Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Solar-based detoxification of phenol and *p*-nitrophenol by sequential TiO_2 photocatalysis and photosynthetically aerated biological treatment

Tamer Essam[a,b], *Magdy Aly Amin*[b], *Ossama El Tayeb*[b], *Bo Mattiasson*[a], *Benoit Guieysse*[a,c,*]

[a]*Department of Biotechnology, Lund University, P.O. Box 124, S-22100 Lund, Sweden*
[b]*Microbiology Department and Microbial Biotechnology Center, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt*
[c]*School of Civil and Environmental Engineering, Nanyang Technological University, Block N1, Nanyang Avenue, Singapore 639798, Singapore*

ARTICLE INFO

Article history:
Received 10 August 2006
Received in revised form
7 December 2006
Accepted 12 January 2007
Available online 9 March 2007

Keywords:
Advanced oxidation processes
Chlorella vulgaris
Industrial hazardous wastewater
Photobioreactor
Solar-based wastewater treatment

ABSTRACT

Simulated solar UV/TiO_2 photocatalysis was efficient to detoxify a mixture of 100 mg phenol l^{-1} and 50 mg *p*-nitrophenol (PNP) l^{-1} and allow the subsequent biodegradation of the remaining pollutants and their photocatalytic products under photosynthetic aeration with *Chlorella vulgaris*. Photocatalytic degradation of phenol and PNP was well described by pseudo-first order kinetics ($r^2 > 0.98$) with removal rate constants of 1.9×10^{-4} and 2.8×10^{-4} min^{-1}, respectively, when the pollutants were provided together and 5.7×10^{-4} and 9.7×10^{-4} min^{-1}, respectively, when they were provided individually. Photocatalytic pre-treatment of the mixture during 60 h removed 50 ± 1% and 62 ± 2% of the phenol and PNP initially present but only 11 ± 3% of the initial COD. Hydroquinone, nitrate and catechol were identified as PNP photocatalytic products and catechol and hydroquinone as phenol photocatalytic products. Subsequent biological treatment of the pre-treated samples removed the remaining contaminants and their photocatalytic products as well as 81–83% of the initial COD, allowing complete detoxification of the mixture to *C. vulgaris*. Similar detoxification efficiencies were recorded after biological treatment of the irradiated mixture with activated sludge microflora or with an acclimated consortia composed of a phenol-degrading *Alcaligenes* sp. and a PNP-degrading *Arthrobacter* sp., although the acclimated strains biodegraded the remaining pollutants faster. Biological treatment of the non-irradiated mixture was inefficient due to *C. vulgaris* inhibition.

1. Introduction

Because mechanical aeration can represent up to 55% of all the energy used during wastewater treatment (Tchobanoglous et al., 2003), algal photosynthesis can be advantageously used to provide O_2 during the aerobic removal of dissolved organic matter (Mara and Pearson, 1986). This also prevents the hazardous spraying of microorganisms and organic pollutants (Bell et al., 1993) and assists the elimination of pathogens by increasing the temperature and the pH (due to CO_2 fixation) of the treated effluent (Oswald, 2003). Finally, microalgae growth enhances the removal of nutrients (Muñoz et al., 2005) and heavy metals (Muñoz et al., 2006) and produces a rich biomass that can be further used as fertilizers

*Corresponding author. School of Civil and Environmental Engineering, Nanyang Technological University, Block N1, Nanyang Avenue, Singapore 639798, Singapore. Tel.: +65 6790 6391; fax: +65 6791 0676.
E-mail address: bjguieysse@ntu.edu.sg (B. Guieysse).

Abbreviations: AC, acclimated culture; AS, activated sludge; COD, chemical oxygen demand; xh-EC50, effective concentration inhibiting 50% of the target population after *x* h of exposure; OECD, Organization for Economic Cooperation and Development; ThOD, Theoretical chemical Oxygen Demand; PNP, *p*-nitrophenol

0043-1354/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2007.01.015

or digested to produce biogas (Muñoz and Guieysse, 2006). Unfortunately, and despite all their advantages, algal–bacterial processes have hitherto been limited to municipal wastewater treatment, probably because of the high sensitivity of the algae towards toxic pollutants that might be found in industrial wastes (Muñoz and Guieysse, 2006). In such cases, sunlight can support wastewater pre-treatment by direct irradiation or photocatalysis in order to convert recalcitrant and/or toxic pollutants into biodegradable and biocompatible products that can then be biologically treated (Sarria et al., 2002, 2003). Significant energy saving could therefore be achieved by combining solar-based pre-treatment with solar-based biological treatment. However, sequential solar-based photocatalysis and photosynthetically aerated biological treatment has, to the best of our knowledge, never been reported.

This study was therefore conducted to compare the potential of simulated solar irradiation (UV) and photocatalysis (UV/TiO_2) to detoxify a mixture of phenol (100 mg l^{-1}) and *p*-nitrophenol (PNP, 50 mg l^{-1}) prior to its biological treatment under photosynthetic aeration. Direct irradiation is easy to perform but can generate toxic photocatalytic products during the treatment of chlorophenols and nitroaromatics (Dzengel et al., 1999; Essam et al., 2006). In comparison, photocatalysis is fast and efficient because it is based on the photoproduction of hydroxyl radicals with high oxidation potential (1.9–2.7 V) (Pandiyan et al., 2002). Unfortunately, recovering the catalyst can be difficult and increases treatment costs. Phenol and PNP were selected for being priority widespread toxic pollutants (ATSDR—Agency for Toxic Substances and Disease Registry, 2005).

2. Materials and methods

All chemicals were reagent grade. Phenol, PNP and anatase TiO_2 (particle size $\leqslant 44\,\mu m$) were purchased from Sigma-Aldrich. All experiments were conducted in triplicate at $23 \pm 2\,^{\circ}C$. When sterile conditions were needed the glassware and the medium were autoclaved and the stock solutions were sterilized by filtration through 0.2 μm sterile membranes.

