Desalination and Water Treatment
www.deswater.com
20 (2010) 209–219
August
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doi: 10.5004/dwt.2010.1171

Inactivation of faecal bacteria in wastewater by methylene blue and visible light

Sonia Sabbahi[a,b]*, Zoubeir Alouini[a], Layla Ben Ayed[a], Meryam Jemli[a], Abdellatif Boudabbous[b]

[a]*Laboratoire de parasitologie, Institut National de Recherche en Génie Rural Eaux et Forêts, B.P. 10, 2080 Ariana, Tunis, Tunisia*
Tel. +216 71719630; Fax +216 71717951; email: sabbahisonia@yahoo.fr
[b]*Laboratoire Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis*

Received 6 April 2009; Accepted in revised form 26 January 2010

ABSTRACT

The high inactivation of faecal indicators [faecal coliforms (FC), *E. coli* and faecal streptococci (FS)] using a combination of methylene blue (MB) with natural sunlight or artificial visible light determined on a small scale, was dependent mainly on the MB concentration, its application process and pH. In order to avoid primarily leaching of the compound into the environment and to further understand the MB photosensitization mechanisms, MB should be properly immobilized within resin. The FC and FS were found to be susceptible to the photodynamic action of MB fixed to the support. The mechanism of faecal bacteria inactivation by MB also seems to be a combination of Type I and Type II processes, and the relative efficiency of each of them depends notably on the experimental conditions. In parallel, the MB stability under light "photobleaching" has been studied by optical absorption spectroscopy. It has been shown that it was dependent essentially on pH, nature of the medium (distilled water and secondary wastewater effluent) and time exposure to light. Practically, all of the MB (10 μM) disappeared from effluent, exposed to sunlight, by the end of a 12 h experiment with a bleaching rate from 92 at neutral pH. Kinetic data indicate that the dye photobleaching efficiency can be approximated by pseudo-first-order reaction.

Keywords: Faecal coliforms; Mechanisms reaction; Methylene blue; Photobleaching; Photosensitization; Pseudo-first-order reaction; Wastewater

1. Introduction

In the last few years, there has been a diversification of water reuse, namely green spaces and crop irrigation [1]. So, an important attention has to be accorded to its microbial quality. In fact, if a more efficient elimination of microorganisms is needed, disinfection of wastewater must be done. The use of chlorination has been decreasing mainly due to mutagenic and/or carcinogenic disinfection by-products (DBPs) and chlorine residual formed in the disinfection process [2,3]. The ultraviolet (UV) efficiently eliminates enteric bacteria, spores, viruses, and parasites (oo)cysts without producing DBPs or other chemical residues [4,5]. The disadvantage of the UV is its lack of bacteriostatic effect and possibility for photoreactivation or dark repair of UV damaged for some microorganisms [6–9]. Moreover, peracetic acid (PAA) or peroxyacetic acid is a strong disinfectant with a wide spectrum of antimicrobial activity. Major disadvantages associated with it are its cost and the increase of organic content in the effluent due to acetic acid enhancing microbial regrowth (acetic acid is already present in PAA mixtures

* Corresponding author.

and is also formed after PAA decomposition) [10]. From another side, the membrane technologies produce a high-quality clarified effluent and do not require the addition of chemical reagent, thus avoiding the formation of harmful by-products [11]. The greatest obstacle of this technology is its cost.

Recently, advanced oxidation processes (AOPs) for wastewater disinfection have been introduced [12,13]. AOPs are based on the utilization of secondary oxidants, such as ·OH radicals, which are typically generated by the interaction of UV irradiation with a chemical disinfectant capable of releasing radicals. Hydroxyl radicals are considered as the most reactive oxidizing agents in water treatment and they can be used for the oxidation of organic and inorganic compounds or for disinfection purposes. Among these AOPs, TiO_2-photocatalysis [14–16] and photosensitization which appears to be particularly promising [17–24]. Photosensitization or photodynamic inactivation (PDI) is a platform technology which uses a combination of a photosensitizer, light and molecular oxygen to achieve selective destruction of a biological target [25,26]. Energy from light is absorbed by the photosensitizer and then passed on to molecular oxygen with the formation of the very reactive singlet oxygen ($^1O_2$). During this process, the photosensitizer is regenerated so that it acts as a kind of catalyst and many molecules of singlet oxygen can be formed from a single molecule of photosensitizer, so long as light and molecular oxygen are present. Upon irradiation with light of an appropriate wavelength and in the presence of molecular oxygen, photosensitizers can initiate a photochemical Type I or a Type II reaction or a combination of both [27]. In a Type I reaction, the activated photosensitizer reacts with a substrate molecule by either an electron or a hydrogen transfer, leading to the formation of radicals. In a Type II reaction, an energy transfer occurs to the ground state of molecular oxygen, leading to the production of the reactive singlet oxygen. As a consequence of both pathways, the photodynamic effect can result not only in selective tissue injury, but also in the elimination of different kind of pathogens if they are present in the direct neighbourhood of the photosensitizer [28]. Both pathways can lead to cell death [29] and pathogens inactivation in wastewater [17,18,22,23,30]. The majority of PDT experiments were carried out with the photosensitizer in solution. However, this approach can be inappropriate for applications where residual traces of photosensitizer in the medium are not acceptable, such as water disinfection. In this way, photosensitizers immobilized on polymeric supports have been proposed to avoid this problem [31–34].

So, the aim of the present work was to investigate the photosensitization of secondary effluent triggered by monocationic methylene blue photosensitizer (MB) selected as the photosensitizing agent for Tunisian secondary wastewater effluent. MB was used at different concentrations against faecal coliforms (FC), *Escherichia coli* and faecal streptococci (FS), under visible light (artificial visible light and natural sunlight). Besides, different pH values were used to determine the MB photodynamic efficiency. In addition, the MB stability under visible light "photobleaching" was investigated by optical absorption spectroscopy.

