Bulletin of Faculty of Pharmacy, Cairo University (2015) **53**, 77–82

HOSTED BY

Cairo University

Bulletin of Faculty of Pharmacy, Cairo University

www.elsevier.com/locate/bfopcu
www.sciencedirect.com

ORIGINAL ARTICLE

Enhanced photocatalytic–biological degradation of 2,4 dichlorophenoxyacetic acid

Reham Samir, Tamer Essam *, Yasser Ragab, Abdelgawad Hashem

Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt

Received 27 February 2015; accepted 23 March 2015
Available online 19 June 2015

KEYWORDS

Sequential;
Photo-catalysis;
2,4-D;
Biodegradation;
Bioremediation

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]

* Corresponding author.
E-mail address: tamer.essam@yahoo.com (T. Essam).
Peer review under responsibility of Faculty of Pharmacy, Cairo University.

http://dx.doi.org/10.1016/j.bfopcu.2015.03.002

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		

withdrawn and centrifuged at 11,000*g* for 5 min and immediately subjected to analysis by HPLC.

2.5. Analysis

2,4-D was analyzed by HPLC, using Shimadzu Japan shinpack HRC-ODS column, Shimadzu SCL-10 AVP system controller, Shimadzu SPD-10AVP UV–Vis detector, Shimadzu LC-10ADVP liquid chromatograph and Shimadzu DGU-12A degasser. The mobile phase was a mixture of water, acetonitrile and acetic acid in a ratio of 59:40:1, respectively, the flow rate was 1 ml min^{-1}. λ_{max} was 283 nm. The 2,4-D was identified and quantified by comparing both the retention time and the area under the curve of the 2,4-D peak in the UV visible spectrum to those of the standard. Phyto-toxicity tests were conducted using *Lepidium sativum* according to El-Rakaiby et al.[19]

3. Results

3.1. Microorganisms

Morphological examination showed that the main members of the isolated consortia were mainly, Gram negative rods and to a less extent Gram negative cocci. Interestingly, the presence of protozoa like microorganisms was observed at the end of the biodegradation process using consortium # 2 (Fig. 1). Molecular identification of the major elements of biodegrading bacterial isolates revealed the predominance of gram negative rods bacteria, where 2 isolates (with the highest biodegradation capacities) were identified as *Pseudomonas* sp. *YT1* and *Stenotrophomonas maltophilia YT2* and submitted to the gene bank (accession numbers JN129496 and JN129495, respectively).

3.2. Photo-catalytic treatment

The photo-catalytic degradation rate increased when the distance from the irradiation source was decreased. The highest rate of photocatalytic removal was recorded at 10 cm distance (Fig. 2). Up to 60% removal was recorded after only 21 h. with no remarkable increase in the removal efficiency after 48 h (Fig. 2).

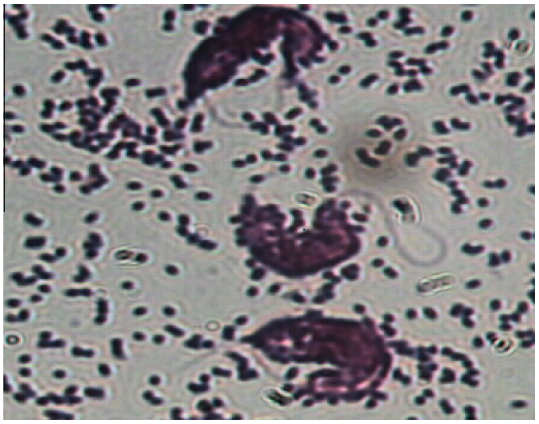

Figure 1 A picture under the light microscope for consortium # 2 after complete biodegradation of 50 mg 2,4-D l^{-1}.

