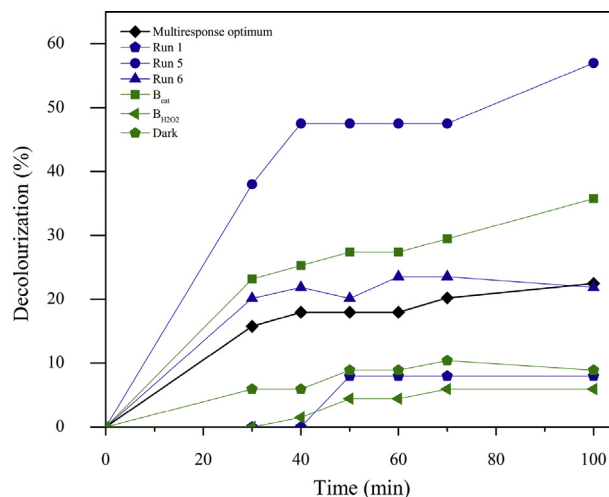
Meanwhile, the  $\text{H}_2\text{O}_2$  blank experiment ( $B_{\text{H}_2\text{O}_2}$ ) in absence of catalyst showed negligible inactivation (Fig. 1), which indicate that the viral inactivation observed in all runs up to 100 min was mainly due to the effect of the CWPO treatments. The most probable pathway of inactivation in the Fe-activated heterogeneous Fenton system is the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  on the catalyst surface producing hydroxyl and hydroperoxyl radical species, but probably also other Reactive Oxygen Species (ROS) responsible of organic degradation and reduction of viral infectivity by means of damage occurred on the single stranded RNA or the capsid and A protein (Giannakis, 2017; Giannakis et al., 2017b; Kim et al., 2010). In the other hand, the inactivation in absence of the synthetic surrogate of NOM ( $B_{\text{NOM}}$ ) was just 0.67-log, which is lower than most of the runs in our experimental design. It has been previously reported that NOM may compete with microorganisms for the ROS produced in the reaction and diminish the inactivation effectiveness (Giannakis et al., 2017b; Tsydenova et al., 2015). However, in our case it seemingly suggest that NOM on the contrary favored inactivation. Some authors have recently reported that humic substances in water may contribute to inactivation of microorganisms in the presence of solar light

acting as precursors of ROS such as superoxide ( $\text{O}_2^-$ ), singlet oxygen ( $^1\text{O}_2$ ), and the hydroxyl radical ( $\text{OH}\cdot$ ) which are able to degrade organic compounds and biomolecules (Carratala et al., 2016; Porras et al., 2018). This could be a reasonable explanation for our results considering that all runs were performed under natural solar light irradiation. In this sense, inactivation under multi-response optimum amounts of peroxide ( $\text{H}_2\text{O}_2$ )<sub>d</sub> and catalyst but in dark conditions ( $B_{\text{Dark}}$ ) allowed us to confirm our hypothesis; although some inactivation occurred, it clearly reached one of the lowest values (Fig. 1, Table 1).

### 3.2. NOM removal

Organic matter removal was studied to account for the interaction taking place between the viral agent and the complex system commonly present in real water sources and their effects on the catalytic inactivation. As it is depicted at Fig. 2, an increase in the decolourization percentage was obtained up to 70 min of assay, being 47.5 % the higher achieved (run 5), whereas NOM mineralization reached its highest percentage in run 10, which was 31.75 %, after 70 min of reaction (Table 1). The potential of CWPO to eliminate color in water could be explained by the high reactivity of HO $\cdot$  toward aromatic compounds (humic and fulvic species), which are one of the main responsible of color in surface waters (Galeano et al., 2012). Similar explanation can be raised for the DOC removal, but formation of other oxidizing species has been also claimed (Matilainen and Sillanpaa, 2010). Fast reaction on NOM has been explained to occur by either addition of HO $\cdot$  on double bonds, addition of an electron from an organic substituent to HO-radicals and by H-atom abstraction and the consequent generation of carbon centered radicals; these species latter react with oxygen forming organoperoxy radicals giving rise to ketones, aldehydes and evolved carbon dioxide (Matilainen and Sillanpaa, 2010). It is remarkable that upon decrease in the catalyst loading (run 5 vs. run 6, Table 1) from 2.27 g/L to 0.66 and identical dose of peroxide, color depletion dropped almost by a factor of 2, whereas the DOC mineralization remained almost not affected; it probably occurred because in the complex synthetic mixture of the NOM surrogate, the degradation of the fraction with heavier molecular weights led to intermediates and by-products without significant losing their content of chromophore groups, although maintaining similar levels of DOC mineralization.

