Electronic Supplementary Material (ESI) for RSC Advances

Novel Claycunbic to eliminate micropollutants and Vibrio fischeri from water

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Supporting Information section contains following (20 pages):

1. Extended experimental section with details on synthesis and characterization of Claycunbic, experimental design and process optimization, adsorption and catalytic activity as well as Vibrio fischeri bioluminescence inhibition test.

2. Thermogravimetric analysis of original montmorillonite clay and Claycunbic.

3. High resolution XPS spectra of each region and atomic composition of Claycunbic based on XPS findings.

4. Chemical structures of MB, MO and DCP.

5. Absorption bands of Methylene blue (MB) at various reaction times.

6. The sorption capacity vs the square root of time for Methylene blue (MB), Methyl orange (MO) and Dichlorophenol (DCP) adsorption onto Claycunbic.

7. Intra-particle diffusion model parameters (k_{id} (mg g^{-1} $min^{-1/2}$, C (mg g^{-1}), R^2).

8. Table that contains experimental matrix: reaction conditions as coded values (hydrogen peroxide dose as x_1, catalytic adsorbent amount as x_2 and Methylene blue (MB) removal efficiency as Y), results and predicted values

9. The predicted vs experimental Methylene blue (MB) degradation plot.

10. Pareto chart showing the standardized effects of independent variables.

11. Inhibition of bacterial growth in the presence of various Claycunbic concentrations.

Experimental

Synthesis

The pH was then adjusted to 2.8 with Na_2CO_3. The resultant slurry was sonicated for 30 min and magnetically stirred at room temperature for 3 hours. Then Claycunbic was filtered, 5 times washed with deionized water to ensure the complete removal of Cl^- ions and dried in air at 60 ^oC for 12 hours. After drying, samples were crushed to a fine powder and subsequently activated at 400 ^oC for a period of 2 hours with 5 ^oC min^{-1} in air [1]. The as-prepared catalysts were stored in closed vials and used when required after overnight activation at 120 ^oC to remove accumulated moisture. To recycle the catalytic adsorbent, spent Claycunbic was subjected to 400 ^oC for 2 hours and reused for the subsequent experiments.

Characterization of the catalyst

The accelerating voltage and applied current were 45 kV and 40 mA, respectively. Full width at half maximum (FWHM) of each diffraction line was determined from the profile measured with a scanning rate of 1/2^o min^{-1}, which was calibrated by standard silicon powder for instrumental broadening.

For the XPS analysis, the samples were fixed on a copper plate under a portable anaerobic chamber before being placed in the analytical chamber (10^{-9} mbar vacuum). All spectra were recorded at a 90° takeoff angle. All binding energies were referenced to the silicon 2s core level of montmorillonite clay at 102.9 eV [2] and the resolution was 1.4 eV. The nominal x-ray beam diameter was 600 µm. Since all the samples were non-conductive, the charge neutralization system, which caused peaks to shift to lower binding energy by ~ 4 – 5 eV was adopted. The typical error bar associated to the

binding energies was ± 0.3 eV.

Experimental design and process optimization

An alpha (α) level of 0.05 (95 % confidence level) was used to determine the statistical significance during all the experimental runs. The independent variables of hydrogen peroxide (x_1) and catalyst (x_2) doses were coded as low (-1), mid (0) and high (1) levels, while degradation of MB was the response (Y) of the system. The real values for independent variables were hydrogen peroxide (0.1 – 1 mL) and catalyst (0.5 – 3 g L^{-1}) doses, as shown in Table 1. Performance of MB degradation was assessed by analyzing the response of degradation efficiency (%). A quadratic model is following:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad \text{(SI1)}$$

where Y is the predicted response of the system, x_1 and x_2 are the variables, β_o is the constant coefficient, β_j, β_{jj}, β_{ij} are the coefficients of linear, quadratic and the second-order terms, respectively ($i < j$) and ε is the error. The quality of the fit of polynomial model was expressed by coefficients of determination R^2 and R_{adj}^{2} [3].

Adsorption and Catalytic activity

At regular time intervals, 5 mL of the aliquot was withdrawn from the solution and filtered through 0.45 μm syringe filter to remove the Claycunbic particles. The concentrations of target contaminants were determined by a UV spectrophotometer (Hewlett Packard 845X) at characteristic wavelengths. After the adsorption equilibrium was reached, a portion of the supernatant layers of suspensions were collected and centrifuged at 3000 rpm for 15 min and then analyzed

spectrophotometrically to determine the concentration of MB, MO and DCP remaining in the solution [4]. The adsorption capacities were determined from the difference between the initial and equilibrium concentration of MB, MO and DCP. To test the catalytic activity, 0.05 g of the Claycunbic was mixed with 50 mL of 100 mg L^{-1} MB, MO or DCP with 0.1 mL, 0.5 mL and/or 1 mL of 30 % H_2O_2 and magnetically stirred for 10 min to 4 hours. To efficiently compare the results and optimize the reaction conditions, 10 min reaction time was adopted and 0.5 g L^{-1}, 1 g L^{-1} and 3 g L^{-1} of as-prepared Claycunbic and various concentrations of hydrogen peroxide were used to degrade 100 mg L^{-1} of MB at room temperature.

