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Microbiological degradation of a spent offset-printing developer

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Abstract

To decontaminate spent offset-printing developer Polychrome 4003, several microorganisms were separated from the soil that has been used for developer dumping for 3 years. Two organism cultures were isolated and identified to genus *Geomyces pannorum* and *Bacteria* spp. These organisms, as well as commercial Septic Gobbler (SG) bacteria, were used to decontaminate the developer. Reduction of both the chemical oxygen demand (COD) and the amount of total identified organic compounds reached 30% after 40 day treatment of waste suspension by *G. pannorum* and *Bacteria* spp. A substantially higher degree of COD reduction by ∼80% and the total amount of identified organic compounds by ∼90% was achieved when SG bacteria have been applied for the same period. According to a rapid electrophysiological test with macrophytic algae *Nitellopsis obtusa*, the toxicity of spent offset-printing developer Polychrome 4003 was classified as extremely toxic (>100 toxic units, T.U.), and it remained at the same level after treatment with *G. pannorum* and *Bacteria* spp. More effective biodegradation with SG bacteria diminished toxicity substantially.

Keywords: Microbiological degradation; Polychrome 4003 developer; *Geomyces pannorum*; *Bacteria* spp.; Septic Gobbler bacteria

1. Introduction

Biological treatment of photoprocessing wastewaters has been described in many publications [1–11]. Most of them deal with Kodak Kodacolor process C-41 and Kodak Ektacolor process RA-4 that include developer, bleach, fixer and stabilizer solutions. It has been shown that a large number of photoprocessing wastewater constituents are degradable and do not impair the growth of activated sludge microorganisms. During the biological treatment of ammonia and thiosulfate solutions, the nitrification and oxidation of thiosulfate to sulfate take place at the same time [9,10]. Aerobic degradation of silver bearing photoeffluents, which assumes silver recovery, is also feasible by the activated sludge process [11]. Removal of photoprocessing wastewater-derived chemical oxygen demand (COD) by 70–85% can be achieved by the activated sludge microorganisms. Therefore, the development of biological systems for the pretreatment of photoprocessing effluents seems to be highly promising.

New photographic solutions of different composition have been developed over the years, thus necessitating the study on photoprocessing wastewater treatment. The problem is that for secrecy reasons, the companies do not reveal the full-scale chemical structure of the developers produced; thus, the composition of the outgoing wastewater solutions after photoplate development is unknown. For example, a widely distributed offset-printing developer, trade-named Polychrome 4003 (T-151), is described by the manufacturer as a 10–20% potassium silicate solution. In the development process, the developer undergoes changes per se as well as being enriched by chemicals that are present on the plate surface, i.e. various organic binders, photosensitive compounds and dyes. As our current investigations show, the cumulative wastewater solution is characterised by the high values of COD in the range 80–120 gO_2/l, a wide spectrum of organic compounds and high toxicity. About

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30.000 l of this developer is consumed annually in Lithuania alone.

Difficulties arise when effluent toxicity is evaluated only on the basis of chemical characteristics such as chemical or biochemical oxygen demand, total organic carbon, volatile suspended solids, etc. A large number of chemicals present in the effluent can be identified by the contemporary analytical methods; however, chemical data itself do not involve direct information on the bioavailability of chemicals and/or their possible synergistic effects. Most of the publications dealing with biological treatment of photoeffluents do not include data on treated waste ecotoxicity. In these works, main attention has been paid to the waste impact upon the viability of activated sludge microorganisms. We could not find any publications about biological degradation of spent offset-printing developers.

In this study, the efficacy to decontaminate the offset-printing developer Polychrome 4003 by different microorganisms separated from the soil that has been used for the developer dumping and commercial Septic Gobbler (SG) bacteria was explored. To check the change in chemical composition and toxicity of photoeffluent, the simultaneous measurements of general chemical indices such as COD, the content of organic compounds by gas chromatography (GC) and Fourier transforming IR spectroscopy (FTIR), as well as of toxicity to macrophytic algae *Nitellopsis obtusa* and crustacean *Daphnia magna*, were employed.

2. Experimental

2.1. Materials and procedures

The photoprocessed Polychrome 4003 (PP4003) solution was procured from "Vilspa" printing house (Vilnius, Lithuania).