2.1. Microorganisms

Activated sludge microflora (AS) was obtained from Lund wastewater treatment plant (Sweden). A phenol-degrading *Alcaligenes* sp. (Genbank accession number DQ120520) and a PNP-degrading *Arthrobacter* sp. (Genbank accession number DQ412707) were isolated from the aeration tank of the wastewater treatment plant of a coke company (Cairo, Egypt). Microbial acclimation, selection and enrichment were performed by successive transfer of suspended-batch cultures at increasing phenol or PNP concentration supplied as sole carbon and energy sources (Essam, 2006). Pure isolates were obtained by cultivating pure colonies withdrawn from agar plates and characterized by partial 16S rRNA sequencing (Essam, 2006). These strains were always used together in a culture referred to as acclimated consortia in the following. A microalgal strain morphologically characterized as *Chlorella vulgaris* was also isolated from the stabilization pond of the same treatment plant.

Screening, isolation and maintenance of all microbial cultures were conducted using a mineral salt medium (MSM) composed of (in mg l^{-1} of deionized water): K_2HPO_4, 4000; Na_2HPO_4, 5200; $(NH_4)_2SO_4$, 1950; $CaCl_2 \cdot 7H_2O$, 10; $MgSO_4 \cdot 7H_2O$, 500; $FeSO_4 \cdot 7H_2O$, 10; $MnCl_2 \cdot 4H_2O$, 5.5; $ZnCl_2$, 0.68; $CoCl_2 \cdot 6H_2O$, 1.2; $NiCl_2 \cdot 6H_2O$, 1.2; $CuCl_2 \cdot 2H_2O$, 0.85; H_3BO_3, 0.0031; $NaMoO_4 \cdot 2H_2O$, 0.012; $NaSeO_3 \cdot 5H_2O$, 0.013; $NaWO_4 \cdot 2H_2O$, 0.0165 (Essam et al., 2006, 2007). The pH was adjusted to 7 with 2 N NaOH. This medium was enriched with 500 mg phenol l^{-1}, 50 mg PNP l^{-1} or 4000 mg $NaHCO_3$ l^{-1} for cultivation of *Alcaligenes*, *Arthrobacter* or *Chlorella*, respectively.

2.2. Phenol and PNP toxicity to the selected microalgae

Test tubes of 12 ml were filled with 9 ml MSM containing phenol (10, 25, 50 or 100 mg l^{-1}) or PNP (10, 20, 25 or 50 mg l^{-1}) and 2 g $NaHCO_3$ l^{-1}, inoculated with 0.54 ml of *C. vulgaris* culture, flushed with N_2 gas (to remove any atmospheric O_2), sealed with plastic screw caps and incubated under continuous agitation (150 rpm) and illumination (4000 lx = 18 μW cm^{-2}, Philips TLD 36 W/840 lamp). After 72 h, 2 ml samples were withdrawn and analyzed to measure the chlorophyll content according to Porra and Grimme (1974). Blanks were conducted without adding any pollutants and algal inhibition (%) was calculated as the reduction of the average chlorophyll content in the test samples compared to that in the blanks. These tests were performed under sterile conditions.

2.3. UV-irradiation treatments

UV-irradiation tests were conducted in MSM to allow for subsequent biodegradation studies (Essam et al., 2006, 2007) and buffer the pH (which always remained approx. 7). To allow enough volume for sampling and biodegradation tests, 25 aliquots of 6 ml of MSM supplied with 50 mg PNP l^{-1} and/or 100 mg phenol l^{-1} were transferred into 25 × 10 ml glass tubes (10 cm length) placed beside each other on a rocking shaker. For photocatalysis tests, 1 g TiO_2 l^{-1} was added to each tube (Essam et al., 2007; Ksibi et al., 2003) and the mixtures were sonicated for 5 min to obtain a homogenous suspension. The tubes were then gently shaken and irradiated at 300 μW cm^{-2} (at 15 cm) with two 18 W UV blue lamps (Sylvania Reptistar, Sylvania, USA, approx. 30% UVA–5% UVB). Light intensity within 250–500 nm measured by potassium ferrioxalate actinometry (Hatchard and Parker, 1956) was 1.15×10^{-5} Einstein s^{-1}. A set of controls pre-treated with TiO_2 alone but not irradiated was performed under the same conditions. Samples were periodically withdrawn from three test tubes randomly selected to monitor the concentration of remaining pollutants (1 ml) and nitrate (0.5 ml) and saved at 4 °C prior to analysis. The liquid fractions from each set of experiment were collected and mixed after 60 h of irradiation and samples were withdrawn for COD (10 ml), phytotoxicity (8 ml) and algal-toxicity (20 ml) analysis and immediately treated. TiO_2 was removed by centrifuging the tubes at 1400 g for 15 min (Mistral 1000) before the supernatants were mixed. This experiment was repeated 3 times to provide triplicates for the biodegradation tests.

2.4. Biological treatment

Glass flasks of 35 ml were filled with 25 ml of irradiated mixture and inoculated with 1 ml *C. vulgaris* culture and either 1 ml of AS or 1 ml of acclimated consortia. The flasks were then flushed with N_2 gas to remove any atmospheric O_2, sealed with rubber septa and aluminum caps and incubated for 14 d under continuous agitation (150 rpm) and illumination (18 $\mu W\,cm^{-2}$, Philips TLD 36 W/840 lamp). Controls consisted of flasks supplied with a pre-treated mixture, inoculated (with each consortia) and incubated in the dark as well as flasks supplied with a non-irradiated mixture, inoculated and continuously illuminated. For chemical analysis, 1.5 ml samples were periodically withdrawn and saved at 4 °C. COD, algal toxicity and phytotoxicity were measured at the end of incubation.