Vibrio fischeri bioluminescence inhibition test

Light production is directly proportional to the metabolic activity of the bacterial population and any inhibition of enzymatic activity causes a corresponding decrease in bioluminescence [5]. Detailed testing procedure is described by Lappalainen and co-workers [6]. In brief, freeze-dried photobacteria, Vibrio fischeri (NRRL B-11177), were rehydrated and stabilized at 4 ^oC for 1 h and then stabilized at 15 ^oC for 30 min prior the measurements. Suspensions containing various concentrations of Claycunbic (0.01, 0.1, 1, 2, 3, 5, 10. 15, 20 and 50 g/L) were subjected to 30 min ultrasonication and then subsequently stabilized at 15 ^oC prior to the toxicity measurements [7]. As color and turbidity reduce the light output recorded by the luminometer similar to toxicity, the bacteria was separated by centrifuging from the sample matrix prior to the final luminescence measurements [8]. Thus, 1 mL of centrifuged suspension was transferred to the vial and 200 µL of the bacterial solution was dispensed into the sample. To evaluate the inhibition of the bacterial growth in the presence of Claycunbic, the light output was recorded at t_0 and t_{30}. All the samples were assayed in duplicates. The control test was carried out by recording the light output of the unstressed bacteria after otherwise identical experimental conditions. The light inhibition percentage (INH %) was calculated according to the standard and kinetic methods:

$$KF = \frac{LIC_t}{LIC_o} \qquad \text{(SI 2)}$$

where KF is the correction factor, LIC_t and LIC_o are luminescence intensity of the control after (t_{30}) and before (t_o) exposition (min), in relative units of luminescence.

$$INH\ (\%) = 100 - \left(\frac{LIT_t}{KF + LIT_o} \right) \times 100 \qquad \text{(SI 3)}$$

where $LICT_t$ and $LICT_o$ are luminescence intensity of the tested sample after (t_{30}) and before (t_o) exposition (min), in relative units of luminescence