To find the microorganisms persistent to Polychrome 4003 waste media, the soil samples were collected from the site, where the waste has been dumped for 3 years. Soil-water suspension was used for microorganism selection. A mixture of cultures was grown for 4 days at 20 °C on YEPD medium [12]: 2% peptone, 2% glucose, 1% yeast extract and 2.5% agar. The grown colonies of microorganisms were doubly selected. Then pure colonies were disseminated repeatedly on the separate plates. Two different microorganism cultures were selected and identified to genus *Geomyces pannorum* (Link) Sigler and Carmichael and *Bacteria* spp. Micromycete inoculum was grown in a YEPD liquid medium for 2 days. Biodegradation assay was carried out in a PP4003/distilled water suspension (1:5); the pH was adjusted to 7.5. The aliquotes of the suspension (100 ml) containing 1% (by volume) of microorganisms inoculum, 0.25% glucose and 0.25% peptone supplements were kept in conical flasks, covered with aluminium foil. The cultures practically did not grow without glucose and peptone supplements. Sulfuric acid was used for solution acidification. For toxicological testing, control cultivations were performed in a synthetic minimal yeast medium (2% glucose, 0.2% K_2HPO_4, 0.1% $MgSO_47H_2O$, 0.01% $(NH_4)_2SO_4$, 2 mkg/l biotyne, 200 mkg/l thiamine). They were grown for 20 days at 20 °C. The viability of microorganism cultures was checked taking samples of the biodegradation mixture every 10 days and subculturing them in YEPD medium to form colony units.

The other attempt to biodegrade photoeffluents was to employ a Septic Gobbler bacteria concentrate, purchased from Cassco American Biotech Sales, USA. The bacteria concentrate ~30 mg/l every 3 days was inserted in 400 ml of a PP4003 wastewater solution diluted by distilled water. The mixture was kept in a chemical flask and periodically bubbled with air.

2.2. Analytical methods

The organic compounds present in the investigated solutions were analyzed by gas chromatography and Fourier transforming IR spectroscopy. GC analyses were carried out on an HP 5890 (Hewlett Packard) gas chromatograph equipped with an HP 5971 mass selective detector and an HP 7673 split/splitless injector. The separation was performed on a silicon capillary column, CP-Sil 8CB (50 m × 0.32 mm, film thickness 0.25 μm). The temperature in a GC oven was programmed as follows: from 60 °C, it was increased to 160 °C at a rate of 5 °C/min and to 250 °C at a rate of 10 °C/min and the final temperature was kept for 5 min. The temperatures of the injector and the detector were 250 and 280 °C, respectively. The flow rate of carrier gas (helium) was 1 ml/min. Mass spectra (MS) in electron mode were generated at 70 eV.

FTIR spectra were recorded using a BOMEM MB (Hartman & Braun, Canada) spectrometer, which operated from 4000 to 400 cm^{-1} at ambient temperature. Background corrections were made using KBr as a reference blank.

Diethylether, dichloromethane, chloroform, benzene and methylene chloride were tested as extrahents for the recovery of organic compounds from solutions. Diethylether was found to be the most suitable extrahent, which enabled to extract the highest amount of organic compounds at pH = 2.

The COD values were established by the potassium dichromate method [13]. Oxidation by dichromate was carried out at 148 °C for 2 h with a subsequent titration using Mohr salt $(NH_4)_2Fe(SO_4)_26H_2O$. pH was measured with a Hydromet ERH-11 type electrode and a pH-meter Elmetron CP 315.