2.5. Analysis

Chemical analysis: All samples were centrifuged during 10 min at 11,300 *g* (Biofuge 13, Heraeus, Germany) and portions of the supernatants were used for subsequent analysis. Phenol and PNP were analyzed by HPLC-UV using a Waters 2690 HPLC system (USA) equipped with an autosampler and a diode-array detector from the same manufacturer and a Supelcosil LC-8 column (Supelco, USA). Elution was performed with a mobile phase composed of methanol/water/acetic acid (30:69:1 v/v). External standards were used to enable quantitative determination at 280 nm. The limit of quantification was 3 $mg\,l^{-1}$ for phenol and 1 $mg\,l^{-1}$ for all other compounds. Catechol and hydroquinone were identified by comparing the retention times and absorption spectra (210–400 nm) of unknown peaks with those of external standards.

COD was measured using LCK 414 test tubes (Dr. Lange, Germany) and a Lasa 100 photometer equipped with an LT 100 heater (Dr. Lange). Nitrite and nitrate were colorometrically analyzed using an FIAStar 5000 analyzer (Foss Tecator, Sweden) according to the manufacturer instruction.

Bioassays: Phytotoxicity was assayed by adding five seeds of *Lepidium sativum* onto a 5.5 cm (∅) filter paper placed in a glass dish filled with 2 ml of sample (pH 7). When necessary, the samples were diluted 4 times and/or centrifuged for 10 min at 1400 *g* (Mistral 1000). The dishes were then covered and incubated in complete darkness for 5 d. Three Petri dishes were prepared for each test and phytoxicity (%) was calculated as the ratio of the reduction of average stem length of the 15 test seeds by the average stem length of the 15 control seeds (tap water). Potential outliers were identified and rejected by using the Grubb's test at the 5% significant level and results were analyzed with one-way ANOVA at 5% significance.

Agal-toxicity assays were performed in triplicates according to the OECD 201 guidelines using the isolated *C. vulgaris* as test microorganism since *Chlorella* often predominates in algal pond systems (Canovas et al., 1996). When necessary, samples were first centrifuged at 11,300 *g* for 10 min. Test tubes of 7 ml were then filled with 5 ml of samples, 0.25 ml of a 40 g $NaHCO_3\,l^{-1}$ sterile stock solution and inoculated with 0.3 ml of algal culture. The tubes were then flushed with N_2 gas, closed with plastic screw caps and incubated for 72 h under continuous agitation (150 rpm) and illumination (18 $\mu W\,cm^{-2}$, Philips TLD 36 W/840 lamp). After incubation, 2 ml samples were withdrawn from each tube to analyze the chlorophyll content according to Porra and Grimme (1974). Blanks were obtained by inoculating and incubating MSM supplied with 2 g $NaHCO_3\,l^{-1}$ under the same conditions. Algal inhibition (%) was then calculated as the reduction (%) of the average chlorophyll content in the tested samples to that in blanks.

To monitor microbial respiration, gas samples of 150 μl were periodically withdrawn from the closed bottles using a syringe and injected on a GC (Agilent 6890N) equipped with a thermal conductivity detector (TCD). The carrier gas was helium at a flow rate of 30 $ml\,min^{-1}$ and the columns used were a Hayesep N 80/100 9 ft 1/8 in and Molesieve 5 A 60/80, 6 ft 1/8 in. The column, injector and detector temperatures were 60, 105 and 150 °C, respectively.

The results given represent the average from 15 (toxicity) or 3 (other tests) replicates ± standard deviation.

3. Results and discussion

Photosynthetically aerated biological treatment was inefficient to remove the contaminants or reduce the toxicity in the non-irradiated mixture (Table 1). However, both the AS and the acclimated consortia were able to biodegrade phenol and PNP when oxygen was externally provided by air bubbling (data not shown). Photosynthetic aeration therefore failed due to algal inhibition, as confirmed by the algal-toxicity assay (Table 1). PNP was more toxic than phenol as it completely inhibited algal growth at 50 $mg\,l^{-1}$, whereas phenol only inhibited the algae by 26% at 100 $mg\,l^{-1}$ (Fig. 1). This agrees with previous studies reporting a phenol 96h-EC50 of 370 $mg\,l^{-1}$ to *C. vulgaris* (Shigeoka et al., 1988) as compared to a PNP 14d-EC50 of 70 $mg\,l^{-1}$ (Madhavi et al., 1995). Nitrophenols are in fact commonly used in the production of herbicides for inhibiting photosynthesis (Umamaheswari and Venkateswarlu, 2004).

UV irradiation was not suitable as pre-treatment option as 60 h irradiation only removed 4 ± 1% and 8 ± 2% of the phenol and PNP supplied in the mixture and 9 ± 1% and 12 ± 1% of the pollutants provided individually. By comparison, 60 h photocatalysis removed phenol and PNP from the mixture by 50 ± 1% and 62 ± 2%, respectively. The initial Theoretical Oxygen Demand (ThOD) introduced by phenol and PNP (338 $mg\,l^{-1}$, based on phenol and PNP ThOD equivalents of 2.38 and 1.61 $g\,g^{-1}$, respectively) was similar to the COD value experimentally measured (359 ± 7 $mg\,l^{-1}$), showing that these parameters can be compared for mass balance analysis. Taking into account the remaining phenol and PNP concentration (54 and 19 $mg\,l^{-1}$, respectively), photocatalysis should have caused a ThOD reduction of 47%. The low COD removal experimentally recorded (11 ± 3%) therefore suggested that the contaminants were only partially oxidized, which was confirmed by the presence of hydroquinone and catechol in irradiated samples (Fig. 2A). The ThOD remaining from the pollutants and their photocatalytic products (16 mg hydroquinone l^{-1} and 13 mg catechol l^{-1}, using a ThOD equivalent of 1.9 $g\,g^{-1}$ for both compounds) was, however,

