Environmental Engineering and Management Journal

February 2015, Vol.14, No. 2, 465-471
http://omicron.ch.tuiasi.ro/EEMJ/

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PRELIMINARY ECOTOXICOLOGICAL EVALUATION OF ERYTHROSIN B AND ITS PHOTOCATALYTIC DEGRADATION PRODUCTS

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Abstract

The class of xanthene dyes has a complex chemical structure, which showed to be toxic for mixed culture of microorganisms (i.e. anaerobic granular sludge). Because of the unwanted effects of Erythrosin B (Ery B) on environmental components and some food confirmed previously, the dye was chosen in this study to evaluate its ecotoxicity. Also, the Ery B photocatalytic degradation products were assessed in terms of their ecotoxicity.
Three-days of seed germination and root growth tests were conducted using a dicotyledonous plant that is the garden cress (*Lepidium sativum* L.), in the presence of different dye concentration and its photodegradation products. Dye affected mostly the roots of the plant. According to toxic effects on root growth, toxicity of the dye indicated a 72h exposition average Effective Concentration EC_{50} value corresponding to 25 mgL^{-1} Ery B.
The presence of the Ery B photocatalytic degradation products in the aqueous solution leads to a higher efficiency on *Lepidium sativum* L. germination, favoring the stem length growth.

Key words: dye degradation, *Lepidium sativum* L., toxicity test

Received: November, 2014; Revised final: February, 2015; Accepted: February, 2015

1. Introduction

Dyes constitute one of the pollutants class causing a variety of environmental problems: they can generate eco-risks beside the dyes itself, by toxic by-products produced naturally trough oxidation or reduction pathways assisted by environmental conditions (Abo-Farha, 2010; Fatta-Kassinos et al., 2010; Gaya and Abdullah, 2008; Luenloi et al., 2011).

Food Red 14 (Erythrosin B), authorised as a food additive in the EU is a water-soluble dye of xanthene class widely used as a colorant since 1973 in different industries, such in foodstuffs (baked goods, fruits cocktails), cosmetics (lipsticks, bubble baths) or drugs (tablet, capsule), but their presence in the environment is of increased worry, since they may produce severe environmental and public health risks due to possible mutagenic or carcinogenic effects (Apostol et al., 2012). This class of compounds has a complex chemical structure, and showed to be toxic for mixed cultures of microorganisms (i.e. anaerobic granular sludge), enzymes, aquatic and terrestrial organisms, fungi, (Apostol et al., 2012; Carpenter et al., 1984; Krasnoff et al., 1999; Mizutani, 2009; Uesugi et al., 2006; Walthall and Stark, 1999). This is the reason for that there are numerous researches addressing the

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removal of dyes from aqueous effluents so as to minimize the ecological and health risks.

The photocatalytic processes have been applied successfully in the removal of organic pollutants from different classes (pesticides, pharmaceuticals and personal care compounds, halogenated compounds, dyes), where biodegradation of recalcitrant pollutants demonstrated to be inefficient due to the compounds toxicity (Caliman and Gavrilescu, 2009; Gavrilescu et al., 2014; Perreira et al., 2009; Van der Zee and Cervantes, 2009).

Heterogeneous photocatalysis has significant advantages over many processes used in the treatment of aqueous effluents polluted with dyes. The majority of the researchers reported that the process can induce the break of the dye molecule, but rarely complete mineralization can be achieved in an economically and environmental friendly way (Betianu et al., 2008; Caliman et al., 2008; Kumar, 2010). Those considerations conducted to a limited number of reported materials regarding the reaction mechanism and intermediates, and open door for new research. Although only few studies reported the total removal of dyes by photocatalysis, the method can result in the mineralization of the hazardous pollutants rather than transferring them to another phase, as in the case of adsorption on different materials (Apostol and Gavrilescu, 2009; Brinza et al., 2009; Satuf et al., 2011). In 2007, two papers were published addressing preliminary studies on Erythrosin B photodegradation, but no information were provided on the toxicity of dye degradation products (Hasnat et al., 2007; Uddin et al., 2007).