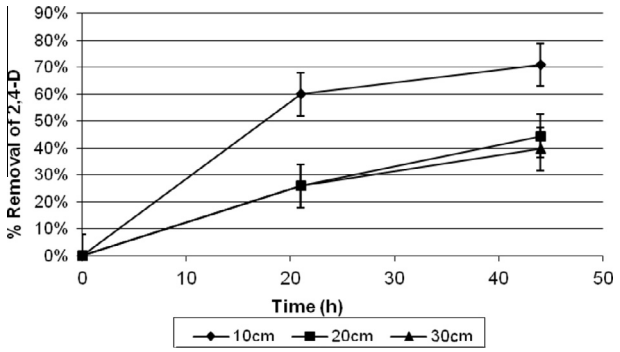

Figure 2 The recorded removal % of 2,4-D in flasks supplemented with 50 mg 2,4-D l^{-1} and irradiated with UV in the presence of 1 g TiO_2 l^{-1} at different distances from the UV lamp. Data are the mean of triplicate experiments. Error bars show the standard deviation.

3.3. Biological treatment

The microbial consortia were able to degrade 50 mg l^{-1} of 2,4-D and reached more than 90% clearance in about 45 h with a lag phase of about 18 h (Fig. 3). However, continuous subculture (~10 subcultures) of the bacterial consortium led to their activation and adaptation where more than 90% removal of 2,4-D was achieved after only 20–24 h and the lag phase was highly diminished (Fig. 3). The isolated consortia were able to biodegrade up to 700 mg 2,4-D l^{-1}, however, the biodegradation failed to remove 2,4-D at concentrations of 800 mg l^{-1} (data not shown).

3.4. Phyto-toxicity tests

The 2,4-D was found to be toxic to the *Lepidium sativum* seeds leading to an 85% inhibition in the growth of the stem. Biodegradation rendered the effluent non toxic with zero inhibition recorded. Moreover, the average stem length was even more than that obtained by the blank that was conducted along with the experiment (Table 2).

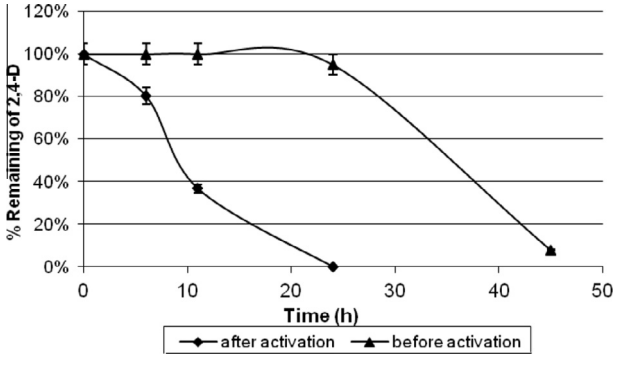

Figure 3 The recorded residual percentage of 50 mg 2,4 D l^{-1} in flasks inoculated with consortia before activation or after activation and incubated for 48 h at 30 °C and 200 rpm agitation rate. Data are the mean of triplicate experiments. Error bars show the standard deviation.

Table 2 The inhibition percent of the stem growth before and after 2,4-D biodegradation in phyto-toxicity test.

	Water	MSM	2,4-D	Treated 2,4-D
Stem length (cm)	1.02 ± 0.49	1.05 ± 0.48	0.18 ± 0.23	1.25 ± 0.32
Inhibition (%)	0	0	85	0

3.5. Factor affecting the rate of degradation

Different biodegradation factors that affect the rate of biodegradation were studied, which are; the initial concentration of 2,4-D, agitation rate, temperature and pH of the media. Table 1 shows the optimum value(s) of each factor affecting the degradation process. Increasing the concentration of 2,4-D had no effect on the biodegradation time up to 200 mg l^{-1}. Higher concentration than 200 mg l^{-1} remarkably elongated the lag phases and consequently the biodegradation time. For instance biodegradation of 700 mg 2,4-D l^{-1} was achieved after 70 h compared to 24 h at 200 mg 2,4-D l^{-1}. Both microbial consortia failed to degrade 800 mg l^{-1}, where this high concentration resisted biodegradation for 28 days.

The degradation rate of the microbial consortia was highly affected by the agitation rate, where biodegradation rate increased and the observed lag phase disappeared. The highest biodegradation rate was observed at 300 rpm (Table 2). The effect of pH had no significant effect on the biodegradation rate of 2,4-D where at all tested pH range a similar pattern was always observed (data not shown). The biodegradation of 50 mg l^{-1} of 2,4-D was not detected at high temperature (42 °C). The biodegradation was highly prolonged at 37 °C. Low temperatures were not also favoured and lag phases were prolonged, where the optimum temperature for the microbial consortia was found to be 30 °C (Table 2).