Table 1 – Photocatalytic-biological treatment of mixture of 50 mg PNP l^{-1} and 100 mg phenol l^{-1} in mineral medium

Treatment	Control[a]	UV/TiO_2[b]	Biol[c]		Combined UV/TiO_2+Biol[d]			
			AC	AS	LAC	LAS	AC	AS
Phytotoxicity (%) $\times 0$[e]	100	100	100	100	48±7	49±6	95±6	96±5
$\times 4$[e]	27±6	0	23±9	22±10	0	0	0	0
Algal toxicity (%) $\times 0$[f]	96±0	54±3	97±0	97±1	0	0	NQ[g]	NQ
$\times 2$[f]	85±2	13±5	79±4	77±4	0	0	NQ	NQ
COD (mg l^{-1})	359±7	319±8	317±12	329±18	70±4	63±3	277±15	289±16
COD removal (%)	0	11±3	12±1	8±1	81±5	83±5	23±1	20±1
Phenol removal (%)	3±0	50±1	14±0	10±0	100	100	59±1	56±0
PNP removal (%)	5±0	62±2	7±2	6±1	100	100	66±2	66±2

[a] Non-irradiated mixture incubated in the dark for 14 d (no inoculation).
[b] Mixture irradiated with UV during 60 h in the presence of 1 g TiO_2 l^{-1}.
[c] Non-irradiated mixture inoculated with AC (acclimated consortia) or AS (activated sludge microflora) with *C. vulgaris* and incubated under continuous illumination (18 μW cm^{-2}) for 14 d.
[d] Combined photocatalytic-biological treatment in flasks supplied with a mixture preliminarily treated by UV/TiO_2 for 60 h, inoculated by *C. vulgaris* with AC or AS and incubated under continuous illumination (LAC and LAS) at 18 μW cm^{-2} for 14 d or kept in the dark (AC or AS).
[e] Dilution factor (in deionized water) of samples submitted to the toxicity test.
[f] Dilution factor (in MSM) of samples submitted to the toxicity test.
[g] Not quantified (NQ) due to bacterial growth and pollutant consumption. The high initial pollutant concentration shows algal toxicity was approx. 50% (value after UV/TiO_2 treatment).

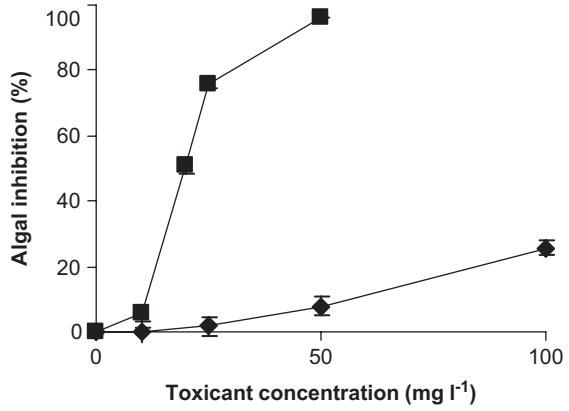

Fig. 1 – Inhibition (%) of chlorophyll synthesis by *C. vulgaris* in 12 ml tubes supplied with different concentrations of phenol (diamond) or PNP (squares), inoculated with 6% v/v of *Chlorella vulgaris* and incubated under continuous agitation (150 rpm) and illumination (18 μW cm^{-2}) during 72 h. Vertical bars represent the standard deviation on triplicates.

still significantly lower than the remaining COD (319±8 mg l^{-1}). Hence, non-aromatic photocatalytic products such as formic and acetic acids might also have been released (Tanaka et al., 1997).

Photocatalytic degradation of phenol and PNP was always well described by pseudo-first order kinetics ($r^2 > 0.98$). Lower removal rate constants recorded when the pollutants were provided together (1.9×10^{-4} and 2.8×10^{-4} min^{-1}, respectively) than when they were individually provided (5.7×10^{-4} and 9.7×10^{-4} min^{-1}) due to competition for hydroxyl radicals in the mixture. Faster first order PNP removal rate constants of 7.2×10^{-3} and 1.2×10^{-2} min^{-1} were reported by Tanaka et al. (1997) and Di Paola et al. (2003). However, these authors added PNP in pure water and used more powerful light sources (500 W and 125 W Hg lamps emitting 18 and 10.5 mW cm^{-2}, respectively). Similarly, faster phenol first order rate constants of 1.55×10^{-2} and 4.9×10^{-3} min^{-1} were achieved by Tanaka et al. (1997) and Sonawane and Dongare (2006) when irradiating phenol in pure water. The lower removal rates recorded in the present study can therefore be explained by the lower irradiation intensity applied and the use of mineral medium as, for instance, SO_4^{2-} is a known inhibitor to phenol photocatalysis (Yawalkar et al., 2001).

Phenol photocatalysis resulted in formation of catechol and hydroquinone (Fig. 2B), which were also detected by Yawalkar et al. (2001) during solar photocatalysis. Hydroquinone, nitrate and traces of phenol and catechol were detected as PNP photocatalytic products (Fig. 2C), which agrees with the observations of Tanaka et al. (1997) and Dieckmann and Gray (1996) during PNP photocatalysis using 500 W super-high-pressure and 450 W medium pressure Hg lamps, respectively. Unidentified peaks, which might have been transition intermediates such as 4-nitrocatechol (Dieckmann and Gray, 1996) or recombination products (Peiró et al., 2001) were recorded only after 12 and 24 h of the PNP photocatalysis. PNP photocatalysis only yielded the release of 0.93 mol of NO_3^- per each mol of PNP degraded, suggesting that other nitrophotocatalytic products were formed. NH_4^+ released has, for instance, been reported during PNP photocatalysis (Tanaka et al., 1997) but this could not be demonstrated here due to the presence of this compound in the medium.