Results and Discussion

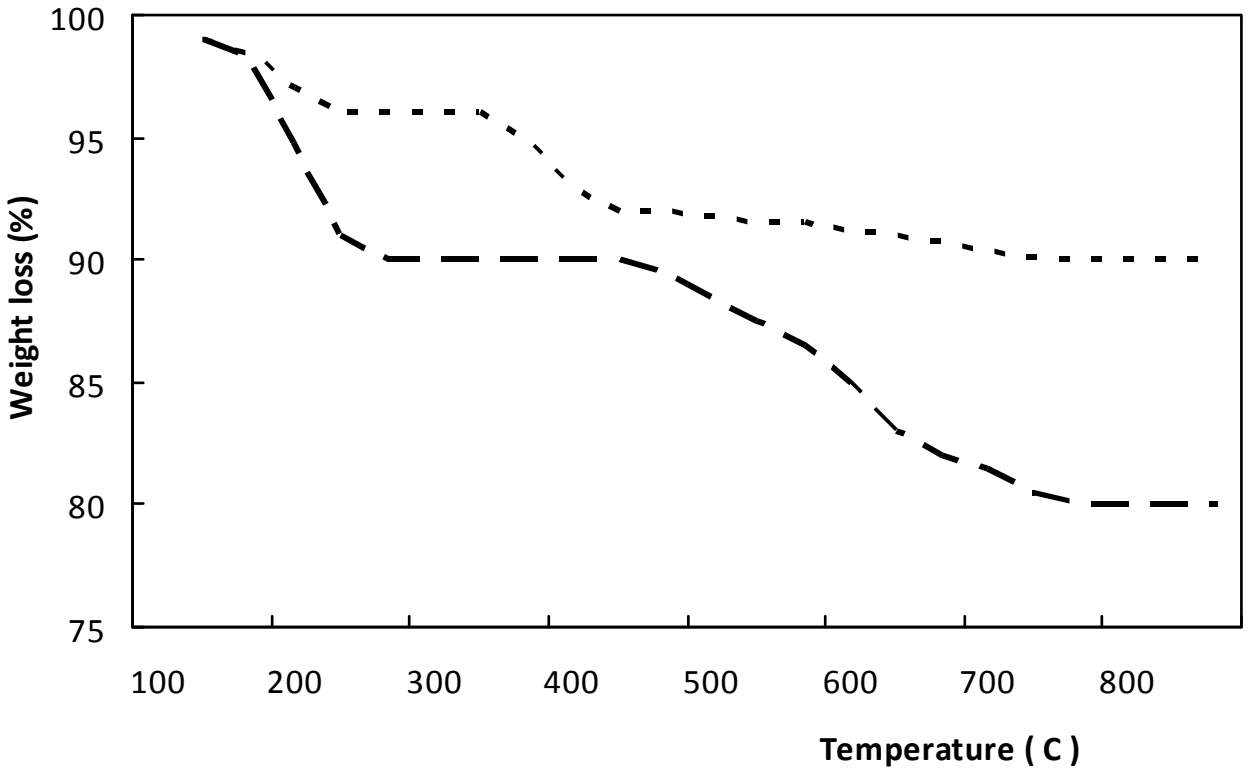
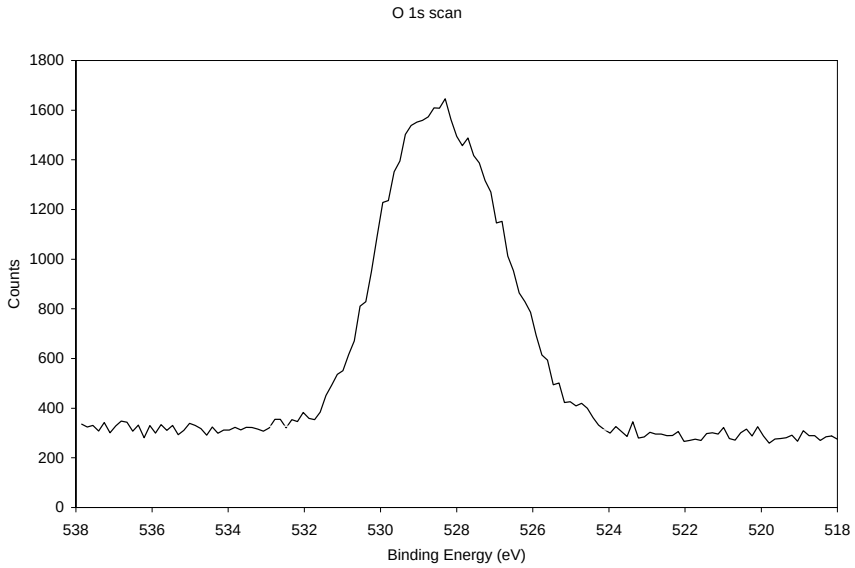
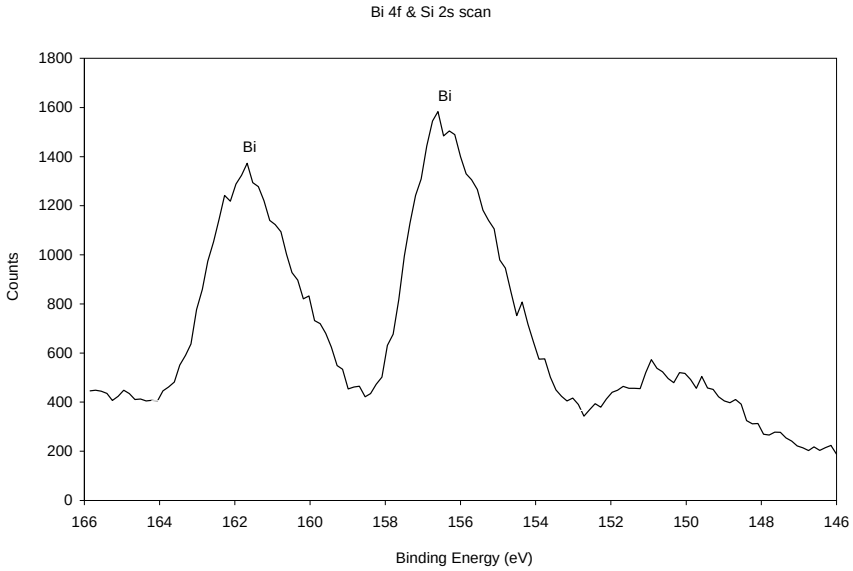


Figure SI1. Thermogravimetric analysis curves for original un-modified montmorillonite clay (long dashed line) and Claycunbic (short dashed line) after calcination

