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ORIGINAL ARTICLE

Enhanced photocatalytic–biological degradation of 2,4 dichlorophenoxyacetic acid

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Abstract 2,4-Dichlorophenoxyacetic acid (2,4-D) is the third most commonly used herbicide all over the world. There is a contradicted opinion about its toxicity and its half life in the environment. In this study the most effective method of its degradation and bioremediation has been studied. Two microbial consortia capable of utilizing 2,4-D as a sole source of carbon were isolated from the Egyptian environment. One of the microbial consortia interestingly contained a certain kind of protozoa as one of the mixed consortia members. Degradation of 2,4-D by the microbial consortia was affected by 2,4-D initial concentration, agitation, pH of the medium and temperature. The two consortia were able to degrade up to 700 mg l^{-1} of 2,4-D. Pre-treatment with UV radiations in the presence of photocatalyst such as TiO_2 accelerates the biodegradation process. The toxic non biodegradable concentration of 2,4-D which was found to be the 800 mg l^{-1}, was degraded by pre-treatment with UV/TiO_2 and a subsequent microbial inoculation. The combined treatment proved to be an efficient mean of biodegradation and detoxification of toxic non biodegradable concentrations of 2,4-D.

1. Introduction

Nowadays the whole world is facing a global pollution problem that affects enormously the living organisms and causes a disturbance in the ecological equilibrium that could endanger the continuity of life on earth.[1] The problem is very apparent in Egypt where the quality of the Nile river water is seriously threatened by untreated industrial and agricultural wastes, sewage, and municipal wastewater. The trapped nutrient-rich silt which is considered to be a natural fertilizer for the country's farmland behind the high dam forced the farmers to make more use of xenobiotics such as chemical fertilizers as well as modern herbicides and pesticides.[2]

2,4-Dichlorophenoxyacetic acid (2,4-D) is the third-most widely used herbicide all over the world and is still applied in Egypt although its use is restricted by the European Community and in many countries.[3,4] The health hazards of 2,4-D to humans include that it may induce a cytogenetic damage in human lymphocytes, an irreversible eye damage, hepatotoxicity and nephrotoxicity,[5,6] besides being classified by the International Agency for Research on Cancer (IARC) as class 2B carcinogen to humans.

Many methods are proposed for treatment of chemical pollution,[7] however, the safest and the cheapest one is the use of microorganisms for biodegradation of these pollutants.[8]

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Previous literature reported that the most biologically active strains against 2,4-D proved to belong to the genera *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Nocardia*, *Streptomyces* and *Aspergillus*.[9,10] However, the presence of halogens in the molecular structure of 2,4-D renders it highly persistent and resistant for biodegradation for decades.[11,12]

The use of UV irradiation with and without the assistance of an oxidizing agent (UV/TiO_2, UV/H_2O_2) alone or combined with the biological methods proved to be very efficient, cheap and sustainable as in case of wastewater treatments.[13,14] Therefore, physical or physicochemical pre-treatments are usually important in case of substances which are highly toxic to the biological degraders or recalcitrant materials where the pre treatment changes these materials into less toxic and biodegradable metabolites.[15–17] In this prospective, the present study investigated the optimum method to detoxify and bioremediate 2,4-D using biological consortia alone or in combination with photo-catalytic pre-treatment against increasing concentration of 2,4-D.

2. Materials and methods

All chemicals were of reagent grade. 2,4-Dichlorophenoxyacetic acid and TiO_2 (anatase powder with particle size of approximately 325 mesh $\leqslant$ 44 μm) were purchased from Sigma–Aldrich. A stock solution of 2,4-D (2 g l^{-1}) was prepared in acetone. Unless otherwise specified, all experiments were conducted in triplicate at room temperature (25 ± 2 °C) and the results are expressed as the mean of the triplicate experiments.