2.3. Electrophysiological algal test (Charatox)

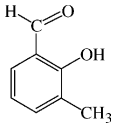
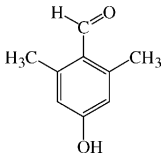
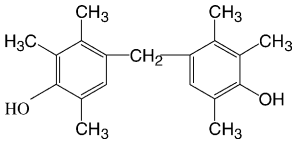
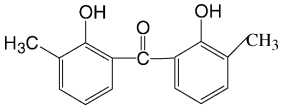
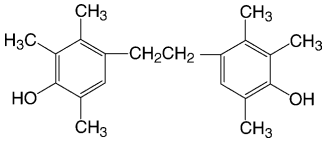
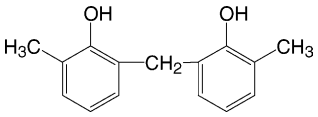
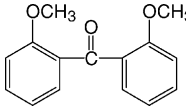
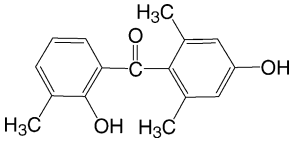
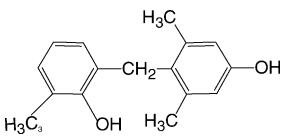
Freshwater charophyte algae, *N. obtusa* (Desv.) J. Groves, were harvested in Lakes Sienis and Švenčius (southern Lithuania) during the vegetation period in 2002–2003. After separation from the bulk, single cells were kept at room temperature in glass vessels with equal parts of tap and lake water and a specific medium [14]. Tests were carried out at room temperature in dim light.

The electrophysiological biotest employs a 45-min EC_{50} cell membrane depolarisation endpoint. The details of the computer-assisted experimental setup, testing procedures for range finding and EC_{50}-determining tests and the methods for measurement of cell transmembrane resting potential (RP) have been published previously [15,16]. Briefly, bioelectrical activity of up to 32 living internodal cells was measured simultaneously according to K-anaesthesia method [17], modified for multichannel recording with extra cellular chlorinated silver wire electrodes [15]. Two independent tests were performed at the same time, by dividing 32 cells into two groups. The discrete RP values from distinct cells were taken every second. For the determination of EC_{50}, the percentage decrease in average RP value**,** in relation to that of untreated cells**,** was calculated for each concentration [15,16]. The EC_{50} value was estimated using a linear regression of the averaged decrease in RP with the logarithms of exposure concentration.

2.4. *D. magna* test (Daphtoxkit F^{TM} *magna)*

D. magna mortality (immobilization) test was performed following the Standard Operational Procedure of Daphtoxkit F^{TM} [18]. All the materials required to perform tests with

Table 1
GC/MS data of etheral extract of the initial waste solution

Number	Retention time, chemical name	Mass spectrometric fragmentation peaks	Compound
1	8.3 3-methyl-2-hydroxybenzaldehyde	136 (*m*/*z*) 135 122 118 107 90 77 63 51	
2	12.6 2,6-dimethyl-4-hydroxybenzaldehyde	150 (*m*/*z*) 149 135 121 107 104 91 77 65 51	
3	28.6 4,4′-methylenebis-[2,3,5-trimethyl] phenol	284 (*m*/*z*) 269 253 223 209 165 149 135 97 83 77 55	
4	28.9 2,2′-dihydroxy-3,3′-dimethylbenzophenone	242 (*m*/*z*) 227 209 199 165 153 135 115 91 77 57 55	
5	29.7 4,4′-ethylenebis-[2,3,5-trimethyl] phenol	298 (*m*/*z*) 283 253 239 175 149 141 115 91 77 57 51	
6	30.7 2,2′-methylenebis-[3-dimethyl] phenol	228 (*m*/*z*) 209 195 165 121 108 91 77 51	
7	31.2 2,2′-dimethoxybenzophenone	242 (*m*/*z*) 221 209 178 165 135 122 121 108 91 77 51	
8	31.5 2,4′-dihydroxy-3,2′,6′-trimethylbenzophenone	256 (*m*/*z*) 241 223 179 165 135 121 107 91 86 77 51	
9	31.6 2,4′-methylenebis-[3,2′,6′-trimethyl] phenol	242 (*m*/*z*) 227 211 181 165 122 107 91 77 51	

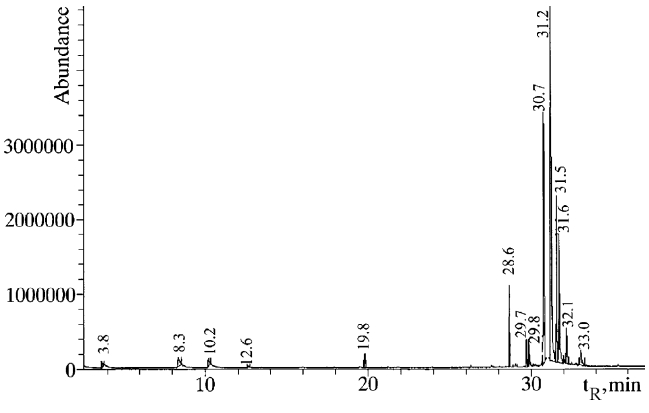


Fig. 1. GC/MS chromatogram of PP4003 etheral extract.

daphnias were purchased from Microbiotests Inc., Belgium. Test organisms, included in a kit in the form of resting eggs (ephippia), were started to hatch 72 h prior to testing. A 48-h LC50 (lethal concentration causing 50% mortality of testing organisms) bioassay was performed in a multiwell testplate.