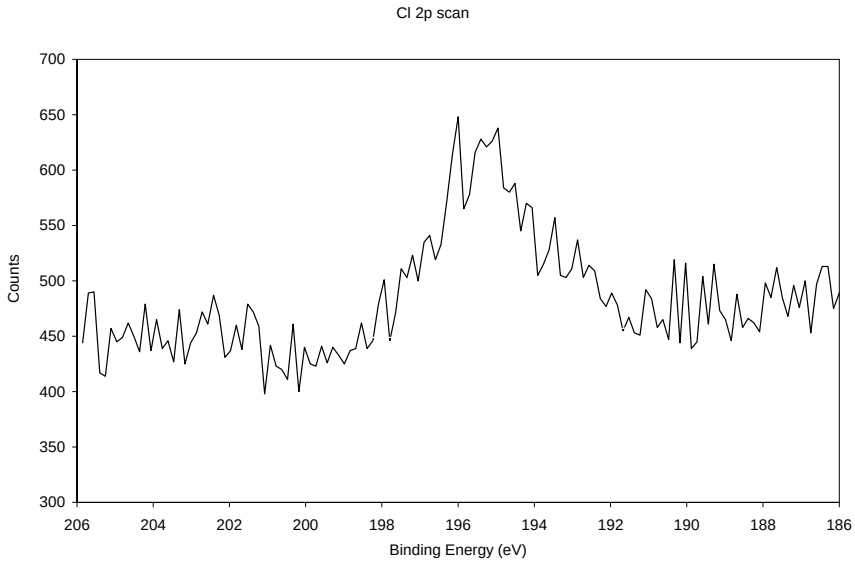
a)



b)


Bi 4f & Si 2s scan
Counts
Binding Energy (eV)
Bi
Bi
1800
1600
1400
1200
1000
800
600
400
200
0
166
164
162
160
158
156
154
152
150
148
146

c)



d)

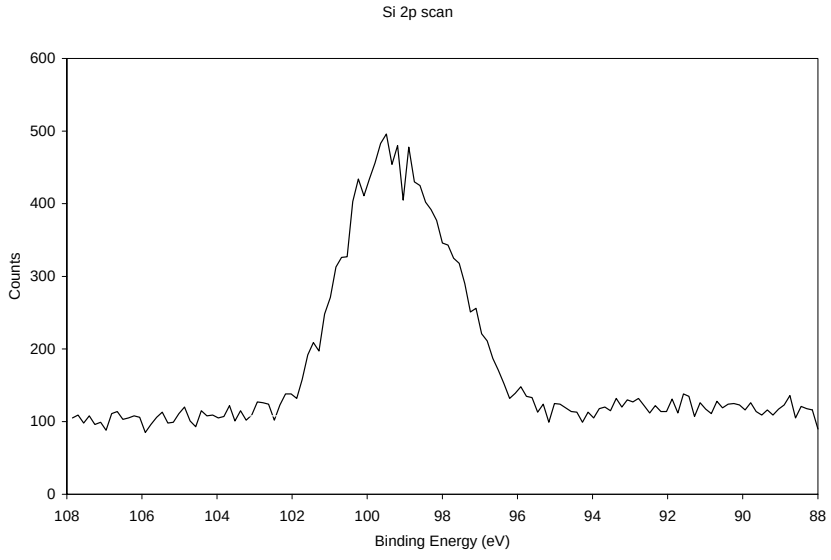
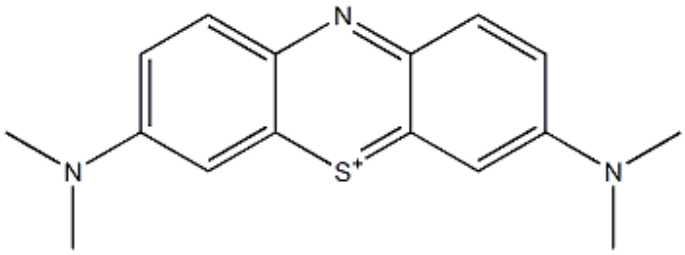


Figure SI2. High resolution graphs for each region a) O, b) Bi, c) Cl and d) Si of XPS spectra.

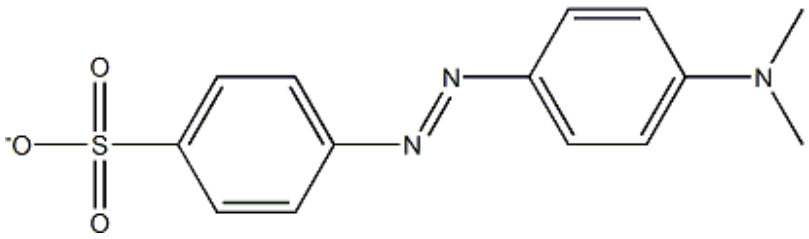
Table SI1. Atomic concentrations of elements in Claycunbic obtained by XPS.