In addition, it also stressed that adsorption affected more significantly decolourization than mineralization, probably because of the microporous nature of the Al/Fe-pillared clay suggesting that low molecular



**Fig. 2.** Color removal throughout the CWPO inactivation of the MS2 coliphage. Multi-response optimum: 0.33 g cat/L  $(\text{H}_2\text{O}_2)_d = 3.36\%$ .  $B_{\text{cat}}$ : 2.27 g cat/L.  $B_{\text{H}_2\text{O}_2}$   $(\text{H}_2\text{O}_2)_d = 3.36\%$ . Dark: 0.33 g cat./L  $(\text{H}_2\text{O}_2)_d = 3.36\%$  without presence of natural solar light irradiation.



weight chromophores got more easily adsorbed. In this sense, blank assay  $B_{\text{cat}}$  displayed 29.46 % of color removal and just only 0.68 % of NOM mineralization in 70 min of reaction. It also showed that  $\text{H}_2\text{O}_2$  is required to achieve higher decolourization, since  $B_{\text{H}_2\text{O}_2}$  blank experiment clearly exhibited lower decolourization and mineralization (5.92 % and 5.19 %, respectively), demonstrating the essential role played by the catalyst in activation of the oxidizing agent. This also means that although there was an effect of NOM adsorption on responses (Galeano et al., 2012), in our case it just marginally might explain NOM mineralization results, which must be then mainly attributed to CWPO catalytic degradation. Although the color removal and NOM mineralization obtained in this study are lower compared to previous reports (Galeano et al., 2012), it is noteworthy that in our investigation no more than 25.5 % of the theoretical amount of  $\text{H}_2\text{O}_2$  required for full oxidation was used, since in this investigation mainly optimization of the CWPO viral inactivation instead of NOM mineralization or color removal was aimed; thus, assessed  $\text{H}_2\text{O}_2$  dosages were set according to previous reports related to viral inactivation by Fenton processes (Kim et al., 2010; Nieto-Juarez et al., 2010). In addition, it was established in preliminary analyses (data not shown) that higher amounts of  $\text{H}_2\text{O}_2$  provided too fast MS2 depletion, which complicated the study of the MS2 inactivation by making difficult its experimental measurement. Finally, in similar behavior observed in the experiments of viral inactivation, Fig. 2 reveals that there was a significant effect of light on the efficiency of decolourization under circum-neutral pH conditions by comparison of Dark experiment with  $B_{\text{H}_2\text{O}_2}$  and  $B_{\text{cat}}$  blank experiments. Absence of light dropped decolourization even below simple adsorption by the solid catalyst under natural solar light ( $B_{\text{cat}}$ ), although still higher than  $B_{\text{H}_2\text{O}_2}$ , effect somehow expectable because in the Dark experiment both catalyst and  $\text{H}_2\text{O}_2$  were present, whereas  $B_{\text{H}_2\text{O}_2}$  was made in absence of catalyst, then just measuring direct attack of not-activated molecular hydrogen peroxide that was more rather negligible under assessed conditions of reaction.

In the other hand, according to previous studies of the Fenton process (Wang et al., 2014), the apparent kinetics of the process involves three or more reactions contributing to NOM decolourization that must be pseudo-first order; the reaction is influenced by either the amount of catalyst or substrate. Given the low loading of active iron in our catalyst (just 0.62 %), within the assessed NOM concentrations higher amounts of catalyst should increase the reaction rate but do not affecting the mechanism. Meanwhile, since the hydroxyl radicals and other short-lived oxidizing species are consumed almost immediately once formed, the net change in the reaction can be assumed to be zero and the constant depends on both (i) the concentration of the substrate at any time and (ii) the concentration of significant intermediates and byproducts generated. Moreover, Fig. 2 shows that main phenomena explaining NOM decolourization were the heterogeneous Al/Fe-PILC catalyzed Fenton, simple adsorption onto the catalyst's surface and probably also a small effect of the homogeneous Fenton process catalyzed by the tiny concentration of the iron leached in the reaction media. Furthermore, the adsorption effect was ruled out from the multi-response optimization by using all responses expressed per mass unit of the solid catalyst in the statistical process.

### 3.3. Fraction of reacted $\text{H}_2\text{O}_2$

As an indirect evidence of the catalytic performance displayed by CWPO activating the oxidizing agent, fraction of reacted  $\text{H}_2\text{O}_2$  was monitored. In most of the cases fraction of reacted  $\text{H}_2\text{O}_2$  was relatively high and even it got fully consumed in some experiments (Table 1). As a general trend fraction of reacted  $\text{H}_2\text{O}_2$  increased along the first stage of the experiments and then it stabilized or even decreased through the last 30 min (Fig. 3). This behavior could be explained because below certain concentration of organic substrates, hydroxyl radicals may be more significantly back-coupled again to form free, molecular  $\text{H}_2\text{O}_2$ . The apparent stabilization in reacted  $\text{H}_2\text{O}_2$  before 40 min of assay mainly in the experiments with the higher peroxide doses could be attributed to the