The current work reported also the immobilization of the MB in resin in order to avoid leaching compounds into the environment and to further understand the MB mechanism photosensitization.

2. Materials and methods

2.1. Wastewater characterization

Wastewater samples used in this study were effluents collected from the wastewater treatment plant (WWTP) of Charguia. Table 1 summarized the main characteristics of the effluent and of this WWTP. The standard faecal indicators: FC, *E. coli* and FS were used. Water samples were collected in 1 L sterile glass bottles and analysed immediately after collection. The presence of thermotolerant coliforms (FC), *E. coli* and FS was studied using most probable number method (MPN). This latter consists of three steps: a presumptive test, a confirmation test and a completed test. In the multiple-tube method, a series of tubes containing a suitable selective broth culture medium is inoculated with test portions of wastewater sample [35]. Results were expressed as the most probable number of bacteria present in 100 mL water, if all samples tubes were negative, the result expressed as <

Table 1
Main characteristics of the Charguia secondary effluent used in this study

Localisation of the plant	District of Tunis, Tunisia	
Treatment process	Primary sedimentation + Activated sludge	
Parameter	Charguia secondary effluent	
	Minimum	Maximum
pH	6.45	7.47
DO (mg L^{-1})	5.7	6.7
COD (mg L^{-1})	60	130
BOD5 (mg L^{-1})	17	28
Colour as Vis-abs at 400 nm (cm^{-1})	0.167	0.216
EC (mS cm^{-1})	2.94	5.4
Suspended solids (mg L^{-1})	27	50
Faecal coliforms (per 100 mL)	104	106

DO: Dissolved oxygen; COD: Chemical oxygen demand; BOD_5: Biological oxygen demand; EC: Electric conductivity; Vis–abs: Visible absorbance. Absorbance at 400 nm was used to characterise apparent colour.

3 CFU per 100 mL water (< 0.5 log) (1 positive tube = 3 CFU according to the number of selected dilutions), four dilutions for faecal indicators in wastewater before and after treatment were done.

Serial dilutions of irradiated with MB and control samples (irradiation without photosensitizer or incubated in the dark with photosensitizer) were performed.

2.2. Photosensitizer and light source

MB (Prolabo) was kept in the dark. A 500 Wt halogen lamp (OSRAM) was the light source, with an intensity of 500 W m^{-2} was employed in the photomicrobial testing experiments. The entire irradiation spectrum of the lamp (range: 500–750 nm; peak: 650 nm). An incident light rate on the sample was approximately 500 W.m^{-2}. The distance between the lamp and the irradiated water container was 45 cm. In all experiments the samples were added with suitable volumes of photosensitizers' solutions to yield photosensitizer concentrations used. Before stating the irradiation, the samples were maintained in the dark for 10 min under moderate stirring to obtain a homogenous reaction medium (average of dissolved oxygen content was to the order of 6.2 mg/L). Experiments were run without stirring or supplementary aeration in plastic and rectangular container (an area of 660 cm^2) containing a 1.8 cm thick layer of water with a volume of 1 L. The same experiments were repeated under solar light to compare the effectiveness of solar disinfection with that of halogen lamp. It was applied in the spring–summer time. For the experiments conducted in sunlight, total solar radiation was measured and recorded over the duration of the experiment. It ranged from 500 to 894 W m^{-2}. Measurements were taken using a solarmeter–pyranometer (Instruments HAENNS messger.A.TE, solar 118) placed on the horizontal directly beside the reactors. Wastewater samples were exposed to the Tunisia sunshine and the experiments were conducted during spring and summer (from April to August). Complete range sunshine without cloudy days conditions was encountered during these experiments. Solar power levels varied from a maximum of 894 W m^{-2} (full sunshine, summer time) to a minimum of 500 W m^{-2} (spring time). The country also benefits from a rate of sunshine (more than 3000 h/y) with a maximum at about 4700 h/y in August. Experimental sets where conducted on different days, and though efforts were made to minimize the differences between sets, both solar radiation and initial contaminant concentrations varied from set to another.

2.3. Laboratory experiments

The pH of the secondary wastewater sample was adjusted with the addition of sulphuric acid or sodium hydroxide. The suspension was first stirred in the dark for 5 min before irradiation.

2.4. Spectroscopic and photophysical studies

MB solution was prepared using sterilised distilled water. MB structure was already illustrated in Sabbahi et al. [36]. Absorbance was obtained with a commercial sample of MB, used without any further purification, was recorded spectrophotometrically in various solvent (distilled water and wastewater) systems. Absorbance was measured with a Spectronic® 20 Genesys TM spectrophotometer.

The MB was directly dissolved in distilled water at a concentration of 10 μM. The concentration was checked spectrophotometrically using the extinction coefficient

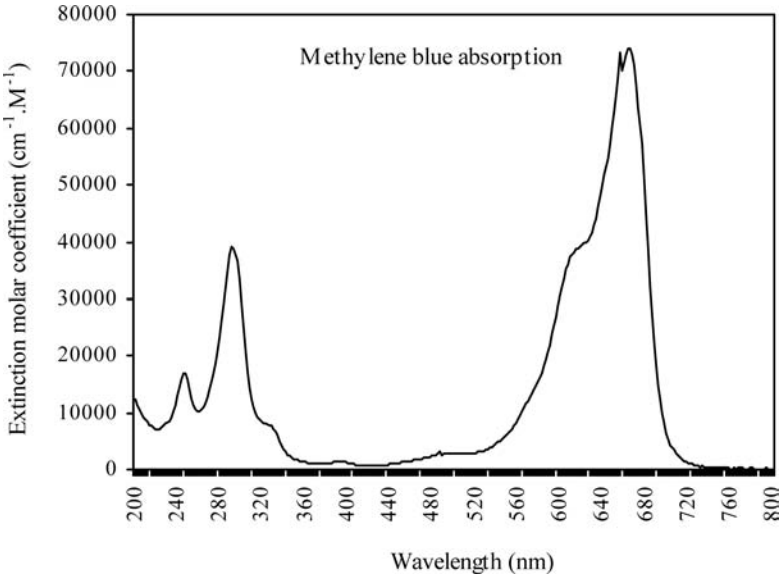

Fig. 1. Methylene blue UV-visible absorbance spectrum in aqueous solution.