3.6. Optimization of the degradation process

Applying the optimized conditions of agitation (300 rpm), pH (5–9) and temperature (30 °C), both bacterial consortia achieved complete biodegradation of 50 mg l^{-1} of 2,4-D in 15–17 h. Interestingly, the lag phase disappeared with

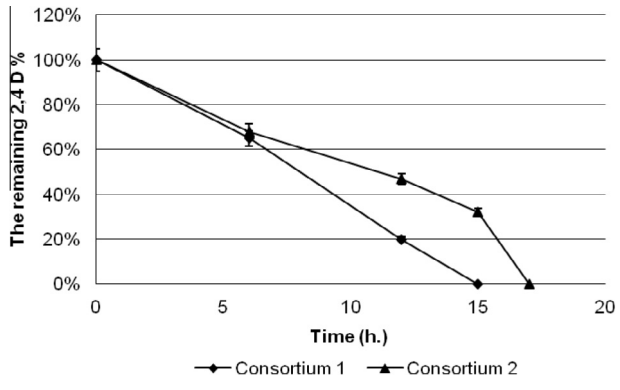

Figure 4 The recorded 2,4-D % of 50 mg 2,4-D l^{-1} using both consortia and the optimized conditions; agitation rate (300 rpm), pH (7) and temperature (30 °C). Data are the mean of triplicate experiments. Error bars show the standard deviation.

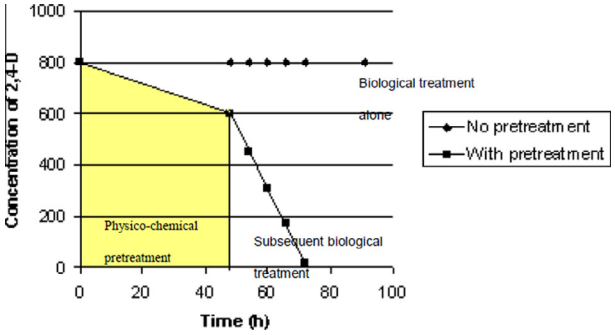

Figure 5 The recorded concentration of 2,4-D in flasks supplemented with 800 mg l^{-1} and inoculated with the consortium alone or after pre-treatment with UV/TiO_2. The flasks were incubated at 200 rpm agitation rate and 30 °C.

significant improvement of the biodegradation rate increasing from 2 to 3.3 mg of 2,4-D h^{-1} (Fig. 4).

3.7. Sequential photo-catalytic (TiO_2/UV)–biological treatment

The photo-catalytic pre-treatment of the concentration of 2,4-D that resisted biodegradation (800 mg l^{-1}) followed by inoculation of microbial consortia resulted in the degradation of 2,4-D in about 24 h (Fig. 5). Biological treatment alone failed to remove 800 mg 2,4-D l^{-1} (Fig. 5). Phyto-toxicity assay showed that sequential photocatalytic-biological treatment completely detoxified the pollutant solution while 100% inhibition was recorded with samples treated by biological mean only.

4. Discussion

2,4-D is extensively used in Egypt to eradicate weeds and to clear and kill Ward El-Neal that blocks the river track. Many studies were reported that 2,4-D may cause cytogenetic damage in human lymphocytes,[5] hepatotoxicity, nephrotoxicity,[6] promotion of lung tumors and non Hodgkin's lymphoma.[20,21] Therefore, this compound was selected as a model of toxic chemical pollutants relevant to Egypt where this study is conducted.