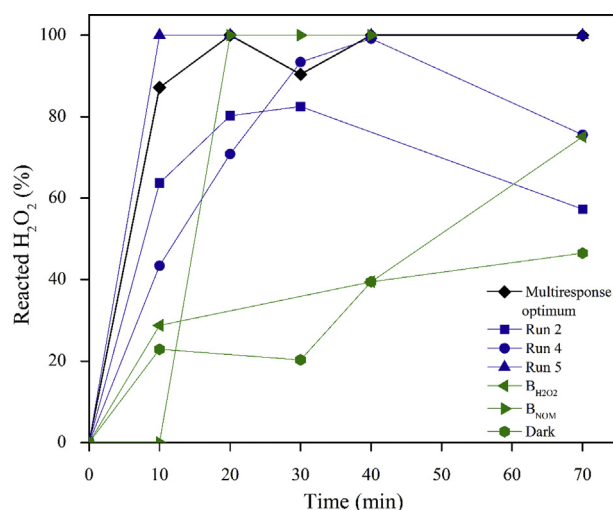


Fig. 3. Fraction of reacted  $\text{H}_2\text{O}_2$  throughout the CWPO inactivation of the MS2 coliphage. Multi-response optimum: 0.33 g cat/L ( $\text{H}_2\text{O}_2$ )<sub>d</sub> = 3.36 %.  $B_{\text{H}_2\text{O}_2}$  ( $\text{H}_2\text{O}_2$ )<sub>d</sub> = 3.36 %.  $B_{\text{NOM}}$ : 0.33 g cat./L ( $\text{H}_2\text{O}_2$ )<sub>d</sub> = 3.36 % without the presence of NOM. Dark: 0.33 g cat/L ( $\text{H}_2\text{O}_2$ )<sub>d</sub> = 3.36 % without the presence of natural solar light irradiation.

compensation between continuous  $\text{H}_2\text{O}_2$  addition and consumption by means of interactions with catalyst, NOM and viruses. In the other hand, this fraction in  $B_{\text{H}_2\text{O}_2}$  reached 39.47 % in absence of catalyst. Such a consumption of  $\text{H}_2\text{O}_2$  probably happened because of decomposition exerted by natural solar light. It is well known that UV light provokes  $\text{H}_2\text{O}_2$  photolysis to form hydroxyl radicals (Penru et al., 2012); moreover, this value was still lower than majority of runs, confirming that its decomposition in experimental runs mainly resulted from the catalytic activation.

It is also noteworthy that even under low ( $\text{H}_2\text{O}_2$ )<sub>d</sub> values, in some experiments high inactivation together with significant reacted fraction of  $\text{H}_2\text{O}_2$  were achieved. It also confirms that stepwise addition of  $\text{H}_2\text{O}_2$  promotes better use of the oxidizing agent by the catalytic system, preventing the radical species to get significantly scavenged by undesired side reactions (Yamal-Turbay et al., 2013).

### 3.4. Effect of non-controllable factors on CWPO MS2 inactivation

P-values about the effect of environmental, non-controllable factors obtained by multiple regressions are shown in Table 4. It has been widely reported that in heterogeneous Fenton processes, the use of Fe supported in solid materials aims to widen the operational pH range by preventing Fe leaching (Galeano et al., 2014; Ramírez and Galeano, 2017; Ribeiro et al., 2016). In spite that Al/Fe pillared clay catalysts has shown high stability against the chemical leaching of the active metal (Fe), even under acid pH values (3–4) typical of Fenton systems (Galeano et al., 2010, 2014), in general some other solid catalyst recently reported activating CWPO have shown to be less stable in terms to metal leaching,

Table 4

P-values\* of covariates from multiple regressions for every analyzed response.

Parameter	MS2 Inactivation	Reacted $\text{H}_2\text{O}_2$	Decolourization	NOM mineralization
Constant	0.19	0.15	0.07	0.34
pH	0.84	0.75	0.50	0.51
Temperature	0.39	0.36	0.46	0.21
Initial DOC	0.07	0.06	0.04	0.46
Initial viral titer	0.23	0.23	0.11	0.82
R <sup>2</sup>	0.489	0.527	0.277	0.477

\* Statistical analyses performed at 95 % of confidence.

in special under low pH values (Munoz et al., 2017b; Ribeiro et al., 2016). Besides, in the case of the Al/Fe-PILCs, the inclusion of Al is an advantage as it establishes a strong interaction with Fe that markedly reduce Fe leaching even at low pH, improving CWPO efficiency (Mirzaei et al., 2017). In our investigation, pH or temperature did not exhibit either a significant statistical effect on any of the analyzed responses (p value <0.05) within the range of circumneutral values and temperatures assessed, respectively, typical of real surface water sources. Although in general the increase of temperature has been reported to enhance the reaction kinetics of the heterogeneous Fenton process (Munoz et al., 2017b), other reported results were more comparable to ours, in which no significant differences were found in Fenton processes operated at either room temperature or 40 °C (Mirzaei et al., 2017). In this sense, the effect of temperature on the Fenton reaction has been widely demonstrated and reported in literature, but mainly above 50 °C (Rueda Márquez et al., 2018), whereas mild temperatures have been more rather scarcely studied. This is a very interesting finding making Al/Fe-PILC-catalyzed CWPO more feasible to be considered as an alternative technology to improve drinking water treatment plants under continuous variation in either input pH or temperature within the range of mild conditions featuring ambient conditions.