(71089 M^{-1} cm^{-1} at 670 nm) shown in Fig. 1 as a theoretical absorption spectrum of 1 M MB. This spectrum was taken with a spectrophotometer using a 1 cm quartz cuvette filled with 1 M solution of MB in Water to convert this data to absorbance *A*, multiply by the molar concentration and the pathlength. To convert this data to absorption coefficient in (cm^{-1}), multiply by the molar concentration and absorbance.

Maximum absorbance wavelength (λ_{max}) for each irradiated sample was recorded to determine the photobleaching of dye. The dye concentration measured from the field control container served as the initial concentration (C_0) for calculating the $C(t)/C_0$ ratio.

2.5. MB immobilization with resin

The wastewater sample was poured onto a container (rectangular shape of ≈ 30×20 cm) which was coated into its sides by mixture [MB + polyester isophtalic non-accelerate resin AROPOL® K514 (Ashland and Scott Bader) + ethanol (Biotechnica)]. The matrix was allowed to dry overnight at ambient temperature. Samples were exposed to the visible light in the container colourwash with the mixture for bacterial testing. The absorption was due to MB because the resin is transparent in the visible region. Such a resin was chosen for several characteristics namely it was insoluble in water with best resistance to chemicals, heat and hydrolysis and non toxic at low doses.

An ANOVA test was carried out to assess homogeneity of variance with a significance level of 5% ($p < 0.05$).

3. Results and discussion

3.1. MB concentration and light source

When secondary wastewater sample (pH ≅ 7) was irradiated with artificial visible light in the presence of MB, the expected disinfection effect was observed for all bacteria with MB concentrations varying from 5 to 70 μM (Fig. 2). The initial concentration of FC was 1.1×10^4 CFU per 100 mL. In the presence of 5 μM MB and artificial visible light, little disinfection occurred. No significant statistical differences (ANOVA, $p < 0.05$) were found between FC counts in the effluent from secondary wastewater and after 2 h using 5 μM MB. This latter was found to be less effective for photochemical disinfection when used under artificial visible light (Fig. 2). In the samples taken after 2 h, there was a steady increase in the percent destruction, which ranged from 78 to >99% as MB concentration increased from 10 to 50 μM. In this time, statistically significant differences were observed between these concentrations used for FC, *E. coli* and FS reduction. In fact, after 2 h at 50 μM MB, the samples exhibited > 99% FC reduction. A slight decrease of CF, *E. coli* and FS reduction under artificial visible light, was noted at 70 μM MB. This latter concentration might hinder light penetration [17,37]. Other studies [38], in the condition of large concentration of dimers, (namely at 70 μM concentration in our work), Type II is shifted to Type I reaction, practically abolishing $^1O_2$ generation.

Complete disinfection did not occur at all in the dark (Fig. 2), although 58% of coliform reduction was observed with ≥ 50 μM MB in the dark vs. 7 to 81% of *E. coli* reduction with respectively 10 and 50 μM MB. For FS, a reduction of approximately 88% with 50 μM MB was observed. The control visible light alone had a coliform reduction of 40% for 2 h contact time (Fig. 2).

The same experiments were repeated under sunlight in order to compare the effectiveness with that of artificial visible light (Fig. 2). The initial sample temperature was about 23°C and after 3 h of irradiation, temperature remains between 28 and 35°C. Spot temperature checks with temperature strips on the outside reactor glass yielded values of 35°C in sunlight, which was considered not sufficient to cause disinfection by pasteurization [20,39,40].

Photodynamic effect on FC, FS and *E. coli*, showed respectively 9, 44 and 67% of reduction after 2 h of sunlight and at 10 μM MB. Only a few FC were detected in the effluent at 50 μM MB and after 6 h of contact time (<0.5 log in samples contained FC with a maximum value of <3 CFU 100 mL^{-1}). From 20 μM MB, inactivation yield of bacteria was favoured which were compared for water reuse standard: WHO stringent guidelines for irrigation, FC <1000 CFU 100 mL^{-1}.

In the absence of MB with the same intensity sunlight and after 2 h, 85% (a 0.8 log) of FC reduction was noted (Fig. 2). The reduction in the viable cell number for the blank (sample without MB) must be attributable to direct solar–UV radiation instead of heating or MB photodynamic action. In order to avoid any ambiguity, the term MB photodynamic action consists in the photoinactivation of bacteria, corresponding to the loss of cultivability caused by light and MB as photosensitizer.

Results also showed a no reduction of some physical/chemical parameters (COD, BOD_5, EC, pH) (data not shown).

3.2. The pH effect on the MB photobactericidal action

In previous studies of MB photodisinfection processes pH values from 8.6 to 10 were found to be optimum [17,30,41,42]. Comparative experiments were performed at three pH values: 5, 7 and 9 in our experiments (Fig. 3). Ten μM MB was used for the pH effect study.