TiO_2 photocatalysis detoxified the mixture to *C. vulgaris* by approx. 50% (Table 1). This enabled algal growth, O_2 production and the subsequent aerobic degradation of the remaining pollutants. Less than 10 mg phenol l^{-1} and 3 mg PNP l^{-1} were removed in the inoculated flasks supplied with the irradiated

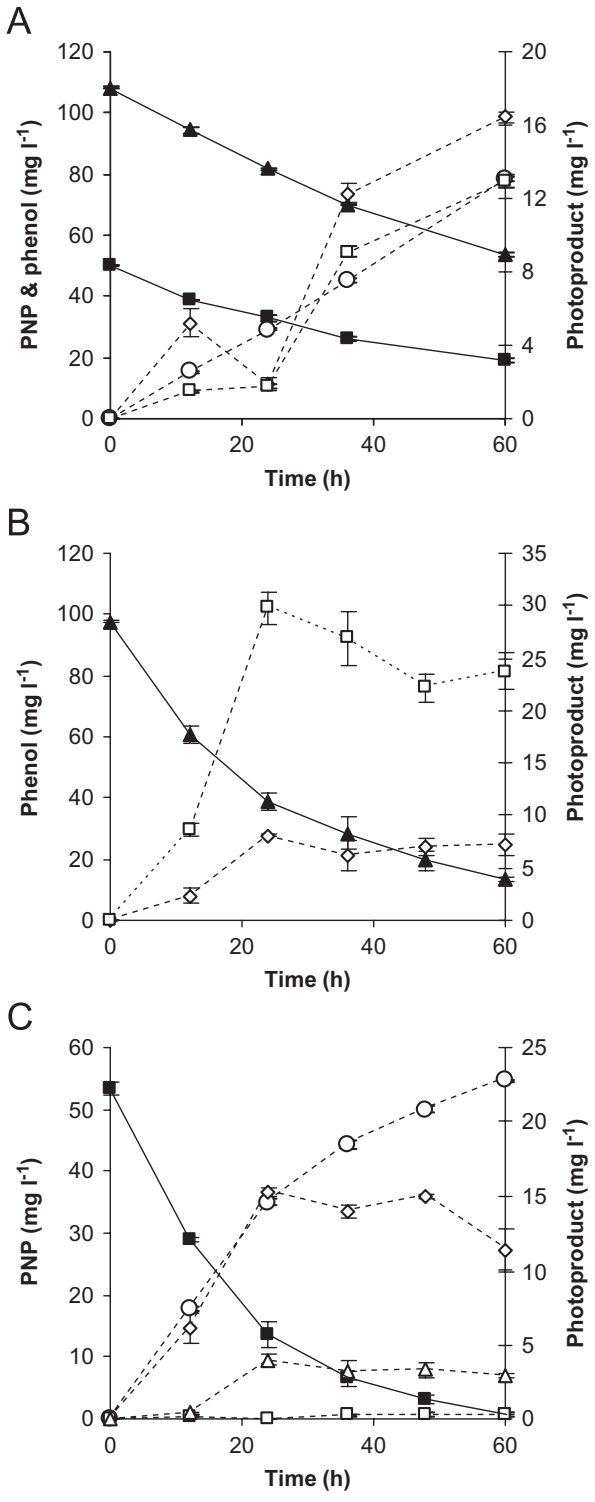

Fig. 2 – Changes in the concentrations (mg l^{-1}) of phenol (closed triangles), PNP (closed squares), nitrate (open circles), hydroquinone (open diamonds) and catechol (open squares) in tubes supplied with 1 g TiO_2 l^{-1} and: (A) 100 mg phenol l^{-1} and 50 mg PNP l^{-1}; (B) 100 mg phenol l^{-1} or (C) 50 mg PNP l^{-1}; and irradiated during 60 h with UV light at 300 μW cm^{-2} (1.15×10^{-5} Einstein s^{-1}). Open triangles (C) show the concentration of phenol released during PNP photocatalysis. Vertical bars represent the standard deviation on triplicates.

mixture and incubated in the dark, showing that photosynthetic oxygen supply was necessary to support microbial activity (Table 1). Complete removal of the remaining pollutants was thus achieved after 7 d of incubation in the illuminated flasks inoculated with the acclimated consortia (Fig. 3A) compared to 10 d when AS was used as inoculum (Fig. 3B). This difference was due to the acclimatization time needed by the AS. Hydroquinone and catechol, which are both readily biodegradable and common microbial metabolites of aromatic compounds (Harbison and Belly, 1982; Smith, 1990; Ye et al., 2004), were completely removed after 5 d of incubation, regardless of the consortia used. Thus, UV/TiO_2 photocatalysis degraded phenol and PNP by ring opening, ring hydroxylation or replacement of nitrate by hydroxyl group,