Similarly, no statistically significant effect was exerted by the initial NOM concentration (DOC) on the recorded responses (p value >0.05), except in the case of decolourization. In this case color removal must be mainly attributed to the high reactivity of radicals toward humic and fulvic species, which are the major contributors to color in real water (Galeano et al., 2012), and in minor extent to adsorption of low molecular-weight fractions of the NOM surrogate within the micropores of the clay catalyst. The effect of initial DOC on the other responses could be explained by the occurrence of two different phenomena in the experiments: in some cases, high concentrations of contaminant could increase the removal kinetics; however instead, the high concentration of contaminant will require more radicals to be removed and this induces decreased efficiency (Mirzaei et al., 2017). Such variability in the effect exerted by starting DOC would account for the lack of statistical significance of this parameter. Moreover, it is remarkable the close-to-significant effect exerted by the initial DOC on the MS2 inactivation (p-value just slightly over 0.05); it probably suggests that ROS other than just hydroxyl and hydroperoxyl radicals, for instance those promoted by the presence of dissolved NOM under circumneutral pH and natural solar light irradiation (let's say, triplet states of the hydrophobic fraction in NOM) may be are playing a role in the viral inactivation, at least within the range of low levels of DOC featuring real surface waters and peroxide doses here evaluated, as recently reported for bacterial disinfection (Porrás et al., 2018).

Regarding initial viral titer, in this study a high viral concentration was used in order to facilitate more accurate viral detection and thus getting the MS2 coliphage disinfection potential truly related to CWPO (Carratala et al., 2016). However, scarce information about the effect of the initial titer has been reported; apparently an increase in the viral concentration would result decreasing inactivation level because a high titer may favor viral aggregation and contribute to protect inner viruses from damage (Cheng et al., 2014) or simply because a major quantity of viral particles would require greater radical concentration to be inactivated. Here we found that different concentrations of viruses did not exert a significant effect over inactivation. Similar results have been reported by other studies in viral disinfection by means of solar light (Carratala et al., 2016). In spite of the high viral concentration used, an important inactivation activity was observed which suggests that CWPO might be able to easily achieve full viral inactivation from real surface water where viral titers usually are pretty much lower ( $10^1$  -  $10^2$  PFU/mL), according to previous microbiological analyses made on our samples of surface water in south-western Colombia.

In principle upon CWPO real-scale application, several non-controllable parameters might play important roles on the overall performance of the process (Mirzaei et al., 2017). Nevertheless, none of the

covariates here studied exhibited significant effect on CWPO responses toward simultaneous MS2 inactivation, color and DOC depletions. It demonstrates that parameters mainly influencing the CWPO viral disinfection are more related to catalyst loading and dose of hydrogen peroxide ( $(H_2O_2)_d$ ), which fortunately can be properly set up in order to improve cost-efficient disinfection together with degradation of dissolved chemicals on typical real surface supplies to produce better potable water.

### 3.5. Statistical optimization of the CWPO viral inactivation

Since the catalyst loading was one of the experimental factors, and simple adsorption as well as the catalytic response depends on this factor, individual and multiple-response optimizations were carried out by first dividing every response of Table 1 by the catalyst amount used in each run in order to rule it out from the statistical analysis (Table 5). Variance analysis showed that catalyst concentration exerted a significant negative linear effect on the MS2 inactivation (Table 6), fraction of reacted  $H_2O_2$  and decolourization; besides, it also displayed a significant positive second order effect on inactivation and reacted  $H_2O_2$ . Meanwhile  $(H_2O_2)_d$  exerted statistically significant effects on decolourization (linear and quadratic). Finally, although the NOM mineralization was positively affected by the two studied experimental factors, their effects were not statistically significant, probably because the peroxide doses assessed were too far lower than theoretical required for full mineralization.

After neglecting statistically non-significant values, results were fitted to the second-order polynomial Eqs. (1, 2, 3, 4). Statistical models for MS2 inactivation and reacted  $H_2O_2$  can be considered adequate for prediction as long as the obtained  $R^2$  values were higher than 0.9 (Table 7). This means that less than 10 % of the total recorded variation in data could not be explained by the models. Likewise, model predicting decolourization was fitted in 0.857 and therefore it could be considered acceptable. In contrast, mineralization data was clearly not significantly adjusted to the model obtained ( $R^2 = 0.334$ ).

$$\text{MS2 Inactivation} = 354.22 - 331.12(\text{cat}) + 0.49(H_2O_2)_d + 81.59(\text{cat})^2 \quad (1)$$

$$\text{Reacted } H_2O_2 = 389.49 - 338.47(\text{cat}) - 4.56(H_2O_2)_d + 84.53(\text{cat})^2 + 0.132(H_2O_2)_d^2 \quad (2)$$

$$\text{Decolourization} = 57.10 - 20.93(\text{cat}) - 2.59(H_2O_2)_d + 4.64(\text{cat})^2 + 0.07(H_2O_2)_d^2 \quad (3)$$

$$\text{NOM mineralization} = 11.84 + 0.142(\text{cat}) + 0.89(H_2O_2)_d - 0.519(\text{cat})^2 \quad (4)$$

The single-response optimization is represented by surfaces and contour plots (Fig. 4). MS2 inactivation reached its higher percentage at the lowest catalyst concentrations, whereas in opposite trend, higher  $H_2O_2$  dosages increased viral inactivation (Fig. 4a). Other study recently reported that low concentrations of Al/Fe-PILC clay catalyst may improve its interaction with  $H_2O_2$  and favor generation of oxidizing radicals (Castro-Ríos et al., 2017) that eventually inactivate bacterial microorganisms. Besides, it has been previously reported that a high MS2 inactivation could be reached in heterogeneous photo-Fenton systems using Fe solid loadings lower than 0.1 mg/L (Giannakis et al., 2017b). Our optimization results can be also attributed to the high performance featuring Al/Fe pillared clays catalyzing CWPO reaction which is governed by characteristics such as the amount of incorporated Fe as truly mixed Al/Fe pillars, high compensation of the starting CEC and high specific surface area represented as microporous content as main attributes of this type of solid catalysts (Giannakis et al., 2017b; Munoz et al., 2017a; Muñoz et al., 2018). Such a set of properties of the catalyst might promote the effective activation of  $H_2O_2$  even at very low doses by facilitating its internal diffusion to the Fe active sites (Munoz et al., 2017a). Furthermore, within the low concentrations (doses) of  $(H_2O_2)_d$  and middle catalyst loadings here studied (indeed, as a function of the Fe

**Table 5**

Experimental conditions of the CWPO treatments and measured responses per unit mass of catalyst.