As shown in Fig. 3, results indicated a significant pH effect for disinfection with MB, corroborating Eisenberg et al. [43] work which reported a very strong correlation with pH values and more efficient MB inactivation at a basic pH. However, this was inconsistent with Cooper and Yogi Goswami [20] findings, which reported no significant pH (7 and 10) effect for water disinfection with MB. After 1 h of irradiation and at pH 7, 77% of FC

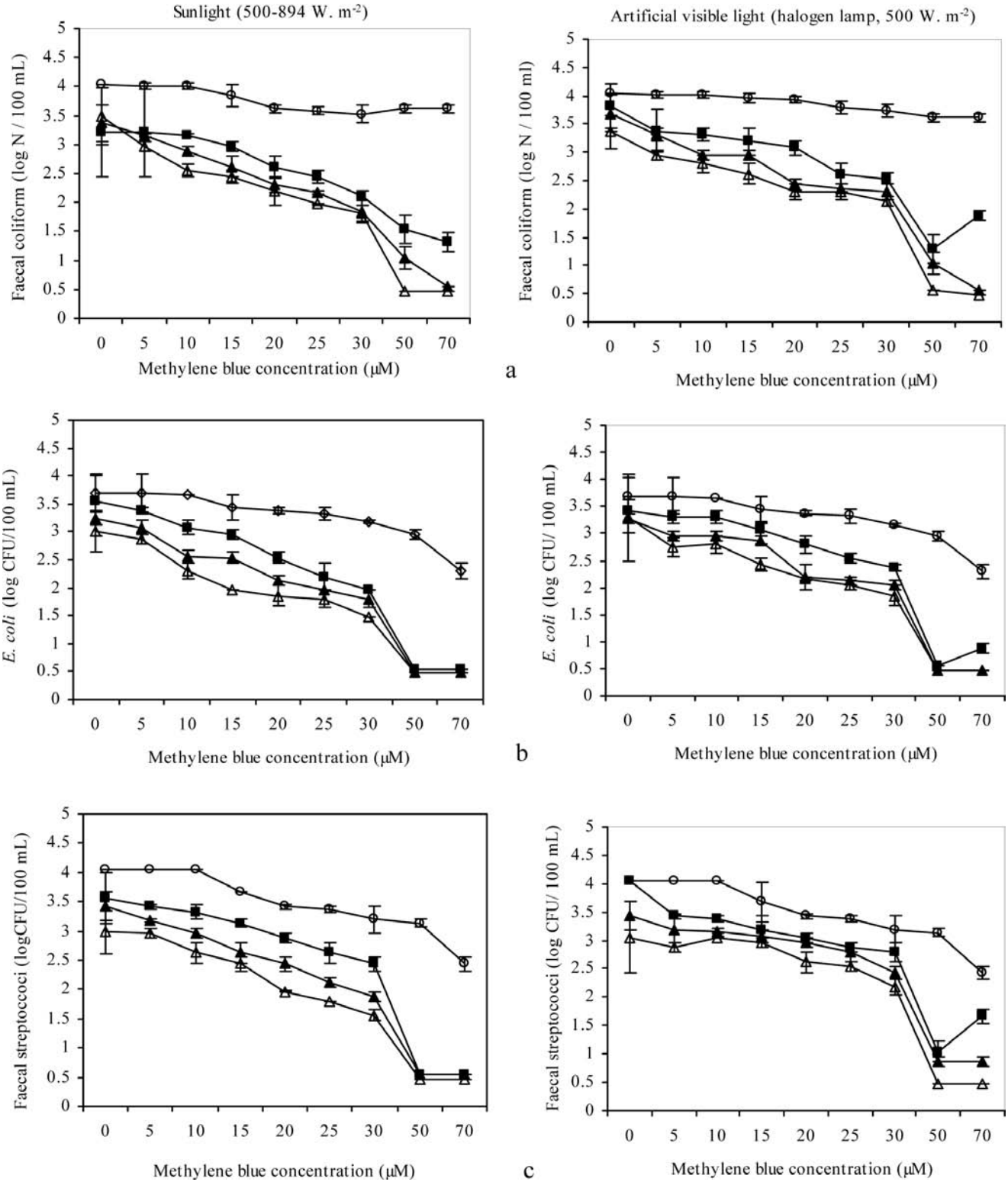

Fig. 2. (a) Faecal coliform, (b) *E. coli* and (c) Faecal streptococci counts (log CFU/100 mL) photoinactivation in secondary wastewater effluent (pH ≅ 7) by methylene blue at different concentrations used by sunlight (I_{avg} = 500–894 W m^{-2}) and artificial visible light using halogen lamp (500 W m^{-2}) after (■) 2 h contact time, (▲) 4 h, (△) 6 h and(○) samples incubated in the dark with methylene blue at different concentrations. Values are means of three replicates.

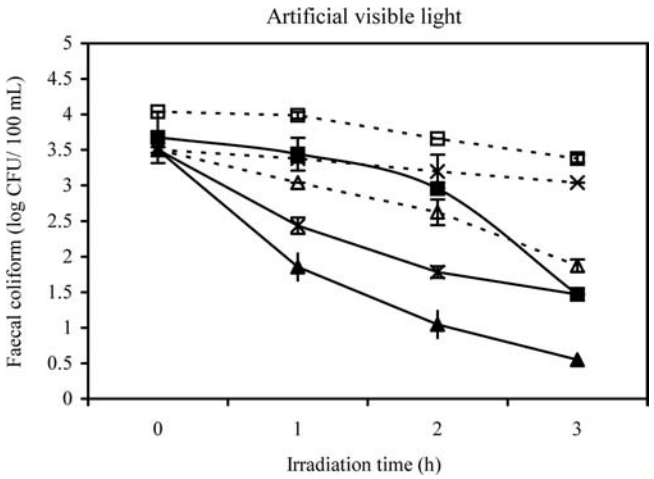

Fig. 3. Faecal coliforms photoinactivation in secondary wastewater effluent by artificial visible light with methylene blue (10 μM) at pH values: (—×—) pH 5; (■) pH 7; (▲) pH 9 vs. artificial visible light without methylene blue at pH values: (- -×- -) pH 5; (- -□- -) pH 7; (- -Δ- -) pH 9.Values are means of three replicates.