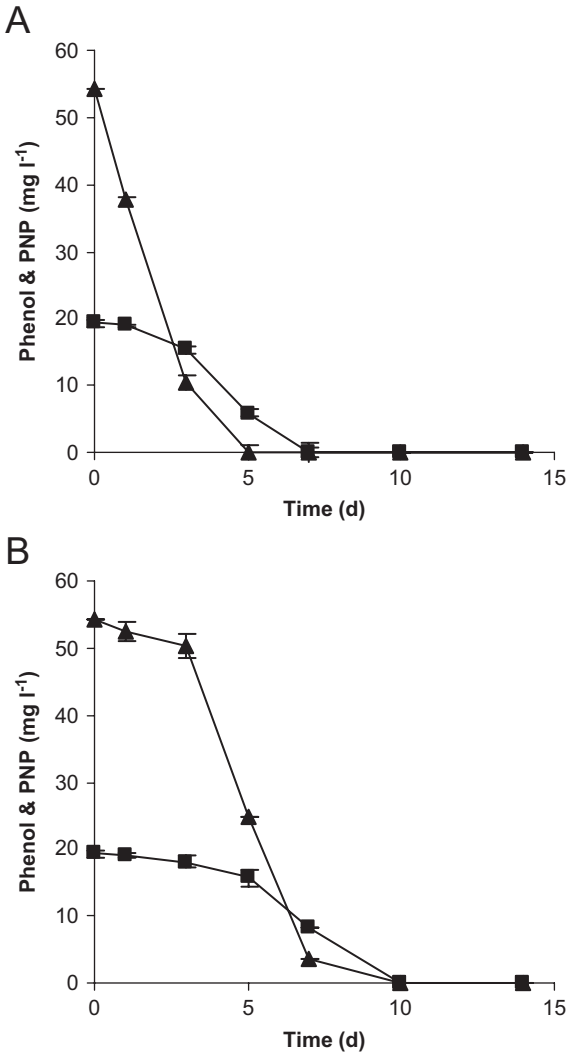

Fig. 3 – Changes in the remaining concentrations (mg l^{-1}) of phenol (triangles) and PNP (squares) in flasks supplied with a mixture of 100 mg phenol l^{-1} and 50 mg PNP l^{-1} pre-treated by UV/TiO_2 photocatalysis for 60 h, inoculated with *C. vulgaris* and an acclimated consortia (A) or activated sludge (B) and incubated under continuous illumination at 18 μW cm^{-2} for 14 d. Vertical bars represent the standard deviation on triplicates.

which generated more biodegradable compounds (Loonen et al., 1999; Goi et al., 2004).

Complete detoxification to *C. vulgaris* was only achieved by sequential photocatalytic-biological treatment (Table 1). Similar phytotoxicity values (at 5% significance level) were recorded in the irradiated mixture treated with the AS or the acclimated consortia. The same level (50% inhibition) was recorded in pollutant-free MSM, showing that the detoxification efficiency of the combined treatment was satisfactory. Similar COD removal efficiencies (5% significance level) were achieved when using AS or the acclimated consortia (Table 1). The remaining COD was likely generated by unknown biorecalcitrant photocatalytic products and/or microbial by-products.

In all inoculated flasks, O_2 was consumed and CO_2 was released during the first 4 d of incubation (Fig. 4). This was likely due to microbial consumption of the dissolved O_2 initially introduced in the flasks (as neither the AS nor the acclimated culture were inhibited by the mixture). Microbial activity, however, stopped in the flasks supplied with the non-irradiated mixture and illuminated as well as in the flasks supplied with the irradiated mixture and incubated in the dark (Fig. 4A). This was caused by O_2 depletion due to algae inhibition in the non-irradiated mixture or lack of photosynthetic activity in the pre-treated mixture, which also limited COD and pollutant removal in these tests (Table 1). In comparison, the levels of O_2 and CO_2 started to increase and decrease, respectively, in the illuminated flasks supplied with the pre-treated mixture as results of photosynthesis. After 10 d of incubation, the O_2 level remained constant whereas CO_2 was undetected, which indicated that microbial activity has stopped due to complete removal of biodegradable pollutants (Fig. 3). *C. vulgaris* therefore supplied the O_2 required by the AS or the acclimated consortia to biodegrade the pollutants, using in turn the CO_2 released during microbial respiration. Hence, all the organic carbon initially present is converted into biomass, which virtually allows to double the yield of CH_4 production from anaerobic biomass digestion as demonstrated by Muñoz et al. (2005) during acetonitrile removal under photosynthetic aeration.

Interestingly, nitrate removal was recorded in all flasks inoculated and supplied with the pre-treated mixture (Fig. 5). This was more likely caused by nitrate reduction than its assimilation since NH_4^+ (initially present at 500 mg l^{-1} in the