Run	Factors		Responses			
	Cat (g/L)	(H <sub>2</sub> O <sub>2</sub> ) <sub>d</sub> (%) <sup>b</sup>	MS2 Inactivation (%/g cat.)	H <sub>2</sub> O <sub>2</sub> reacted (%/g cat.)	Decolourization (%/g cat.)	NOM Mineralization (%/g cat.)
1	0.33	14.2	296.5	274	25.1	7.60
2	1.46	14.2	57.7	21.5	10.9	15.0
3	1.46	14.2	54.3	35.1	18.0	9.71
4	0.66	22.2	151.3	154	24.8	37.6
5	2.27	6.30	36.7	45.1	21.4	6.84
6	0.66	6.30	148.8	155	37.2	27.0
7	2.27	22.2	40.0	27.2	10.6	4.22
8	1.46	3.00	49.7	69.8	25.2	9.95
9	1.46	25.5	67.4	47.5	19.5	10.0
10	2.6	14.2	35.8	27.7	12.9	12.5
11	1.46	14.2	48.6	55.3	14.4	12.2

**Table 6**

Regression coefficients and variance analysis for every studied response.

Term	MS2 Inactivation		Reacted H <sub>2</sub> O <sub>2</sub>		Decolourization		NOM mineralization	
	Coeff.	p-value*	Coeff.	p-value*	Coeff.	p-value*	Coeff.	p-value*
Constant	354.22	-	389.49	-	57.10	-	11.84	-
A:Catalyst	-331.12	0.00	-338.47	0.00	-20.93	0.01	0.14	0.13
B: (H <sub>2</sub> O <sub>2</sub> ) <sub>d</sub>	0.49	0.64	-4.56	0.40	-2.59	0.03	0.89	0.77
AA	81.59	0.00	84.53	0.00	4.64	0.12	-	-
AB	-	-	-	-	-	-	-0.52	0.51
BB	-	-	0.13	0.36	0.07	0.03	-	-
R <sup>2</sup> adjusted	0.946	-	0.962	-	0.857	-	0.334	-

\* Statistical analyses performed at 95 % of confidence.

**Table 7**

RSM single-response and desirability-based multi-response optimized values for Al/Fe-PILC catalyzed CWPO inactivation of the MS2 coliphage.

Response	Catalyst concentration (g/L)	(H <sub>2</sub> O <sub>2</sub> ) <sub>d</sub> (%)	Modeled single-response optimum (%)	Modeled multi-response optimum (%)	Experimental multi-response optimum (%)
MS2 Inactivation	0.33	25.5	87.8	84.3	88.4
Fraction of reacted H <sub>2</sub> O <sub>2</sub>	0.33	3.00	90.6	90.1	100
Decolourization	0.33	3.00	14.4	14.1	22.2
NOM mineralization	0.33	25.5	9.95	9.95 <sup>a</sup>	14.2
Desirability <sup>a</sup>	0.33	25.5	-	0.85	-
Desirability <sup>b</sup>	0.33	3.36	-	0.94	-

<sup>a</sup> Multi-response optimization including NOM mineralization.<sup>b</sup> Excluding NOM mineralization.

active in the clay-catalyst), an increasing amount of Fe available to interact with H<sub>2</sub>O<sub>2</sub> promoted higher scavenging effect of Fe<sup>2+</sup> consuming hydroxyl radicals instead than improving the catalytic activation of the oxidizing agent. Meanwhile, once exceeded around 2.5 g/L of catalyst, such a modeled response surface again predicts an increased inactivation in the positive direction of the amount of catalyst irrespective the dose of hydrogen peroxide (Fig. 4a); it probably also arises to (Fe<sub>active</sub>/H<sub>2</sub>O<sub>2</sub>) ratios closer to optimal values widely reported for Fenton systems around or below than 0.3 (Deng, 2007; Galeano et al., 2012; Mansoorian et al., 2014; Youssef et al., 2016) either activated in homogeneous or heterogeneous phase. This analysis is also supported in a very similar response surface obtained as a function of the reacted fraction of hydrogen peroxide (Fig. 4b), where those regions exhibiting optimal MS2 inactivation perfectly fit those with higher degrees of peroxide consumption. It proves that the MS2 inactivation was mainly driven by hydrogen peroxide, previously involved its catalytic activation (Fig. 1 shows that viral inactivation achieved just by peroxide-natural solar light irradiation in absence of the clay catalyst B<sub>H2O2</sub> was the lowest measured).