Most recent studies including the present study were conducted on biodegradation using microbial mixtures and not pure isolates to imitate natural conditions as used by Kye-Heon et al.[22] and Celis et al.[23] who preferred to work with microbial consortia isolated from their environments since it is believed that microbial mixtures are more realistic and would maintain their activities over a wider range of conditions that would affect the rate of the biodegradation. Besides, previous studies like Loos et al.[9] and Ka et al.[10] identified certain strains as potential candidates for degradation of 2,4-D like *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Nocardia*,

Streptomyces and Aspergillus. This was the case in the present study where the most active members of the consortia were *Pseudomonas* sp. and *S. maltophilia* (former *Pseudomonas*). Interestingly, protozoa were observed at the end of the treatment process using consortium # 2. Although, previous studies stated that the role of protozoa in the process of biodegradation was unclear, Jouany and Martin[24] suggested that protozoa can participate in the degradation process since some protozoa have been shown to produce extracellular enzymes and hence play a direct role in biodegradation. However, a large number of protozoan species play only a predatory role and mainly consume bacteria.[25] On the other hand, it has been shown reproducibly that predatory protozoa enhance the efficiency of bacterial biodegradation.[26–28] Protozoa are suggested to stabilize the pH of the medium[25] where better pH regulation has been demonstrated in the presence of protozoa.

In the present study, with continuous subculture (~10 subcultures) of the microbial consortia the 90–100% removal of 2,4-D was achieved after only 20–24 h. The decrease in both degradation time and lag phase by continuous exposure of the bacteria to 2,4-D may be attributed to that 2,4-D degrading enzymes are not constitutive but inducible enzymes which means that the substrate (2,4-D) must be present all the time for the production of these enzymes. This phenomenon was emphasized by Gonod[29] who concluded that the genetic structure of bacterial communities was significantly modified in response to 2,4-D application, but only during the intense phase of 2,4-D biodegradation. The bioremediation of 2,4-D was assured by applying phyto-toxicity test which was conducted for the first time on 2,4-D. The result showed the complete detoxification of the 2,4-D. Moreover the by-products enhanced the growth of the stem compared to the MSM and water alone.

Different factors affected the rate of degradation, where the initial concentration of 2,4-D, the temperature at which the biodegradation process took place, the rate of agitation of the media and the effect of photo-catalysis on biodegradation had a great impact on the degradation process, while the pH of the culture media did not affect the degradation process. These factors were chosen because they simulate the different environmental conditions to which 2,4-D is usually exposed to. Both the agitation rate and the photo-catalysis pretreatment were the most important factors affecting the degradation rate as they highly diminished the time needed for the removal of 2,4-D. This may be due to the mechanism of biodegradation which depends on the step of oxidation of the benzene ring prior to its cleavage that plays a major role in the biodegradation of 2,4-D. The importance of oxygen to the biodegradation of 2,4-D was also proven by Shaler and Klecka[30] who stated that dissolved oxygen concentrations below 1 mg l^{-1} may be a rate limiting step for the biodegradation of chlorinated aromatic compounds.

Our microbial consortia were capable of degrading concentrations up to 700 mg l^{-1} in about 70 h, but failed to degrade 800 mg l^{-1} up till 28 days. This exceeded the study done by Celis et al.[23] who were able to degrade up to 500 mg l^{-1} only by aerobic biomass in 30 days, but failed to degrade 700 mg l^{-1} and exceeded also Santacruz et al.[31] who also were only able to degrade 500 mg l^{-1} of 2,4-D.

The pretreatment of the concentration of 2,4-D that resisted biodegradation by the microbial consortia (800 mg l^{-1}) with UV radiations in the presence of TiO_2 enabled the consortia to degrade 2,4-D in about 24–45 h. Photo-catalysis was able to decrease the concentration of 2,4-D from 800 mg l^{-1} to 600 mg l^{-1} which was no longer toxic to the consortia, so the consortia were able to degrade the remaining 2,4-D reaching zero concentration. The time of biodegradation of the remaining 600 mg l^{-1} after the photocatalysis pretreatment was highly diminished compared to that obtained when the initial concentration of 600 mg l^{-1} of 2,4-D was degraded without previous pretreatment. This may be attributed to that the photo-catalysis process transformed 200 mg l^{-1} out of 800 mg $l^{-1}$of 2,4-D into easily degradable byproducts that increased the biomass of the consortia and induced the production of 2,4-D degrading enzymes in big amount that accelerated the biodegradation of the remaining 600 mg l^{-1} of 2,4-D in relatively shorter time.