In order to evaluate the toxicity of the degradation products of Erythrosin B in our study, the dye was subject of UV photocatalytic degradation in the presence of the catalyst Degusa TiO_2 Aeroxide P25. In the first step, the experiments were conducted in order to evaluate the influence of different parameters on the removal efficiency of the dye (data not shown). The resulted effluent was used to perform toxicity tests, carried out using *Lepidium sativum L.* as the test organism and food chain indicator.

2. Materials and methods

2.1. Materials

The compound investigated in this study (Ery B) was chosen because of its use in different industries, in particular the food industry, despite the toxic and carcinogenic effects demonstrated in the context of research conducted on this topic. The acid dye, Erythrosin B is a red odourless powder or granules with a calculated Log P (octanol-water) of 4.95 at 25°C (Molinspiration, 2007), soluble in water (≤ 9% w/w) and ethanol. The chemical was purchased from Sigma–Aldrich (C.I. Acid Red 51, 45430).

Phytotoxicity tests were carried out with garden cress seeds *(Lepidium sativum L.)* from commercial source (AGROSEL garden cress seeds).

2.2. Methods

2.2.1. Toxicity tests

The growth of *Lepidium sativum* L. roots is a parameter frequently used to assess the ecotoxic risks from river pollution, wastewater sources, and leachates of industrially polluted soils (Studzińska and Buszewski, 2009). *Lepidium sativum* L. was selected as the test organism because of the advantage offered by some morphological and physiological properties (small, high growth rates and vegetative propagation) and due to its capacity to grow in a wide range of pH-values (Gyekye, 2013; Pavel et al., 2013).

Toxicity tests were carried out according to a standardized protocol (ISO/DIS 15 799, (1999): three-days of seed germination and root growth tests were conducted using the dicotyledonous garden cress *Lepidium sativum* L. as indicator. This method was tested first for the assessement of the effects of substances contained in residues applied to soil on plant emergence and growth.

The sensitivity of *Lepidium sativum* L. germination to Erythrosin B and its degradation by-products was investigated using dye solutions of different concentration. The toxicity tests were conducted in triplicate using 20 seeds each for germination on Petri dishes. Volumes of 3mL solution of a certain concentration of Ery B, and the corresponding degradation products, respectively were used to wet the paper supporting the seeds. The Petri dishes were kept at room temperature (~25ºC) for 72 hours. The number of seeds germinated, formed biomass, stem length and root length were measured to determine the germination degree.

The dye was tested in a concentration series (1 to 50 mgL^{-1}) in order to obtain the 72 h exposition average Effective Concentration (72 h-EC_{50}), which is the concentration where 50% of the plants were affected.

2.2.2. Separation of Ery B degradation products from reaction mixture

In order to identify the by-products resulted from Erythrosin B photodegradation, 500mg L^{-1} Ery B were degraded for 8h in the presence of 5 g L^{-1} TiO_2 according to the procedure described previously (Pereira et al., 2013). All chemicals and solvents were obtained from Sigma-Aldrich, Merck and Chemical Company. The final solution was purified by column chromatography using silica gel 60.70-230 mesh. The resulted solution was filtered using a 0.2 μm filter in order to remove the catalyst particles. First, the filtered solution containing the degradation by-products was treated by liquid-liquid extraction with ethyl acetate (Iovu and Nicolescu, 2009). The following step consisted in a solid-liquid extraction on silica gel.

The thin layer chromatography (TLC) screening was conducted and several solvents were tested in order to prepare the sample for NMR analysis (Iovu and Nicolescu, 2009; Tataru and Vata, 1999). Visualization was performed in short- and long- wavelength UV-light. The samples were refrigerated at 4°C and kept in the dark from the time of collection until analyzes.

3. Results and disscution

3.1. Ecotoxicological tests

Toxicity tests were conducted in order to evaluate the ecotoxicity of Ery B dye and its photodegradation products using an indirect acut toxicity bioassay on the dicotyledonous plant *Lepidium sativum* sp. according to the standard procedure. The number of seeds germinated, formed biomass, stem length and root length were measured to determine the germination degree (Fig. 1).