The RSM optimization of Color removal displayed a general trend similar to both precedent surfaces, where the maximum was found toward lowest both catalyst concentration and (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> (Fig. 4c). In the other hand, optimization of NOM mineralization response was not

possible, probably because of the rather too low doses studied in comparison with the theoretical stoichiometric amount required for full mineralization; a linear increase can be seen in this response toward increasing (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> and the lowest catalyst concentration.

As above mentioned, the single-response optimization analyses suggested that even when a low mineralization was reached under the range of conditions evaluated, stronger catalytic conditions such as higher (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> and reaction time are needed to achieve higher color and NOM removals, as previously proposed (Galeano et al., 2012). However, it must be emphasized that current study mainly aimed at optimizing the viral inactivation; in this context, Al/Fe-PILC catalyzed CWPO has shown to require very low dosages of both H<sub>2</sub>O<sub>2</sub> and catalyst to significantly inactivate water-borne viruses like the MS2 coliphage, which is an excellent result seeking for proper adaptation of the CWPO in order to enhance microbiological parameters of drinking water.

In the other hand, two multi-response optimization analyses were performed, taking or not into account the NOM mineralization response. Second one is depicted by means of a unified multi-response surface (desirability function) and a contour graph at Fig. 5. Both analyses indicated that the optimal catalyst concentration was the lowest assayed (0.33 g/L), in strong correlation with the previous single-response surfaces. However, optimal conditions for factor (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> differed

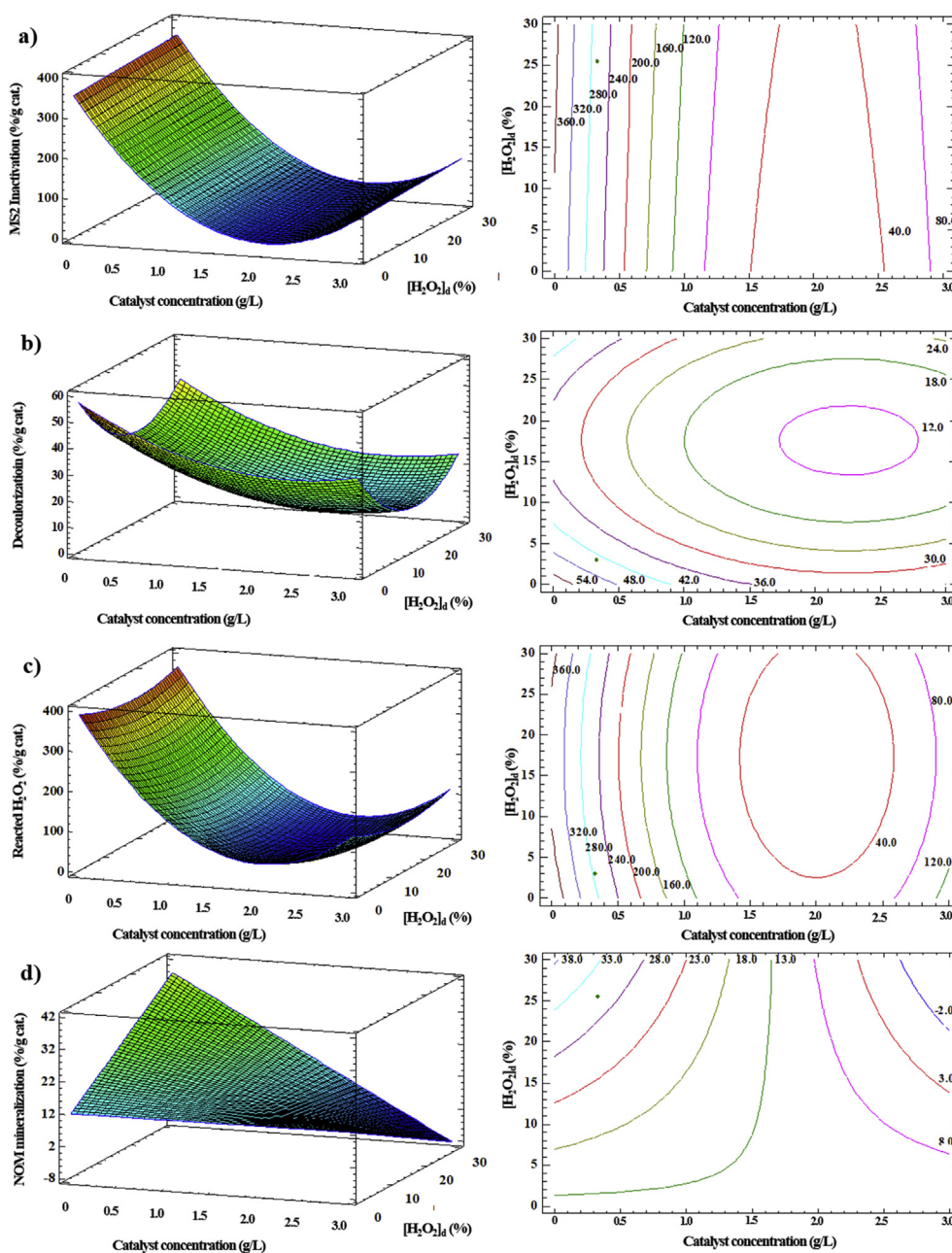


Fig. 4. Response surfaces and contour graphs of the CWPO experimental factors for each response normalized to the amount of catalyst in the inactivation of the MS2 coliphage: (a) MS2 Inactivation; (b) Decolourization; (c) Reacted  $\text{H}_2\text{O}_2$ ; (d) NOM mineralization.

substantially from both approaches, the optimum dosage in the analysis including mineralization was the highest evaluated (25.5 %), whereas excluding this response was close to the lowest dose (3.36 %) (Table 7, Fig. 5). Excluding mineralization of multiple responses optimization resulted in a significant improvement of the Desirability function from 0.84 to 0.94 out of 1.0.