By applying phyto-toxicity test, the toxicity of 2,4-D remained and was not completely detoxified after the photocatalytic pre-treatment only. On the other hand, complete detoxification was achieved after microbial biodegradation. Therefore, using photo-catalytic pre-treatment before applying the biodegradation process, the toxic concentration of 2,4-D (800 mg l^{-1}) that resisted biodegradation alone was able to be degraded and detoxified which proved that the combination between photo-catalysis (TiO_2/UV) and biological method was a successful mean and a safe guard against concentrations of 2,4-D that resist biodegradation.

5. Conclusions

The present study introduced potential microbial consortia to biodegrade 2,4-D at relatively high concentration up to 700 mg l^{-1}. However, both consortia failed to degrade 800 mg l^{-1}. Sequential photo-catalytic–biological treatment proved to be an efficient tool to bioremediate and detoxify high concentrations of toxic and persistent pollutants.

6. Conflict of interest

We have no conflict of interest to disclose.

References

1. Pautrel X. Pollution and life expectancy: how environmental policy can promote growth. *Ecol Econ* 2009;**68**:1040–51.
2. Dekov VM, Komy Z, Araujo F, Van Put A, Van Grieken R. Chemical composition of sediments, suspended matter, river water and ground water of the Nile (Aswan-Sohag traverse). *Sci Total Environ* 1997;**201**:195–210.
3. Chinalia FA, Killham KS. 2,4-Dichlorophenoxyacetic acid (2,4-D) biodegradation in river sediments of Northeast-Scotland and its effect on the microbial communities (PLFA and DGGE). *Chemosphere* 2006;**64**:1675–83.
4. Rosales-Conrado N, León-González ME, Pérez-Arribas LV, Polo-Díez LM. Multiresidue determination of chlorophenoxy acid herbicides in human urine samples by use of solid-phase extraction and capillary LC-UV detection. *Anal Bioanal Chem* 2007;**390**:759–68.
5. Soloneski S, González NV, Reigosa MA, Larramendy ML. Herbicide 2,4-dichlorophenoxyacetic acid (2,4-D)-induced cytogenetic damage in human lymphocytes in vitro in presence of erythrocytes. *Cell Biol Int* 2007;**31**:1316–22.