2.5. Toxicity data conversion and sample ranking

The toxicity data obtained as 50% effect endpoint values (in percent of dilution) were converted into toxic units by the formula [19]: T.U. = $(1/EC_{50}) \times 100$. In case of low toxicity, i.e. below 50% effect level, T.U. were calculated as parts of 50% effect (e.g. the 40% effect obtained with the highest sample concentration has 40/50 of 1 T.U., i.e. 0.8, since the 50% effect corresponds to 1 T.U.). The effect values below 20% were assigned for the zero toxicity [20,21].

For the ranking of samples, the classification scale in T.U. proposed by Persoone et al. [22] was used. According to this arbitrary scale, the samples are classified as “not toxic” (nt, <0.4 T.U.), “slightly toxic” (st, 0.4–1), “toxic” (t, 1.1–10), “very toxic” (vt, 11–100) and “extremely toxic” (et, >100).

3. Results and discussion

The GC/MS analysis of the etheral extract of a PP4003 solution’s at pH = 2 has shown the presence of several compounds, nine of which were identified based on their molecular ion (M^+) and mass spectrometric fragmentation ions (Table 1). The most abundant compounds in the etheral extract were 2,2′-methylenebis-[3-dimethyl] phenol with M^+ 228, retention time (t_R) 30.7–22%, and 2,2′-dimethoxybenzophenone with M^+ 242 and t_R 31.2–35%. The amount of other compounds was in the range 1–12%, and still 5–11% being unidentified. The chromatogram of the etheral extract of initial solution is shown in Fig. 1.

The FTIR spectrum (Fig. 2) bands emerged in 1450–1600 cm^{-1} range are due to the C=C bond stretching vibrations which confirm the presence of aromatic compounds. A strong band at 1700 cm^{-1} is typical of the aromatic aldehydes C=O bond stretching vibration and confirms its presence in the tested solution. Some overlapped bands at 1200 cm^{-1} may be attributed to the C–O stretching vibrations of phenol derivatives as well as to the 1,2- and 1,4-substituted benzene ring C–C bonds deformation vibrations.

pH of PP4003 solution was 12–12.5; its COD ranged 70,000–120,000 mgO_2/l. The COD value decreased after acidification, due to the formation of silicic acid precipitate, which, probably, adsorbs a certain part of organic compounds. The liquid phase COD values at pH 9, 7, 6 and 5 were 13,000, 9000, 5000 and 5500 mgO_2/l, respectively.

The change in the area of liquid phase chromatographic peaks occurring by waste acidification is depicted in Table 2. The amount of organics in solution decreased with the drop in pH. The increase in peaks area at pH = 5 and simultaneous rise in COD may be explained by partial desorption of the organics from the precipitate due to the increased acidity. The sediments formed by the acidification were analyzed using the GC/MS method. The organic compounds disappeared in liquid phase and were found in the precipitate. Evidently, both the total waste organics and the COD value were unaffected.

During the biodegradation experiments with *G. pannorum* and *Bacteria* spp., the microorganisms remained viable and multiplied. The samples for the COD and GC/MS analysis were taken as suspensions, resultant after thorough mixing of an acidified solution.