An optimal experimental run was then performed under conditions given by the second multi-response analysis (Fig. 6). The achieved experimental responses for MS2 inactivation, fraction of reacted  $\text{H}_2\text{O}_2$ , decolourization and even mineralization were all higher than those predicted by the RSM statistical models (Table 7, Fig. 6). It is remarkable that our results under optimal multi-response conditions still showed a decreasing trend of the MS2 titer arriving to 100 min of experiment (70 min of reaction, Fig. 6), which suggests that increasing the time of treatment could lead to even higher MS2 inactivation. The optimal experimental conditions corresponded to the lowest ones evaluated in

this investigation. The use of adequate concentration of reagents in the heterogeneous Fenton-like process is essential in order to improve the reaction efficiency and then, to reduce the operational cost of the CWPO. As explained, an excess of solid catalyst loading could lead to excessive adsorption effect preventing proper activation of the oxidizing agent, whereas an excess of  $\text{H}_2\text{O}_2$  could either increase the possibility of recombination of the oxidizing radicals, make more likely the loss of them by scavenging species in the liquid phase or promote production of less active ROS such as  $\text{HO}_2\cdot$ ; besides,  $\text{H}_2\text{O}_2$  can also deactivate  $\text{HO}\cdot$  radicals to  $\text{H}_2\text{O}$  and  $\text{O}_2$  (Tehrani-Bagha and Balchi, 2018), reaction more significant as the pH increases.

As the optimal  $\text{H}_2\text{O}_2$  concentration greatly depends on active Fe concentration provided by the solid catalyst, a key parameter to be recorded is the ratio ( $\text{Fe}_{\text{active}}/\text{H}_2\text{O}_2$ ) (Deng, 2007; Mansoorian et al., 2014); an optimum ratio may contribute to reduce the consumption and waste of reagents (Mirzaei et al., 2017). Our optimal multi-response



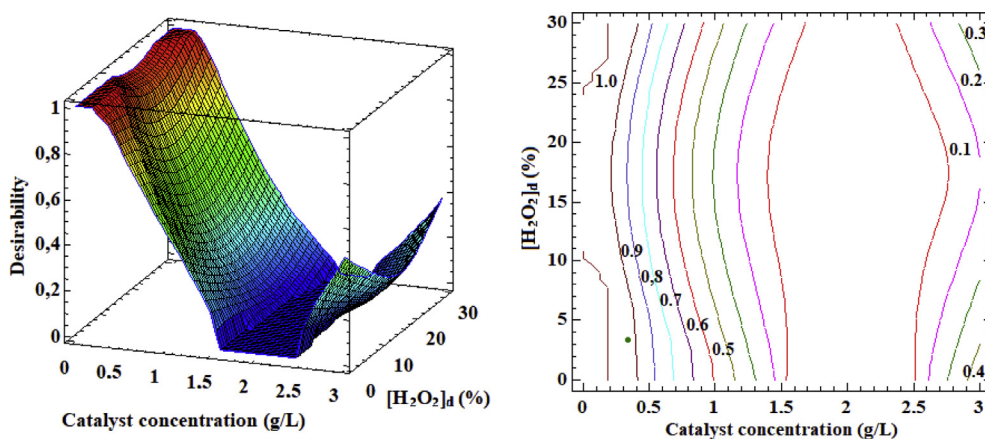


Fig. 5. Response surface and contour graph of multiresponse optimized CWPO treatment in terms of MS2 inactivation, decolourization and reacted  $\text{H}_2\text{O}_2$ .