Element	C	O	Cl	Bi	Si	Al
Atomic concentrations (%)	1.3	63.6	3.9	3.6	23.8	3.0

a)



b)



c)

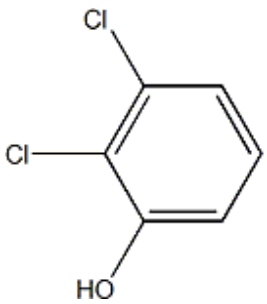


Figure SI3. The chemical structures of a) MB, b) MO and c) DCP.

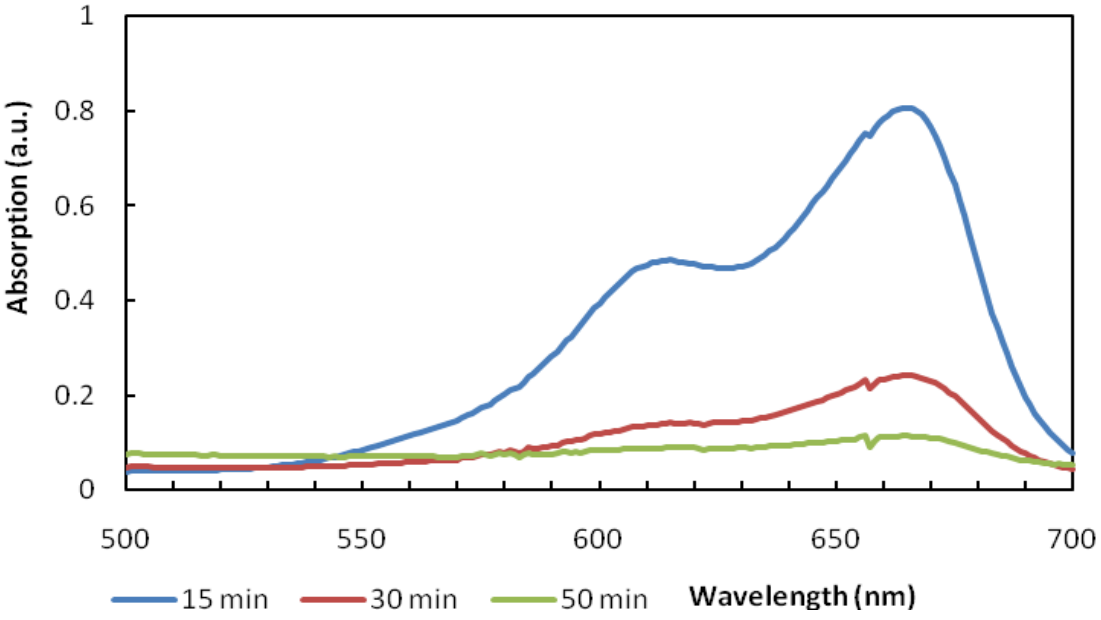


Figure SI4. Absorption bands of Methylene blue (MB) at various reaction times.