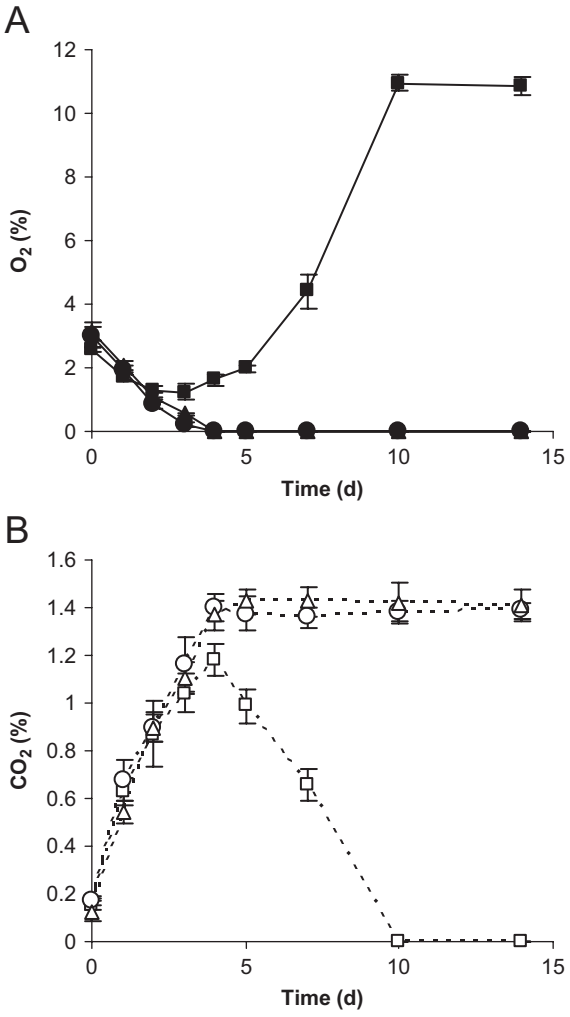

Fig. 4 – Changes in the O_2 (A) and CO_2 (B) levels (%) in the headspaces of flasks supplied with (1) a mixture of 100 mg phenol l^{-1} and 50 mg PNP l^{-1} in MSM pre-treated by UV/TiO_2 photocatalysis during 60 h, inoculated with *C. vulgaris* and activated sludge and incubated during 14 d in the dark (triangles) or under continuous illumination at 18 μW cm^{-2} (squares); or (2) the same mixture non-irradiated, inoculated with the same consortium and incubated under continuous illumination (circles). Vertical bars represent the standard deviation of triplicates. Similar changes were recorded in the flasks inoculated with *C. vulgaris* and an acclimated consortia.

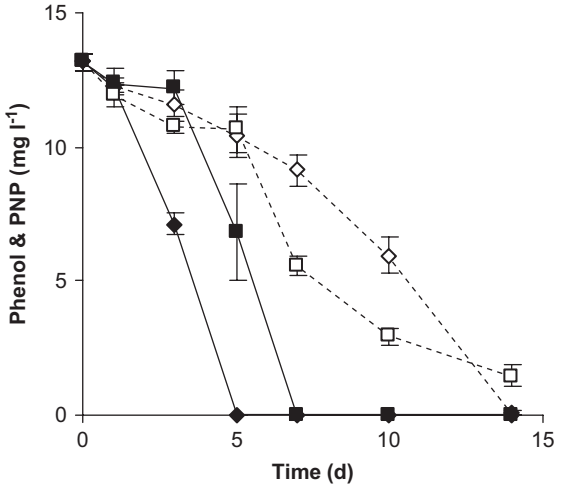

Fig. 5 – Change in nitrate concentrations (mg l^{-1}) in flasks supplied with a mixture of 100 mg phenol l^{-1} and 50 mg PNP l^{-1} pre-treated by UV/TiO_2 photocatalysis during 60 h, inoculated with *C. vulgaris* together with activated sludge microflora (diamonds) or acclimated consortia (squares) and incubated for 14 d in the dark (closed symbols) or under continuous illumination at 18 μW cm^{-2} (open symbols). Vertical bars represent the standard deviation of triplicates.

medium) is usually preferred as N-source for microbial growth, which also explains why nitrate removal was faster in the flasks incubated in the dark where no oxygen was photosynthetically produced. Denitrifiers are common in activated sludge and both *Alcaligenes* and *Arthrobacter* sp. were capable to reduce nitrate. Denitrification did, however, not significantly increase pollutant removal, which remained very low in the flasks inoculated and incubated in the dark (Table 1). Neither pollutant removal nor detoxification was recorded in samples treated with TiO_2 alone or in the irradiated mixture inoculated only with the microalgae (data not shown), showing that algae did not biodegrade the pollutant themselves but were necessary to provide O_2 to the bacteria.

4. Conclusions

UV/TiO_2 photocatalysis was efficient to reduce the toxicity of a mixture of 100 mg phenol l^{-1} and 50 mg PNP l^{-1} to *C. vulgaris*, allowing the subsequent biodegradation of the remaining pollutants and their photoproducts under photosynthetic aeration. Hence, sunlight could serve to power the sequential photocatalytic-biological treatment of toxic effluents in algal–bacterial bioreactors. This enlarges the potential of algal–bacterial systems for the treatment of toxic effluents (Muñoz and Guieysse, 2006).

Acknowledgments

Dr. Jiri Neustupa, from the Department of Botany of the Faculty of Science of Charles University of Prague, Czech Republic, is gratefully acknowledged for characterizing the microalgae strain. This project was supported by funds from the Swedish International Development and Cooperation Agency (SIDA) under the Swedish-MENA research link program. This work falls within the framework of the Egyptian Academy of Scientific Research and Technology's National Strategy for Biotechnology and Genetic Engineering Program whose support of the Microbial Biotechnology Centre is acknowledged.

REFERENCES

ATSDR—Agency for Toxic Substances and Disease Registry, 2005. CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) priority list of hazardous substances, 〈http://www.atsdr.cdc.gov/cercla/〉.

Bell, J., Melcer, H., Monteith, H., Osinga, I., Steel, P., 1993. Stripping of volatile organic compounds at full-scale municipal wastewater treatment plants. Water Environ. Res. 65, 708–716.

Canovas, S., Picot, B., Casellas, C., Zulkifi, H., Dubois, A., Bontoux, J., 1996. Seasonal development of phytoplankton and zooplankton in a high-rate algal pond. Water Sci. Technol. 33 (7), 199–206.

Di Paola, A., Augugliaro, V., Palmisano, L., Pantaleo, G., Savinov, E., 2003. Heterogeneous photocatalytic degradation of nitrophenols. J. Photochem. Photobiol. A—Chem. 155, 207–214.

Dieckmann, M.S., Gray, K.A., 1996. A comparison of the degradation of 4-nitrophenol via direct and sensitized photocatalysis in TiO_2 slurries. Water Res 30 (5), 1169–1183.

Dzengel, J., Theurich, J., Bahnemann, D.W., 1999. Formation of nitroaromatic compounds in advanced oxidation processes: photolysis versus photocatalysis. Environ. Sci. Technol. 33 (2), 294–300.

Essam, T., 2006. Solar-based physicochemical–biological processes for the treatment of toxic and recalcitrant effluents. Ph.D. Thesis, Biotechnology Department of Lund University, Lund, Sweden, ISBN 91-89627-49-0.