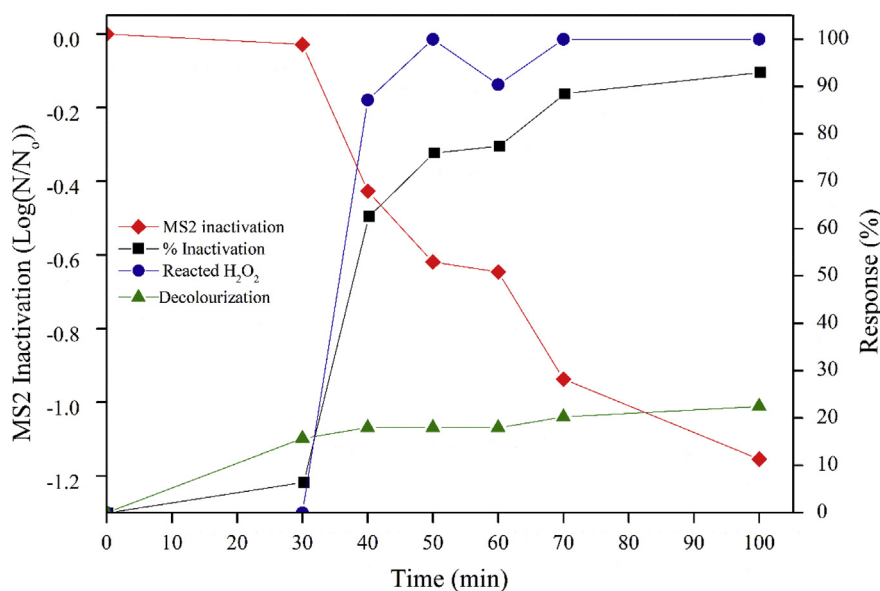


Fig. 6. Experimental evaluation of the multi-response optimal conditions of the CWPO reaction in terms of MS2 inactivation, fraction of reacted  $\text{H}_2\text{O}_2$  and decolourization: 0.33 g cat/L; 3.36 %  $(\text{H}_2\text{O}_2)_d$ , 25.0 °C,  $\text{pH}_0 = 7.07$ ,  $\text{DOC}_0 = 16.06$  mg C/L and  $10^6$  PFU/mL.

experiment was made with a  $(\text{Fe}_{\text{active}}/\text{H}_2\text{O}_2)$  ratio of 0.46, which is comparable with previous reports about photo-Fenton inactivation of F-RNA coliphages, where a ratio of 0.50  $(\text{Fe}_{\text{active}}/\text{H}_2\text{O}_2)$  was used to achieve an inactivation near to 100 % during around 3.5 h of treatment at circumneutral pH (Agulló-Barceló et al., 2013). It suggests that full MS2 inactivation could be easily reached in the presence of the Al/Fe-PILC activated CWPO if reaction time is slightly increased over let's say 90 min of reaction, since the trend of inactivation after 100 min of treatment (70 min of reaction) was still upwards (Fig. 6). Some studies have proven that under an optimum  $(\text{Fe}_{\text{active}}/\text{H}_2\text{O}_2)$  ratio, the amounts of Fe and  $\text{H}_2\text{O}_2$  can be considerably reduced preventing extra-costs of operation by excess of reagents (Mirzaei et al., 2017; Tehrani-Bagha and Balchi, 2018). Furthermore, it could also reduce environmental impacts referring to processes at higher scales (Yamal-Turbay et al., 2013).

Finally, it has been demonstrated that optimal CWPO inactivation of MS2 viral loading in a matrix resembling actual dissolved NOM in real surface waters may be achieved under pretty low levels of both Al/Fe-PILC clay catalyst and dose of  $\text{H}_2\text{O}_2$ . Thus, in the general purpose of applying CWPO in order to enhance both, removal of organic matter to prevent formation of disinfection by-products and disinfection itself, the optimal conditions of viral inactivation realized in this study suggest to be pretty lower than those expected levels for catalyst and oxidizing

agent required to promote higher decolourization, and eventually, maximal NOM mineralization under lowest possible consumption of hydrogen peroxide, what suggests that under such a set of conditions full viral inactivation might be safely achieved.

#### 4. Conclusions

Optimized Al/Fe PILC-activated CWPO treatment showed excellent MS2 inactivation under pretty low both concentration of the clay-catalyst and dose of hydrogen peroxide. Since the effects of non-controllable variables under real conditions of operation (covariates) pH, temperature, and initial viral titer did not show statistical significance on either (1) viral inactivation, (2) decolourization, (3) fraction of reacted  $\text{H}_2\text{O}_2$  or (4) NOM mineralization as responses, the treatment could be efficiently performed under variable environmental conditions. Meanwhile, initial content of dissolved organic carbon interestingly showed positive effect promoting higher levels of three out of four responses in the presence of natural solar light irradiation, just excluding NOM mineralization; it could be attributed to the role played by triplet states of dissolved NOM and other ROS relevant at circumneutral pH enhancing oxidation levels. Multi-response optimization via desirability function based on responses (1) to (3) released excellent fitting (0.94 out of 1.0) and following set of

optimal experimental conditions and corresponding responses: 0.33 g Al/Fe-PILC/L and 3.36 % (H<sub>2</sub>O<sub>2</sub>)<sub>d</sub> (theoretical amount of H<sub>2</sub>O<sub>2</sub> assumed to be required for full oxidation of the initial DOC loading in each experiment calculated from the Chemical Oxygen Demand of our synthetic NOM surrogate) (Fe<sub>active</sub>/H<sub>2</sub>O<sub>2</sub>) = 0.46, giving rise to 93.0 % of MS2 inactivation and 100 % of reacted H<sub>2</sub>O<sub>2</sub> at pH 7.07, 25.0 +/- 0.1 °C, 16.06 mg C/L as starting NOM concentration, for an MS2 starting titer of 10<sup>6</sup> PFU/mL after just 70 min of reaction. Optimal conditions of catalyst loading and dose of oxidizing agent here realized for viral inactivation are clearly lower than those expected required for high levels of either decolourization or NOM mineralization.

## Declarations

### Author contribution statement

Alejandra Ordoñez-Ordoñez, Dolly Revelo-Romo: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ana-Maria Garcia-Mora: Contributed reagents, materials, analysis tools or data.

Arsenio Hidalgo-Troya: Conceived and designed the experiments.

Luis Alejandro Galeano: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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