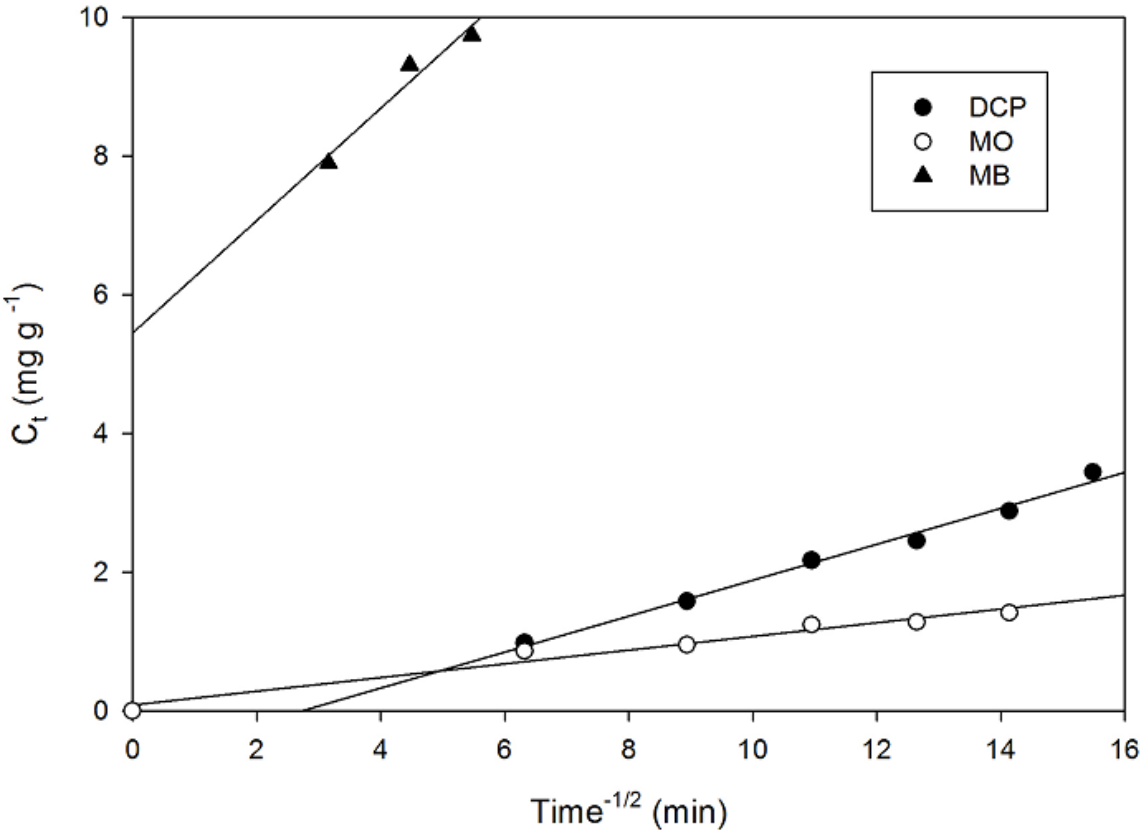


Figure SI5. The sorption capacity vs the square root of time for Methylene blue (MB), Methyl orange (MO) and Dichlorophenol (DCP) adsorption onto Claycunbic.

Table SI2. Intra-particle diffusion model parameters (k_{id} (mg g^{-1} $min^{-1/2}$, C (mg g^{-1}), R^2).

	k_{id} (mg $g^{-1}min^{-1/2}$)	C (mg g^{-1})	R^2
DCP	9.2×10^{-2}	0.95	0.98
MO	1.2×10^{-2}	1.42	0.99
MB	0.4	6.2	0.99

Table SI3. Experimental and coded values of low (-1), medium (0) and high (1) level of variables (catalyst amount and hydrogen peroxide dose)

Independent variables	Coded values		
	Low level (-1)	Medium level (0)	High level (1)
Catalyst amount (g L^{-1})	0.5	1	3
Hydrogen peroxide dose (mL)	0.1	0.5	1

Table SI4. Experimental matrix: reaction conditions as coded values (hydrogen peroxide dose as x_1, catalytic adsorbent amount as x_2 and Methylene blue (MB) removal efficiency as Y), results and predicted values

Run	Hydrogen peroxide dose (x_1, mL)	Catalytic adsorbent amount (x_2, g L^{-1})	MB removal efficiency, exp. (Y, %)	MB removal efficiency, pred. (Y, %)
1	-1	-1	15	21
2	-1	0	100	91
3	-1	1	100	103
4	0	-1	12	12
5	0	0	79	80
6	0	1	100	98
7	1	-1	17	12
8	1	0	74	80
9	1	1	100	99

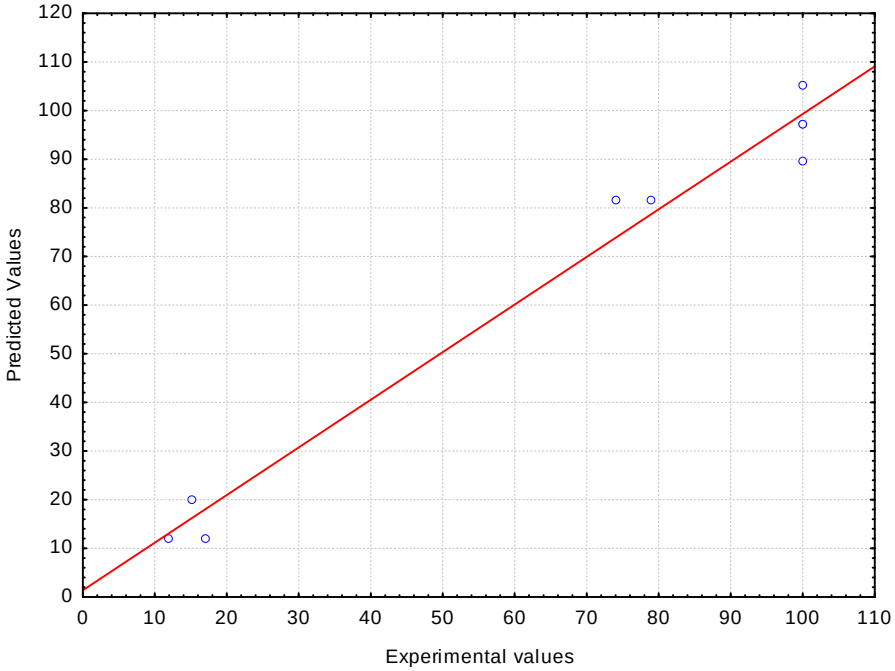


Figure SI6. The predicted vs experimental Methylene blue (MB) degradation plot.