2.1. Microorganisms

Two microbial consortia isolated from the Egyptian environment were used to conduct all the biodegradation experiments. The active isolated consortia were maintained for short time period by periodical cultivation on fresh 2,4-D solid media and kept at 4 °C. Another set was stored through addition of 25% v/v sterilized glycerol to the microbial suspension at the late exponential phase and then transferred into sterile 1.5 ml Eppendorf tubes and stored at −20 °C.

2.1.1. Molecular identification using partial 16S rRNA sequence analysis

The most relevant bacterial isolate was subjected to molecular identification using partial 16S rRNA sequence analysis according to Essam et al.[18] using 2 universal primers 28f 5′AGAGTTTGATCCTGGCTCAG-3′ (positions 8–28 in *E. Coli* numbering) and 1512R 5′ACGGCTACCTTGTTAC GACT-3′ (positions 1512–1493 in *E. Coli* numbering).

2.2. Photo-catalytic treatment

Photo-catalytic UV/TiO_2 tests were conducted in minimal salts medium (MSM) supplemented with the desired concentration of 2,4-D to allow for subsequent biodegradation studies. The phosphate buffer also prevented pH variation during various treatments (the pH value in the medium after UV irradiation and biodegradation tests always remained to approx. 7).

MSM was supplemented with various concentrations of 2,4-D (50 and 800 mg l^{-1}). To each solution TiO_2 was then added at 1 g l^{-1} as catalyst, the mixtures were first sonicated for 5 min to obtain a homogenous suspension. Aliquots of 5 ml of the prepared 2,4-D suspensions were transferred into 25 × 8 ml-screw capped test tubes. These tubes were then mechanically agitated using a rocking shaker and irradiated during 48 h with UV lamp irradiating wave length 256 nm placed at a distance of 10 cm. A control set pre-treated with TiO_2 alone but not irradiated was performed under the same conditions. Samples of 1 ml were periodically withdrawn to monitor the concentration of remaining pollutants.

The liquid fractions from each set of tubes were collected and mixed at the end of the treatment then TiO_2 was first removed by centrifuging the tubes at 1400*g* for 15 min and the supernatants were mixed. This experiment was repeated until sufficient volume of the treated solution is obtained for the subsequent biodegradation tests.

2.3. Biological treatment

The initial biodegradation set of experiments was conducted in 250 ml Erlenmeyer flasks containing 50 ml MSM supplemented with the desired concentration of 2,4-D and inoculated with 5% v/v consortium from an overnight inoculum adjusted to an optical density of 0.08 ± 0.02 at 600 nm. The flasks were covered with cotton plugs and incubated at 30 °C and 200 rpm. Non-inoculated flasks were used as negative control to estimate the potential abiotic removal. Subsequent biodegradation tests were conducted in 100 ml Erlenmeyer flasks supplemented with 25 ml of photo-catalytically pretreated solutions and inoculated with 5% v/v of consortium 1 or 2 with absorbance equals 0.08 ± 0.02 nm of an overnight culture. Flasks were then covered with cotton bulges and incubated at 30 °C and 200 rpm.

In all biodegradation tests samples of 1.5 ml were periodically withdrawn and centrifuged at 11,000*g* for 5 min and immediately subjected to analysis by HPLC. The remaining pollutant and phyto-toxicity were measured at the end of incubation period.

2.4. Study of the effect of common factors affecting the biodegradation efficiency

The impact of various factors affecting the 2,4-D biodegradation was conducted using the same experimental assembly mentioned above in the biological treatment with different levels of variable factors as per Table 1 using one variable at the time approach. Samples of 1.5 ml were periodically

Table 1 A list of the tested factors affecting 2,4-D biodegradation with the recorded optimum value in bold.

Factor	Values of factors studied						
2,4-D concentration	**20**	**50**	**100**	**200**	400	700	800
Agitation	50	100	200	**300**			
pH	**5**	**6**	**7**	**8**	**9**		
Temperature	20	25	**30**	37	42		