6. Lindh CH, Littorin M, Amilon A, Jönsson BA. Analysis of phenoxyacetic acid herbicides as biomarkers in human urine using liquid chromatography/triple quadrupole mass spectrometry. *Rapid Commun Mass Spectrom* 2007;**22**:143–50.
7. Li SX, Zheng FY, Cai WL, Han AQ, Xie YK. Surface modification of nanometer size TiO_2 with salicylic acid for photocatalytic degradation of 4-nitrophenol. *J Hazard Mater* 2006;**135**: 431–6.
8. Essam T, Zilouei H, Amin MA, Ossama ET, Bo M, Benoit G. Sequential UV-biological degradation of chlorophenols. *Chemosphere* 2006;**63**:277–84.
9. Loos MA. Phenoxyalkanoic acids*Herbicides: chemistry, degradation, and mode of action*. 2nd ed. New York: Marcel Dekker; 1975.
10. Ka JO, Holben WE, Tiedje JM. Genetic and phenotypic diversity of 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacteria isolated from 2,4-D-treated field soils. *Appl Environ Microbiol* 1994;**60**:1106–15.
11. Fontmorin JM, Huguet S, Fourcade F, Geneste F, Floner D, Amrane A. Electrochemical oxidation of 2,4-dichlorophenoxyacetic acid: analysis of by-products and improvement of the biodegradability. *Chem Eng J* 2012;**195**:208–17.
12. He M, Wang H, Lin C, Quan X, Guo W, Yang Z. Distribution of persistent organochlorine residues in sediments from the Songhuajiang River Northeast China. *Environ Technol* 2008;**29**:03–14.
13. Essam T. *Solar-based physicochemical–biological*. ISRN LUTKDH/TKBT-06/1102-SE; 2006.
14. Essam T, Amin M, ElTayeb O, Mattiasson B, Guieysse B. Solar-based detoxification of phenol and p-nitrophenol by sequential TiO_2 photo-catalysis and photo-synthetically aerated biological treatment. *Water Res* 2007;**41**:1697–704.
15. Sarria V, Parra S, Adler N, Peringer P, Pulgarin C. Recent developments in the coupling of photo assisted and aerobic biological processes for the treatment of biorecalcitrant compounds. *Catal. Today* 2002;**76**:301–15.
16. Minsker L, Pulgarin C, Peringer P, Kiwi J. Integrated approach useful in the mineralization of non biodegradable, toxic p-nitrotoluene sulfonic acid via photocatalytic biological processes. *New J Chem* 1994;**18**:793–800.
17. Green CE, Stansberry A, Sczechowski J, Nelson Y. *Enhanced biodegradation of hydrocarbon contaminants using photocatalytic pre-treatment*; 2001. Website: http://works.bepress.com/ynelson/6/.
18. Essam T, Amin M, ElTayeb O, Mattiasso B, Guieysse B. Characterization of highly resistant phenol degrading strain isolated from industrial wastewater treatment plant. *J Hazard Mater* 2010;**173**:783–8.
19. ElRakaiby M, Essam T, Hashem A. Isolation and characterization of relevant algal and bacterial strains from Egyptian environment for potential use in photo-synthetically aerated wastewater treatment. *J Biorem Biodegrad* 2012, 10.417212155-6199.S8-001.
20. Gandhi R, Snedeker SM. *Pesticides and breast cancer risk: an evaluation of 2,4-D*. Fact Sheet no.14. Program on Breast Cancer and Environmental Risk Factors. Cornell University, College of Veterinary Medicine. Vet Box 31, NY: Ithaca, 14853-6401; 1998.
21. Lynch MJ, Stretesky P. Toxic crimes: examining corporate victimization of the general public employing medical and epidemiological evidence. *J Crit Criminol* 2001;**10**:153–72.
22. Kye-Heon OH, Tuovinen OH. Degradation of 2,4-dichlorophenoxyacetic acid by mixed cultures of bacteria. *J Ind Microbiol Biotechnol* 1990;**6**:275–8.
23. Celis E, Elefsiniotis P, Singhal N. Biodegradation of agricultural herbicides in sequencing batch reactors under aerobic or anaerobic conditions. *Water Res* 2008;**42**:3218–24.
24. Jouany JP, Martin C. Effect of protozoa in plant cell wall and starch digestion in the rumen. In: Onodera R, Itabashi H, Ushida H, Yano H, Sasaki Y, editors. *Rumen microbes and digestive physiology in ruminants*. Japan: Sci. Soc. Press; 1997. p. 11–24.
25. Jouany JP, Ushida K. The role of protozoa in feed digestion. *AJAS* 1999;**12**:113–28.
26. Jouany JP. Effect of rumen protozoa on nitrogen utilization by ruminants. *J Nutr* 1996;**126**:1335S–46S.
27. Biagini GA, Finlay BJ, Loyd D. Protozoan stimulation of anaerobic microbial activity: enhancement of the rate of terminal decomposition of organic matter. *FEMS Microbiol Ecol* 1998;**27**:1–8.
28. Jouany JP, Mathieu F, Senaud J, Bohatier J, Bertin G, Mercier M. The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on the digestion of the cell wall fraction of a mixed diet in defaunated and refaunated sheep rumen. *Reprod Nutr Dev* 1998;**38**:401–41.
29. Gonod LV, Martin-Laurent F, Chenu C. 2,4-D impact on bacterial communities, and the activity and genetic potential of 2,4-D degrading communities in soil. *FEMS Microbiol Ecol* 2006;**58**:529–37.
30. Shaler TA, Kleck GM. Effects of dissolved oxygen concentration on biodegradation of 2,4-dichlorophenoxyacetic acid. *Appl Environ Microbiol* 1986;**51**:950–5.
31. Santacruz G, Erick R, Bandala ER, Torres LG. Chlorinated pesticides (2,4-D and DDT) biodegradation at high concentrations using immobilized *Pseudomonas fluorescens*. *J Environ Sci Health Part B* 2005;**40**:571–83.