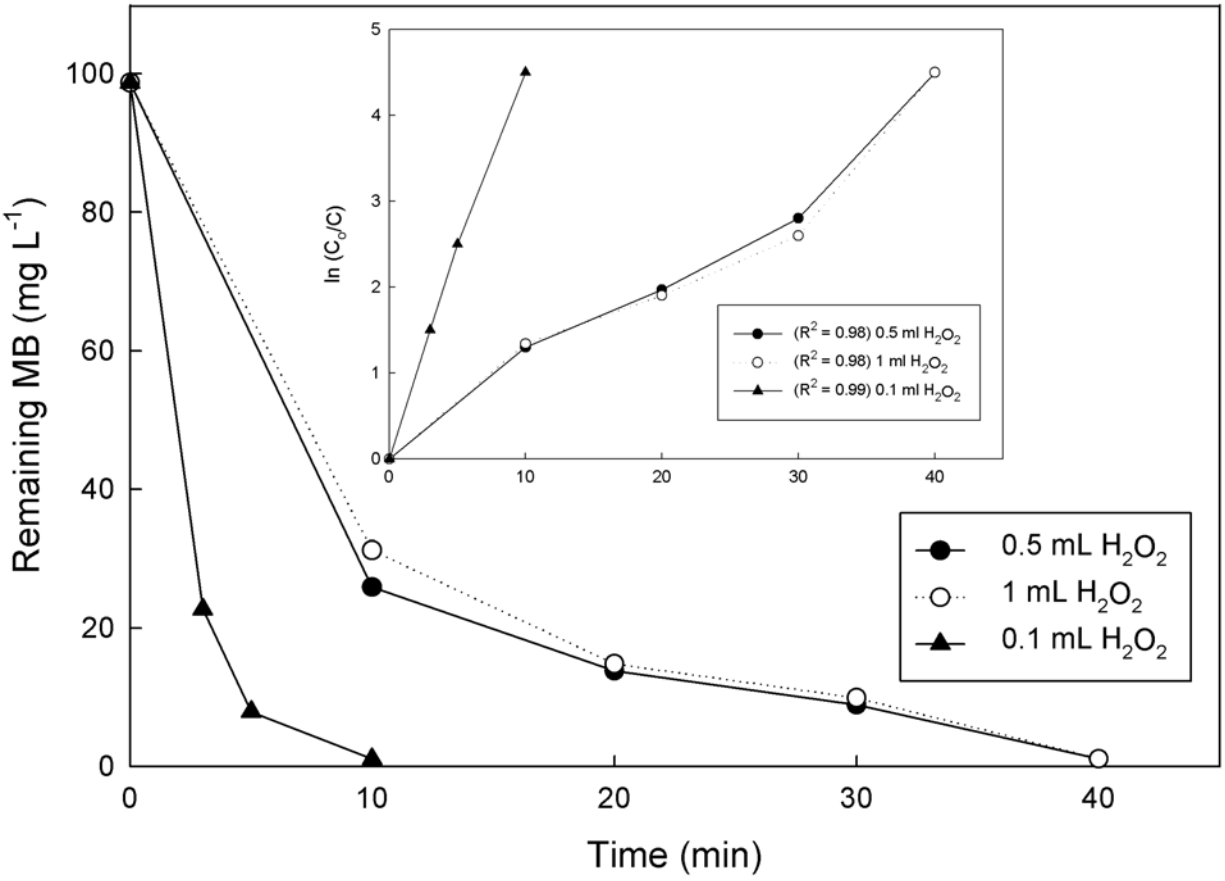


Figure SI7. The effect of the contact time on degradation of Methylene blue (MB) in the presence of optimized Claycunbic concentration (1 g L^{-1}) and various H_2O_2 doses (the values of k_d were determined from the slope of the linear plot of ln [C_o /C] vs t and given as inset).