reduction was observed with 10 μM MB; comparatively, only 17% reduction was attained with artificial visible light alone. At pH 9, MB (10 μM) resulted in at least 98% FC reduction after 1 h of artificial visible light, compared to 65% reduction with light alone. At pH 9, the presence of MB increased the inactivation rate compared with the absence of the photosensitizer (Fig. 3). With MB practically complete coliform destruction was achieved after 3 h of irradiation. In this case, statistically significant differences with a level of 5% ($p < 0.05$) were observed between FC reductions from the pH 9. Survival of FC was reduced under photodynamic treatment at indices of pH 5 and 9 in comparison to the survival at pH 7. The results of control samples kept in the dark with sensitizer showed a small significant decrease of bacteria in wastewater.

It should be known that we have not recorded significant fluctuations of the pH values during the wastewater photosensitization using MB (data not shown).

Presumably, the photobactericidal effect is due too to the relative charges of the bacteria and the MB at high pH. Since the MB is quaternized [44] it has a constitutive positive charge that will not be affected by pH. The bacteria on the other hand have ionizable groups (carboxylates and phosphates) that will be much more negatively charged at high pH, therefore the interaction between bacteria and MB is expected to be greater at high pH. Another works demonstrated that a high pH may increase the penetration of toluidine blue (TBO) into the cells [45]. It is commonly known that methylene blue and toluidine blue have a similar chemical structure and photochemical properties [46]. Wakayama et al. [47] reported higher uptake of TBO molecules by *E. coli* at a slightly basic pH. Higher pH values may also promote the production and effectiveness of cytotoxic molecules. MB has been extensively used for photooxidation of natural and synthetic molecules. Two major photochemical pathways are usually observed: type II where the triplet energy is transferred to oxygen forming singlet oxygen ($^1O_2$, Reaction (2), Fig. 4) and Type I where reducing agents donate an electron to the MB triplet, forming the semi-reduced radical (MB, Reaction (3), Fig. 4). In aqueous solution, the efficiency of $^1O_2$ is dependent on the pH [48]. Tardivo et al. [49] who worked at pH 4, showed that MBH^{2+} triplets had a higher energy level and consequently they reacted with oxygen with a smaller rate constant. They live longer in homogeneous solution and they may produce less $^1O_2$ in the presence of other reaction pathways. The effect of pH on the efficiency of photoinduced process will depend on the sensitizer. In the case of MB, the triplet will react faster with oxygen with the increase in pH, forming more singlet oxygen, that can engage in several types of reactions including getting reduced and end up forming hydroxyl radical in the presence of iron (Reaction (7–9), Fig. 4) [38,50]. Therefore, the pH of the solution may certainly affect the efficiency of Type I and Type II photosensitization mechanism. Furthermore, the behaviour of the MB in such conditions, leads to a contribution of Type I/Type II mechanisms.

3.3. Study of the MB photodegradation under light: MB photobleaching

One drawback to the use of MB as a disinfectant for secondary wastewater was the presence of the dye. Since dyes have been known to be photosensitized [20,51]. The study of the MB photobleaching under light for a long period of time was achieved. The present study was conducted in distilled water and secondary wastewater effluent by different pH values (5, 7 and 9).

The MB photobleaching used was studied spectrophotometrically. Practically, all of the MB at 10 μM disappeared, by visual inspection from container in secondary wastewater exposed to sunlight, by the end of a 12 h experiment, with a photobleaching efficiency of 92% at pH 7 and 98% at pH 5 and 9 (Fig. 5a). In this condition, some slight color was still visible at 10 μM MB. In distilled water, we attained from 48 to 90% respectively at pH 5 and 7 after 6 h vs. 97% at pH 9. The MB photobleaching in distilled water by the sunlight was more rapid than in the secondary wastewater. Spectroscopy showed that MB was photoreduced after irradiation with sunlight and that oxidized form is more notably consumed than reduced form, which is colourless (formation of a non-active colourless MB leuco-form, i.e., LMB) [17,52, 53]. The reduced form of MB, LMB, had typically a λ_{max} at 256 nm [54]. The same photoreduction process was observed even in distilled water (absence of organic matter), indicating that MB itself could act as an electron acceptor consent