Table 1 presents the results of the toxicity tests. It can be observe that dye affect especially the roots of the plant. The presence of the by-products in the aqueous solution leads to a higher efficiency on Lepidium sativum sp. germination if the growth stem length is considered.

Toxicity data are usually expressed either as a concentration causing a specific effect (e.g. death or growth) in 50% of the tested organisms (Effect Concentration, EC_{50}). EC_{50} stands for the concentration of a test compound that reduces root growth to 50% of control (Enderlein et al., 2007; Pavel et al., 2013).

The experimental data were analyzed by regression analysis following logarithmic transformations and EC_{50} values for Erythrosin B were expressed as toxicant concentrations. According to toxic effects on root growth, toxicity of the dye indicated EC_{50} corresponded to 25 mg L^{-1} Ery B.

3.2. Separation of the products from reaction mixture

Two compounds were isolated by liquid-liquid extraction. P1 is a pale yellow compound with a crystalline structure, which is soluble in acetone. The TLC chromatogram indicated that the product P1 is not a pure compound.

P2 is a white powder compound. It was eluted with different mobile phases: chloroform, ethylic ether, acetone, ethyl acetate, hexane. The mixture of n-hexane with ethyl acetate was the best for migration on silica - gel plate in TLC analysis.

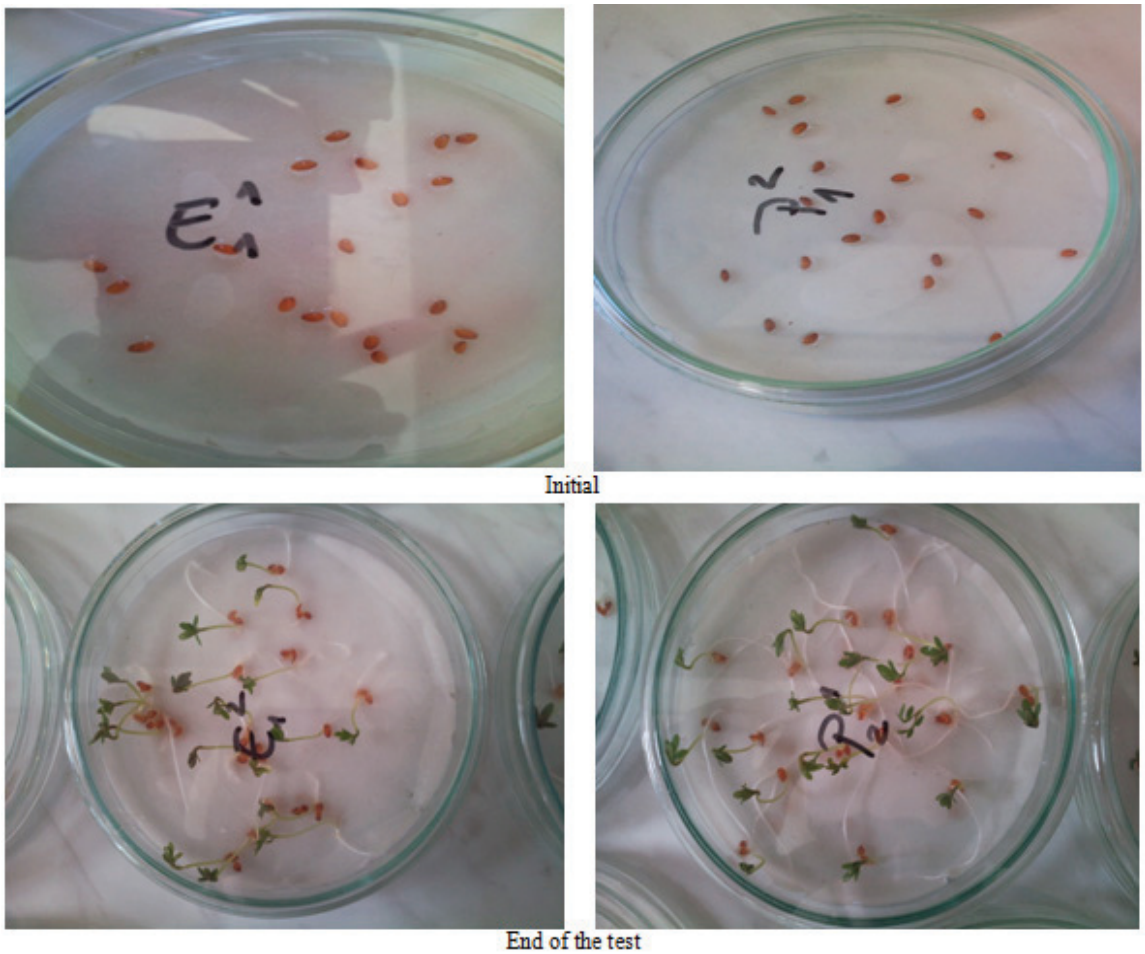

Fig. 1. *Lepidium sativum sp* germination in the presence of Erythrosin B and photodegradation products

Table 1. Erythrosin B and photodegradation by-products effects on *Lepidium sativum* L.

Tested substance	*Stem length (mm)*	*Root length (mm)*		*Biomass (g)*	
			Stem	*Root*	*Total*
Blank	16.0333 ± 0.55	34.9833 ± 1.06	0.1009 ± 0.007	0.0316 ± 0.017	0.3674 ± 0.04
Ery B_1 (25 mg L^{-1})	15.0667 ± 0.93	17.6333 ± 1.53	0.1086 ± 0.008	0.0332 ± 0.006	0.3239 ± 0.02
Ery B_2 (50 mg L^{-1})	10.35 ± 0.46	5.9333 ± 0.48	0.0848 ± 0.006	0.0142 ± 0.002	0.1717 ± 0.146
By-products (25 mg L^{-1})	18.2833 ± 3.41	32.9833 ± 9.07	0.1182 ± 0.021	0.0563 ± 0.011	0.3806 ± 0.06
By-products (50 mg L^{-1})	18.8833 ± 2.53	32.3333 ± 2.23	0.1238 ± 0.014	0.0556 ± 0.006	0.3718 ± 0.03