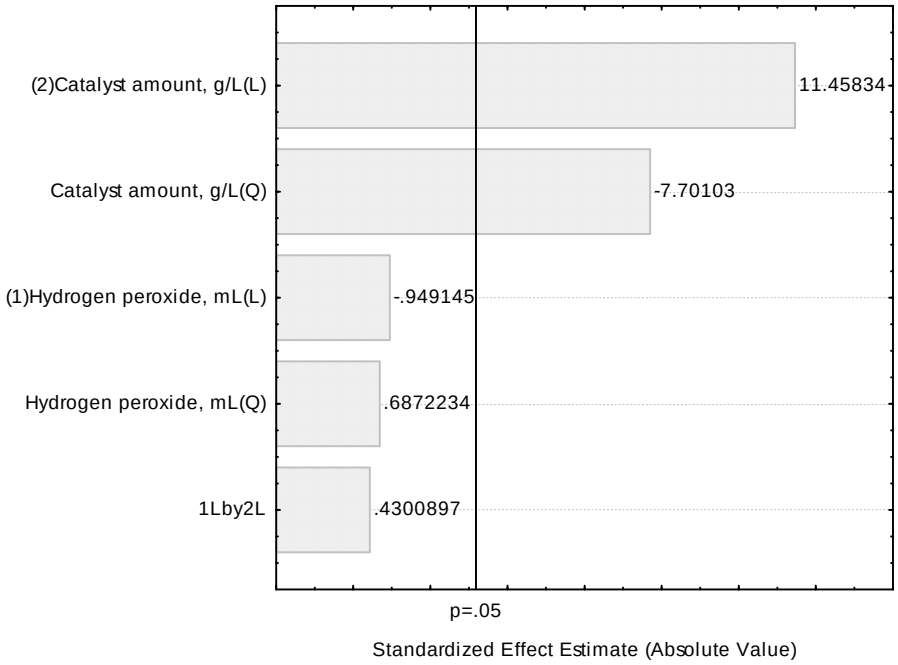
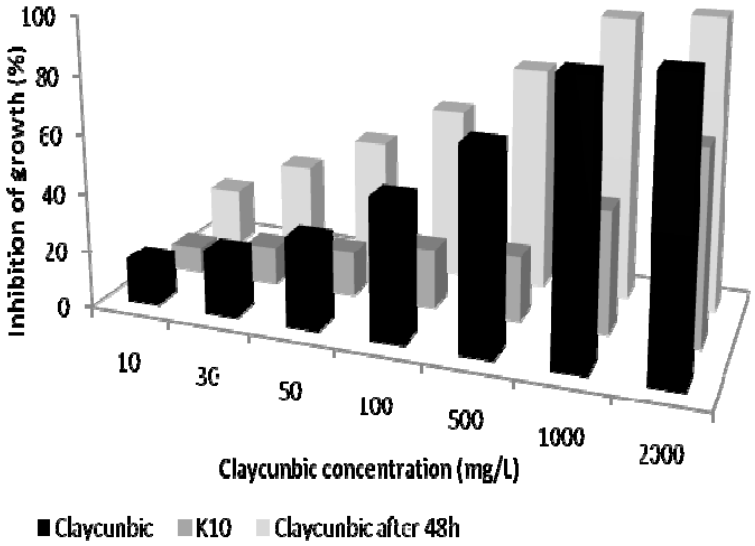


Figure SI8. Pareto chart showing the standardized effects of the independent variables (catalyst amount and hydrogen peroxide dose) and their interactions on the response (Methylene blue (MB) removal efficiency) of the system.

A)



B)

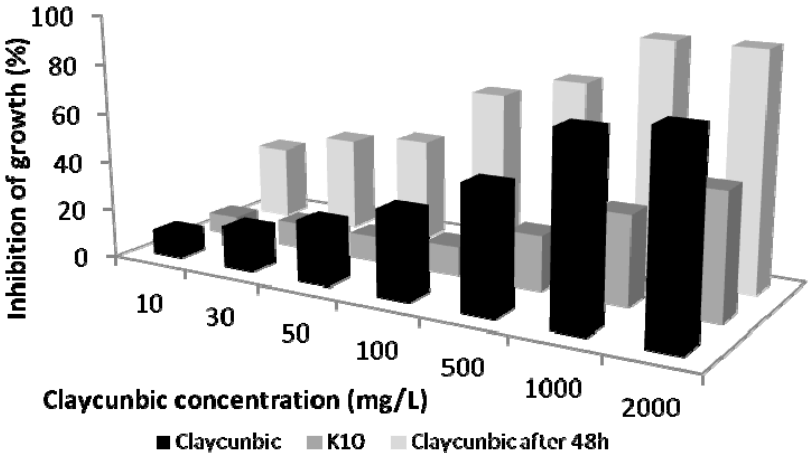


Figure SI9. Inhibition (%) of the luminescence of *V. fischeri* as a function of exposure to various concentrations original un-modified montmorillonite clay K10 (grey bars), Claycunbic (black bars) and

inhibition after 48 h of exposure to Claycunbic (pale grey bars): A) original and B) recycled Claycunbic.

References

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