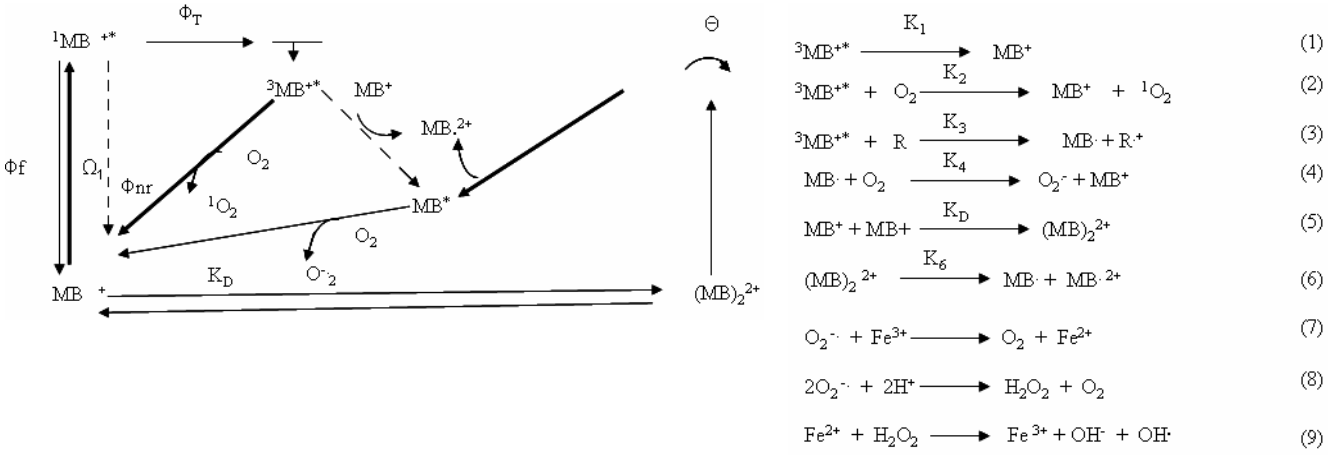

Fig. 4. Methylene blue photochemical reaction routes where MB^+, $^1MB^{+*}$, $^3MB^+$ are methylene blue ground state, singlet and triplet excited states, respectively, MB. And MB^{2+} are methylene blue semi-oxidized radicals, respectively, Ω_1 is light absorption, Φf, Φnr, Φ_T, are fluorescence, nonradiative and triplet quantum yield. Reactions (1)–(4) represent the deactivation routes of MB^+ excited state and radical species where (1) is the $^3MB^+$ spontaneous decay, (2) is the reaction of $^3MB^+$ with molecular oxygen, (3) is the redox suppression of $^3MB^+$ by reducing agents, (4) is the oxidation of MB by molecular oxygen returning the ground state dye and forming superoxide, (5) is the ground state dimerization constant, (6) is the redox suppression of ($^3MB^+$------MB). after exciting ground state dimers, (7)–(9) are Fenton reactions. The relative position of the species presented in this scheme does not represent their actual energy level. Modified from [38] reported by Tardivo et al. [49].

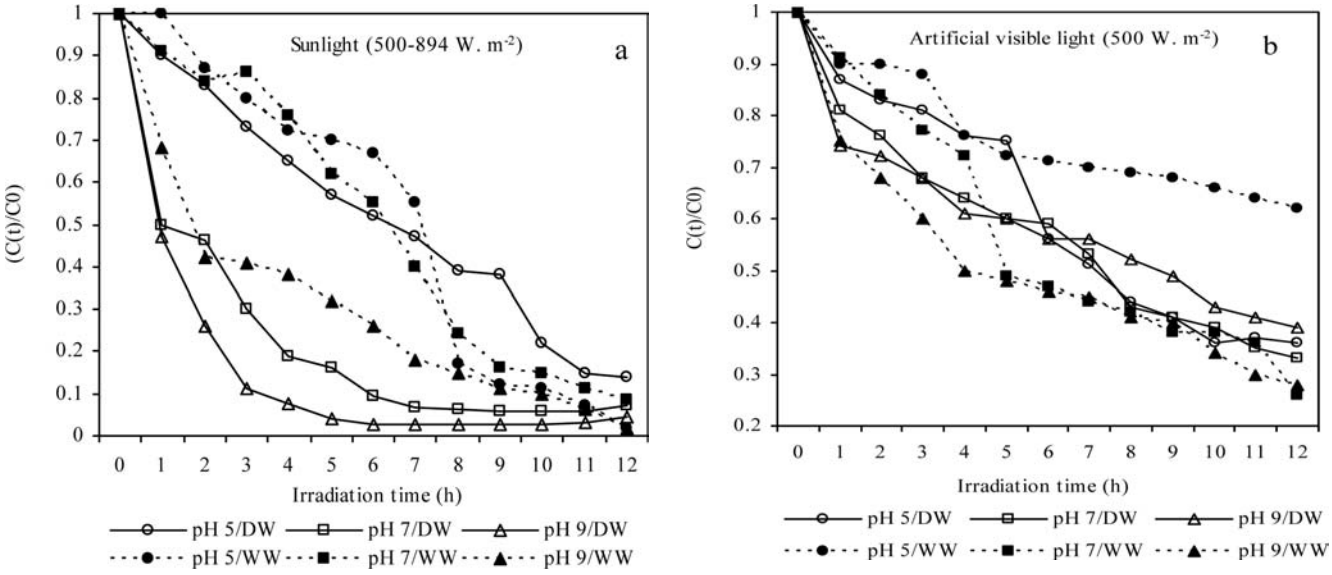

Fig. 5. Methylene blue photobleaching efficiencies at a concentration of 10 μM in distilled water (DW) and secondary wastewater (WW) effluent at different pH values under (a) sunlight and (b) artificial visible light.

to Mills and Wang [54]. The bleaching, as exemplified by the MB photobleaching data, can be approximated as pseudo-first-order kinetics. Fig. 6 shows the linear fit between the $\ln(C(t)/C_0)$ and irradiation time that supports this conclusion. A plot of $\ln(C(t)/C_0)$ vs. time, at different pH, was linear and followed pseudo-first-order kinetics (Fig. 6a). So, our MB photobleaching rate in secondary wastewater, by natural sunlight irradiation, followed pseudo-first-order kinetics with coefficients of determination $R^2 = 0.83$, 0.93 and 0.91 respectively under acidic, neutral and basic pH. Under artificial visible light, practically the same coefficients of determination were obtained