Unfortunately the NMR data were not encouraging because of the sensitivity of the methods and the compound could not be identified.

3.3. UV-Vis analysis

The UV-Vis analysis of the sample containing the dissolved by-products was performed between 200 and 700 nm with a T60 UV-Visible spectrophotometer. The sample was diluted with distilled water. The adsorption spectra showed two distinct adsorption peaks, at ~224 nm and ~ 524 nm (Fig. 2). The band A_0 represents the UV-Vis spectrum of initial Erythrosin B solution before starting the photocatalytic degradation. After the beginning of the degradation process it is observed a decrease in peak intensity corresponding to 524 nm. As the reaction progress, at the value of 224 nm a new peak is formed, that increases in intensity while the rection time increases. The monitorization stopped after 8 h of irradiation. In this solution the presence of I^- ionic species was also evidenced by reaction with $AgNO_3$ (Fig. 3).

Because the data obtained from our analysis does not allow a proper identification of the products formed during photocatalytic degradation, a literature analysis was carried out for xanthene dyes available mechanism in order to propose a degradation patway for Erythrosin B. This survey addressed the following frameworks: the products isolated and identified by chromatographic methods in conjuction

with a spectral study performed by Jain et al. (2005), resulted from electrochemical degradation of Erythrosin B; the possible mechanisms for azo dye X-GRL degradation by three processes based on the degradation intermediates detected by GC/MS reported by Zho and He (2007); the study of Mai et al. (2007) on the reaction products and intermediates of 2,4,6-triiodoresorcinol formed during the synthesis of color additive FD&C Red No. 3 (Erythrosin) using high performance liquid chromatography; mechanism and pathway intermediates resulted from the degradation of Rhodamine B in MPC in the presence of H_2O_2 detected by GC/MS by He at al. (2009); the study of Mehrdad and Hashemzadeh (2009) for the determination of activation energy for Rhodamine B degradation in the presence of hydrogen peroxide and some metal oxide.