Essam, T., Zilouei, H., Amin, M.A., El Tayeb, O., Mattiasson, B., Guieysse, B., 2006. Sequential UV-biological degradation of chlorophenols. Chemosphere 63, 277–284.

Essam, T., Amin, M.A., El Tayeb, O., Mattiasson, B., Guieysse, B., 2007. Sequential photochemical–biological degradation of chlorophenols. Chemosphere 66, 2201–2209.

Goi, A., Trapido, M., Tuhkanen, T., 2004. A study of toxicity, biodegradability, and some by-products of ozonised nitrophenols. Adv. Environ. Res. 8, 304–311.

Harbison, K.G., Belly, R.T., 1982. The biodegradation of hydroquinone. Environ. Toxicol. Chem. 1 (1), 9–15.

Hatchard, C.G., Parker, C.A., 1956. A new sensitive actinometer. II. Potassium ferrioxalate as a standard chemical actinometer. Proc. R. Soc. London 235A, 518–536.

Ksibi, M., Zemzemia, A., Boukchina, R., 2003. Photocatalytic degradability of substituted phenols over UV irradiated TiO_2. Photochem. Photobiol. A—Chem. 159, 61–70.

Loonen, H., Lindgren, F., Hansen, B., Karcher, W., Niemelä, J., Hiromatsu, K., Takatsuki, M., Peijnenburg, W., Rorije, E., Struijs, J., 1999. Prediction of biodegradability from chemical structure: modeling of ready biodegradation test data. Environ. Toxicol. Chem. 18 (8), 1763–1768.

Madhavi, D.R., Umamaheswari, A., Venkateswarlu, K., 1995. Effective concentrations of nitrophenolics towards growth yield of selected microalgae and cyanobacteria isolated from soil. Ecotoxicol. Environ. Saf. 32, 205–208.

Mara, D.D., Pearson, H., 1986. Artificial freshwater environment: waste stabilization ponds. In: Rehm, H.J., Reed, G. (Eds.), Biotechnology. vol. 8. Verlagsgesellschaft mbH, Weinheim, pp. 177–206.

Muñoz, R., Guieysse, B., 2006. Algal–bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40 (15), 2799–2815.

Muñoz, R., Jacinto, M., Guieysse, B., Mattiasson, B., 2005. Combined carbon and nitrogen removal from acetonitrile using algal–bacterial bioreactors. Appl. Microbiol. Biotechnol. 67 (5), 699–707.

Muñoz, R., Alvarez, T., Muñoz, A., Terrazzas, E., Guieysse, B., Mattiasson, B., 2006. Sequential removal of heavy metals ions and organic pollutants using an algal–bacterial consortium. Chemosphere 63, 903–911.

Oswald, W.J., 2003. My sixty years in applied algology. J. Appl. Phycol. 15 (2–3), 99–106.

Pandiyan, T., Martinez, R.O., Martinez, J.O., Amezcua, G.B., Martinez-Carrillo, M.A., 2002. Comparison of methods for the photochemical degradation of chlorophenols. J. Photochem. Photobiol. A—Chem. 146 (3), 149–155.

Peiró, A.M., Ayllón, J.A., Peral, J., Doménech, X., 2001. TiO_2-photocatalyzed degradation of phenol and *ortho*-substituted phenolic compounds. Appl. Catal. B Environ. 30, 359–373.

Porra, R.J., Grimme, LH., 1974. A new procedure for the determination of chlorophylls *a* and *b* and its application to normal and regreening *Chlorella*. Anal. Biochem. 57 (1), 255–267.

Sarria, V., Parra, S., Adler, N., Péringer, P., Pulgarin, C., 2002. Recent developments in the coupling of photoassisted and aerobic biological processes for the treatment of biorecalcitrant compounds. Catal. Today 76, 301–315.

Sarria, V., Kenfack, S., Guillod, O., Pulgarin, C., 2003. An innovative coupled solar–biological system at field pilot scale for the treatment of biorecalcitrant pollutants. J. Photochem. Photobiol. A—Chem. 159 (1), 89–99.

Shigeoka, T., Sato, Y., Takeda, Y., Yoshida, K., Yamauchi, F., 1988. Acute toxicity of chlorophenols to green algae, *Selenastrum capricornutum* and *Chlorella vulgaris* and quantitative structure–activity relationships. Environ. Toxicol. Chem. 7, 847–854.

Smith, M., 1990. The biodegradation of aromatic hydrocarbons by bacteria. Biodegradation 1 (2–3), 191–206.

Sonawane, R.S., Dongare, M.K., 2006. Sol–gel synthesis of Au/TiO2 thin films for photocatalytic degradation of phenol in sunlight. J. Mol. Catal. A. Chem. 243, 68–76.

Tanaka, K., Luesaiwong, W., Hisanaga, T., 1997. Photocatalytic degradation of mono-, di- and trinitrophenol in aqueous TiO_2 suspension. J. Mol. Catal. A. Chem. 122 (1), 67–74.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill, New York, NY.

Umamaheswari, A., Venkateswarlu, K., 2004. Impact of nitrophenols on the photosynthetic electron transport chain and ATP content in *Nostoc muscorum* and *Chlorella vulgaris*. Ecotoxicol. Environ. Saf. 58 (2), 256–259.

Yawalkar, A.A., Bhatkhande, D.S., Pangarkar, V.G., Beenackers, A.A.C.M., 2001. Solar-assisted photochemical and photocatalytic degradation of phenol. J. Chem. Technol. Biotechnol. 76 (4), 363–370.

Ye, J., Singh, A., Ward, O.P., 2004. Biodegradation of nitroaromatics and other nitrogen-containing xenobiotics. World J. Microbiol. Biotechnol. 20 (2), 117–135.