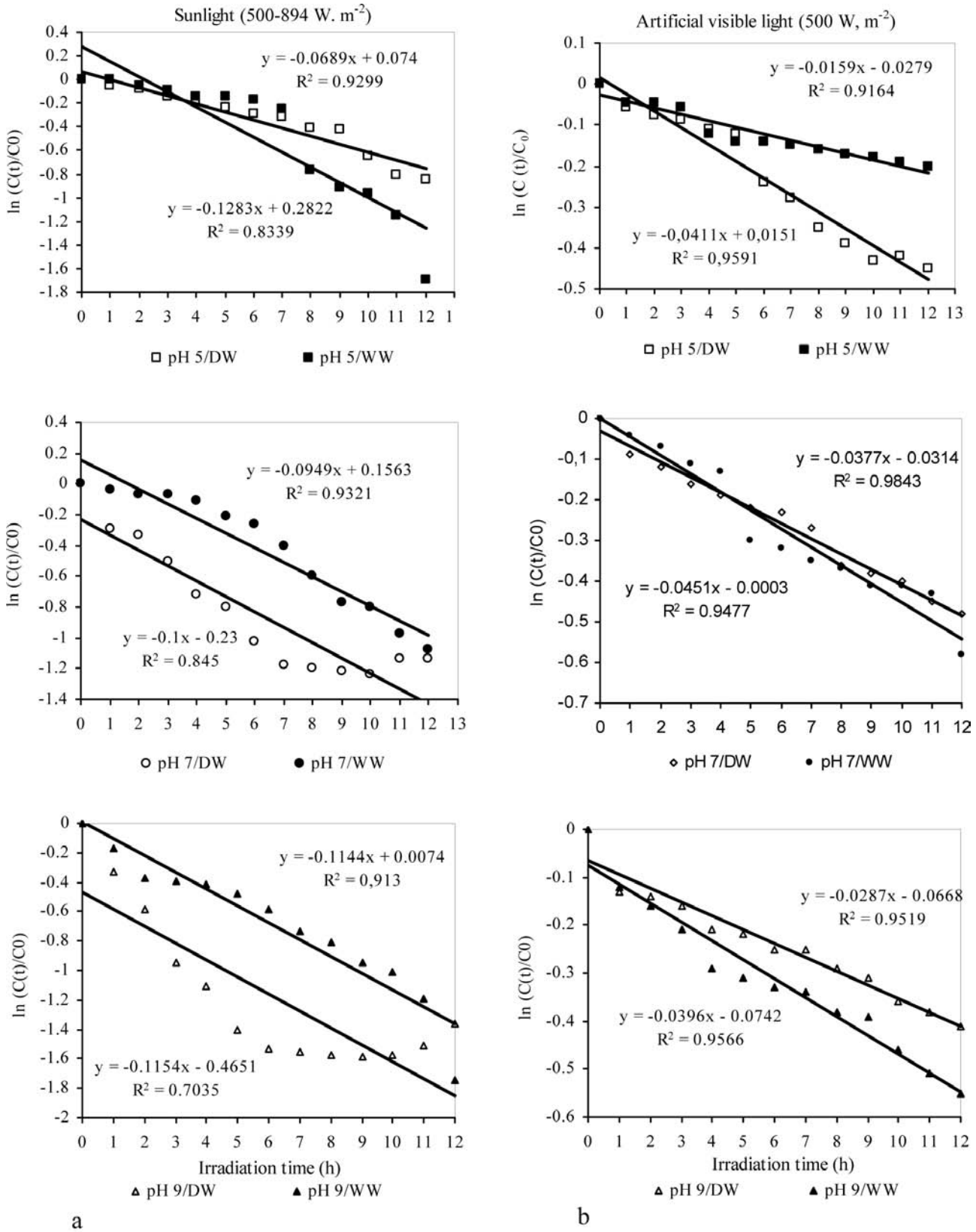

Fig. 6. Straight-line relationship between the ln ($C(t)/C0$) and the irradiation time indicates that photobleaching efficiency of methylene blue (10 μM) in distilled water (DW) and secondary wastewater (WW) effluent at different pH values under (a) sunlight and (b) artificial visible light, can be approximated by a pseudo-first order reaction.

(Fig. 6b), explained by the fact that MB (lmax = 662 nm) absorb strongly in the visible light range of the halogen lamp used (range: 500–750 nm; peak: 650 nm). In addition, the photobleaching process in secondary wastewater at acidic conditions was found to be significantly slower under artificial visible light (Fig. 6b) than sunlight.

As mentioned above, while all of the MB disappeared, by visual inspection from container according to the MB dose used, light source, pH and time exposure to light, the separation of the dye from the water might be done.

3.4. MB immobilized with the polymeric resin

The current work reported on the fixing of the MB with resin and the initial investigation of the photosensitizing properties and biocidal activities of the matrix. Such material might be use in order to avoid leaching of the compound into the environment and to further understand the MB mechanism reaction. In this case, the secondary wastewater effluent was renewed each 3 h.

In practice, this entailed Type II photosensitization mechanism (production of singlet oxygen on illumination) [55] rather than Type I electron transfer redox reactions (production of reactive free radicals). In addition, various types of photosensitizers have been shown to be powerfully bactericidal via the intermediary of singlet oxygen [29]. Due to its considerable singlet oxygen yield (0.5–0.6) [56,57], the present work was based on the production of matrix derived from MB. The mixture containing MB and resin was prepared successfully giving clear blue film. The intensity was depending on MB concentration. The geometry and the source of the light used in those experiments were the same than those used when MB was dissolved in the sample. In addition, the reduction in the viable cell number for the blank (sample with only the resin, without MB) was the same extend than that obtained without resin or MB, must be attributable to direct artificial visible light instead of heating or MB immobilized photodynamic action. For this, three different MB concentrations were tested increasingly, called C_1 (0.005 g MB/0.5 mL ethanol/0.5 mL resin); C_2 (0.01 g MB/0.5 mL ethanol/0.5 mL resin) and C_3 (0.05 g MB/1 mL ethanol/0.5 mL resin). For the disinfection tests, 1 L of effluent was collected from the secondary clarifier of the Charguia biological process and renewed each 3 h for MB photosensitization under visible light. The results indicated that FC and FS were inactivated by the three concentrations used after the first 3 h of irradiation (Fig. 7). From the results obtained, C_2 could be considered as the most important concentration under these conditions. Hence, after 2 successive wastewater renewals (which corresponds to 6 h of visible irradiation), the depletion of the complex (resin-methylene blue) was noted. Therefore, the complex stops functioning after the first 6 h of irradiation for the MB concentration C_1 marked