The results obtained from UV-Vis analysis, TLC products characterization and the literature data helped us to propose a degradation mechanism for Erythrosin B (Fig. 4). The compounds proposed as Erythrosin B photodegradation by-products were subject of some ecotoxicological studies.

In order to explain the effect of the solution containing the Ery B photodegradation by-products on *Lepidium sativum* Table 2 present some tested species and the effects of Ery B degradation products on their development. For example, phthalic acid in solution determines population/ biomass growth of *Scenedesmus subspicatus* and *Chilomonas paramecium*.

A solution of 4-hydroxybenzoic acid inhibits the organisme growth and induces the death of *Oncorhynchus mykiss* (Rainbow trout) and of *Daphnia magna*, but in higher concentrations compared to those resulted from Erythrosin B degradation. A similar behaviour is found for terephtalic acid solutions (Table 2).

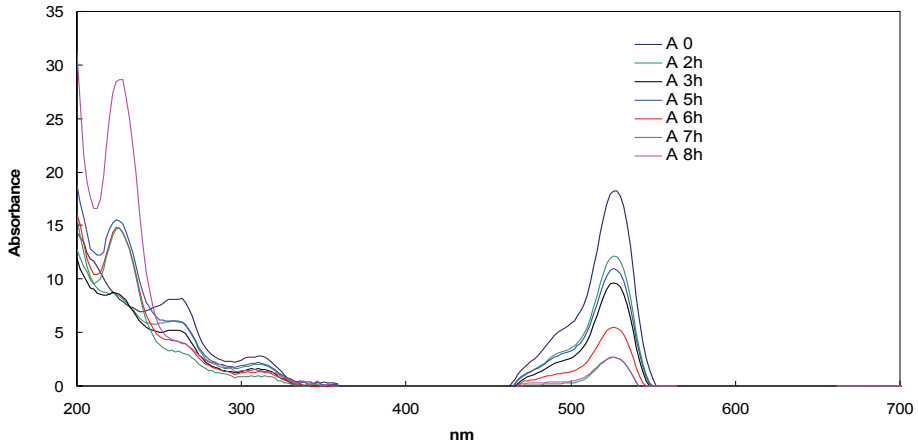

Fig. 2. UV-Vis spectra of final photodegradation solution of Erythrosin B

Reaction of initial solutions

Reaction of final solution

Fig. 3. The presence of I^- in solution after Erythrosin B photodegradation

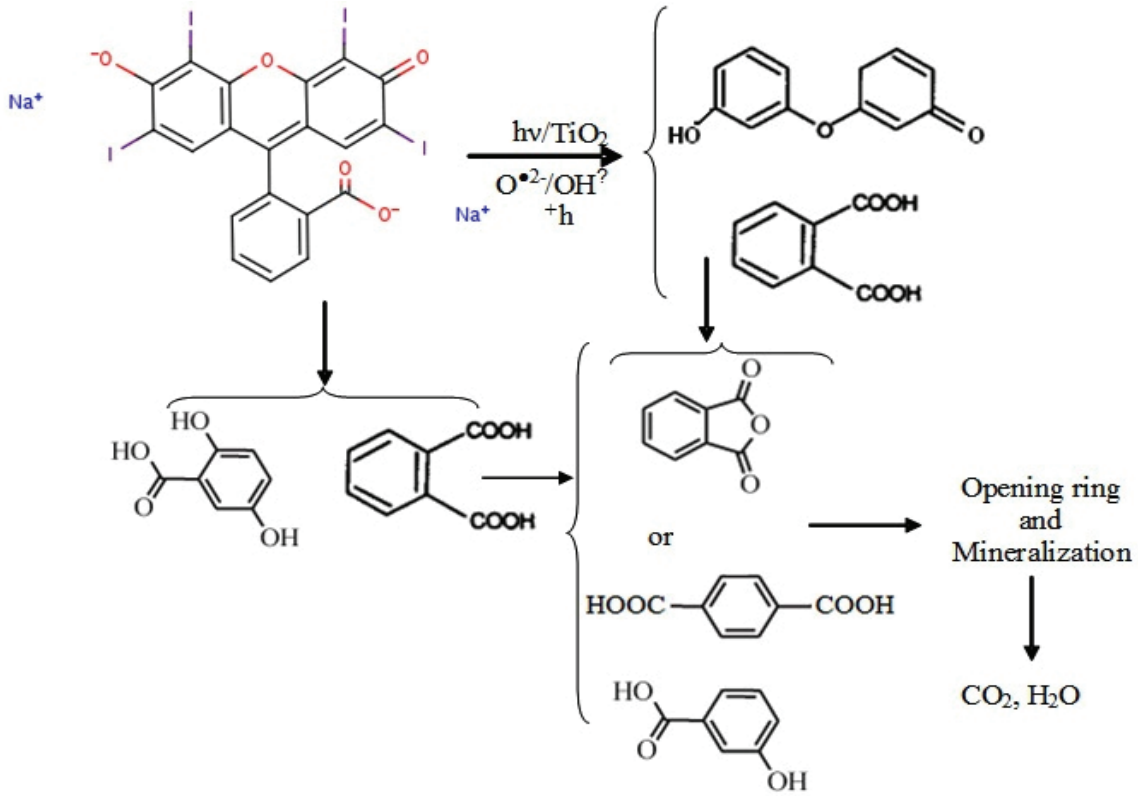

Fig. 4. The most probable degradation mechanism of Erythrosin B in the presence of UV/TiO_2

Table 2. Relevant ecotoxicity studies of Erythrosin B potential photodegradation byproducts

Compound	***Tested species***	***Effects***	***Reference***
2,5-dihydroxybenzoic acid	*Hydrilla* sp.	Growth, general	Martin and Martin (1988)
	Chlorella vulgaris	Population changes, general	Dedonder and Van Sumere (1971)
phthalic acid	*Scenedesmus subspicatus*	Population/ Biomass Growth	Kuhn and Pattard (1990)
	Chilomonas paramecium	Population growth rate	Bringmann and Kuhn (1981)
phthalic anhydride	*Selenastrum capricornutum*	Population – Biomass Growth	Michael et al. (1989)
	Oedogonium cardiacum	Accumulation	Lu and Metcalf (1975)
terephtalic acid	*Tetrahymena pyriformis (Ciliate)*	36-h IC_{50} = 55 mg/L	Yoshioka et al. (1985)
4-hydroxybenzoic acid	*Oncorhynchus mykiss* (Rainbow trout)	72-h EC_{50} = 68.5 mg/L	Verschueren (2001)
	Daphnia magna	48-h EC_{50} = > 100 mg/L	Kuehn (1989)

The main compounds resulted from the degradation process which have similar effects to those found in other studies correspond to phthalic anhydride/phthalic acid and 2,5-dihydroxybenzoic acid.

4. Conclusions

The toxicity of Erythrosin B dye against the garden cress *Lepidium sativum* L. was evaluated in a preliminary trial. The experiments performed using seeds cultivated on Petri dishes showed that the dye affected especially the roots of the plant.

Also, Ery B was the subject of UV photocatalytic degradation in the presence of the catalyst Degusa TiO_2 Aeroxide P25 in order to evaluate the toxicity of its degradation products. Photocatalytic degradation of Erythrosin B led to a complete detoxification of the compounds but the complete mineralization was not achieved. The presences of at least two compounds isolated by liquid-liquid extraction, as main by-products in the aqueous solution, led to a higher efficiency on *Lepidium sativum sp* germination according to stem length growth.

This study confirmed the opportunity of toxicological assays related to dye decolorization experiments. The results are the basis for further investigations essential to obtain the data necessary in the assessment of ecological and health risks.

Acknowledgements

This paper was elaborated with the support of a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCE-2011-3-0559, Contract 265/2011. The scientific support of Professor Dan Scutaru is highly acknowledged.

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