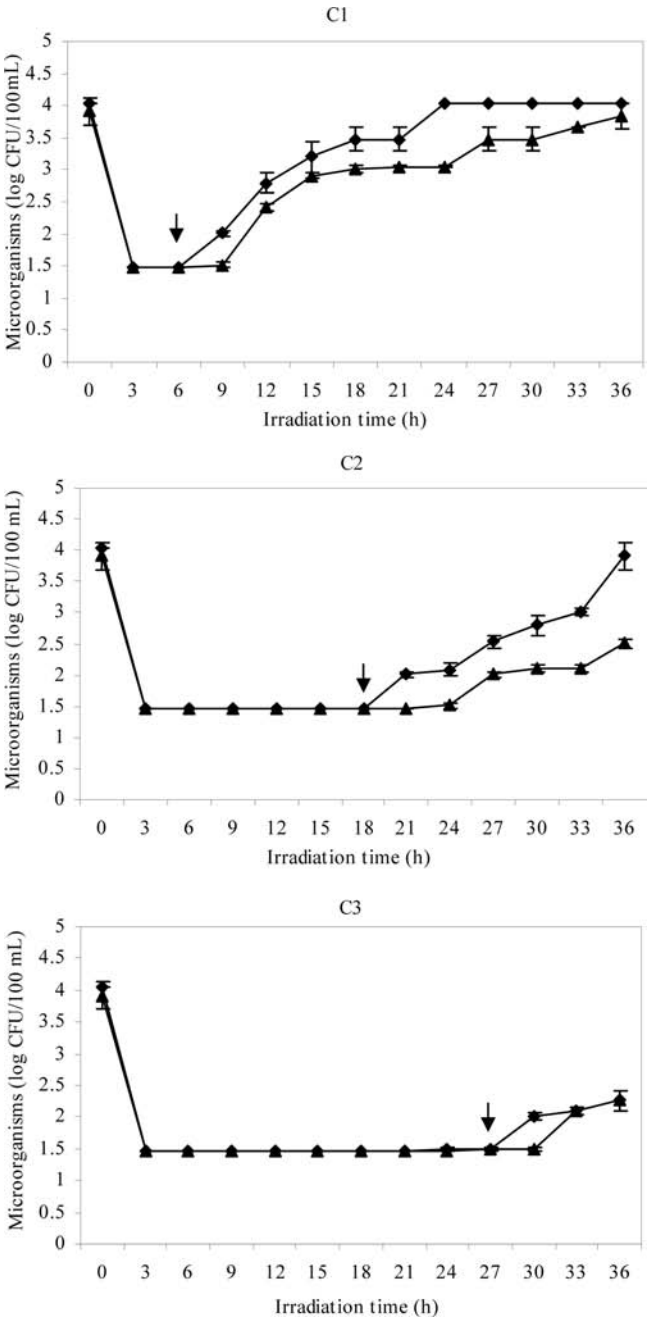

Fig. 7. Antibacterial activity of the methylene blue-resin matrix on (◆) faecal coliform and (▲) faecal streptococci with varying methylene blue concentration and visible irradiation time on the matrix exhaustion. Values are means of three replicates.

by a constant bacterial load without abatement for times greater than 6 h.

The C_3 was allowing a good MB photodynamic action with a long period of about 27 h (9 successive wastewater renewals) and this before reaching the mixture exhaustion; but a release of MB in the medium was recorded just after 1 min exposure to light while concentration C_2

did not demonstrate any release of the MB. In addition, this concentration C_2 made a high time of matrix exhaustion of about 18 h contact time (6 successive wastewater renewals). Interestingly, both Gram-type organisms were susceptible to the photodynamic action of the film.

4. Conclusions

The results for indicators led to good conclusion in effluent photosensitised by MB. In fact, from the experimental results obtained at small scale, significant reductions of FC have been observed. Results varied according to pH values, MB concentrations, MB stability, application process, type and time of irradiation. In view of this greater emphasis should be placed in our control, particularly in the agricultural reuse where their presence may trigger a public health problem.

After 12 h of sunlight, MB overall disappeared and consequently it was photoreduced to its leucoform. Natural sunlight can be used to photobleach MB under these conditions. In fact, separation of the dye from the wastewater was attempted. Physical separation of the photosensitizer and the bacteria eliminated the possibility of direct interaction between bacteria and photoexcited sensitizers.

Most importantly, for disinfection purposes, the photosensitizer should be properly immobilized within the polymeric matrix in order to avoid the leaching of the compound it self into the environment. Additional work must be undertaken to test various types of photosensitizers, which had been shown to be powerfully bactericidal via the intermediary of singlet oxygen. In addition to the polymer type, which require a non-leaching of the photosensitizer by simple mixing/dispersion with the polymer or a strong chemical bond between the two molecules (polymer and photosensitizer).

Furthermore, the behaviour of the MB leads to a Type II mechanism contribution and a Type I/Type II combination for MB being concluded.

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