Alterations in suspensions COD in the course of time are shown in Table 3. The blank sample contained 0.25% glu-

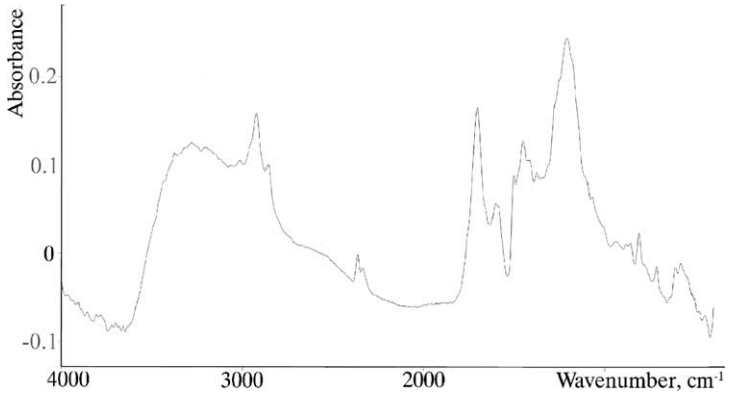


Fig. 2. FTIR spectrum of PP4003 vaporized etheral extract.

Table 2
Dependence of the waste liquid phase etheral extract chromatographic peaks area on pH

pH	Retention time								
	8.3	12.6	28.6	28.9	29.7	30.7	31.2	31.5	31.6
Initial									
12.5	1922	1386	5822	5737	13758	21916	10713	13829	5689
9.0	238	478	394	165	73	388	143	678	137
7.0	134	249	200	43	–	81	37	430	129
6.0	87	184	137	29	–	39	–	315	74
5.0	99	255	459	–	–	–	–	340	79

Table 3
Alteration of COD[a] ($mgO_2/l \times 10^{-3}$) in waste suspension (diluted by distilled water 1:5, pH = 7.5) throughout 40 days treatment by *Geomyces pannorum* and *Bacteria* spp.

Microorganism	Time (days)						COD
	0	5	10	20	30	40	reduction (%)
Geomyces pannorum	117.6	115.0	100.0	91.2	88.0	82.3	30.0
Bacteria spp.	117.6	110.0	100.5	95.2	90.0	89.7	23.7
Blank pattern[b]	117.6	115.0	114.2	114.0	104.2	101.0	14.1

[a] COD values recalculated to undiluted solutions.
[b] Waste suspension diluted by distilled water 1:5.

cose and 0.25% peptone without a microorganism supplement. COD values slightly changed during 40 days and their reduction did not exceed 30%. The decrease in the blank pattern's COD may be explained by the action of indigenous microorganisms. It should be stressed that the all COD values in this work were calculated for undiluted solutions.

The etheral extracts of pattern were analyzed by the GC/MS method after 18 days and 40 days of exposure to micoorganisms. The chromatograms have shown that the amount of organic compounds slightly decreased with time, but its percentage remained practically unchanged. The compounds, corresponding to t_R 8.3 and 12.6, disappeared totally. The peak areas of chromatograms of the patterns exposed for 40 days were reduced approximately at the same order as that of COD (∼25–30%).

According to toxicity data generated by Charatox, which has been evaluated as relatively sensitive test toward complex wastewater samples [20], the initial waste solution as well as that treated by *G. pannorum* and *Bacteria* spp. was classified as extremely toxic (111–200 T.U., Table 4). It was also found that the microorganisms separated from the soil were highly poisonous to algae used. Although these bacteria on synthetic yeast medium were not so toxic to *N. obtusa* as being admixed with PP4003, the toxicity stemming from the microorganisms itself was characterised as very toxic (42–50 T.U., Table 4). So, despite the partial biodegradation of the organics and COD decrease, the toxicity problem remained.

The use of SG bacteria met the task of waste decomposition more effectively. The COD reduction in 1:5 and 1:10 diluted PP4003 suspension samples (pH = 7.5) reached ∼40 and ∼81%, respectively, in 40 days (Table 5). GC/MS analysis has shown the disappearance of compounds with

Table 5
COD[a] ($mgO_2/l \times 10^{-3}$) change of waste suspension (pH = 7.5) treated with Septic Gobbler bacteria and blank samples of wastes

Dilution	Time (days)						COD reduction (%)
	0	5	10	20	30	40	
1:10	83.2	55.1	40.0	32.2	32.0	15.9	80.9
1:5	81.0	71.4	63.3	63.4	55.5	49.0	39.5
1:5 (blank)	81.0	81.0	81.3	79.1	77.0	76.9	5.1

[a] COD values recalculated into undiluted solutions.

Table 4
Charatox test toxicity data

Sample	Biotesting results		Toxicity class[a]
	EC_{50}	T.U.	
PP4003, pH = 7.5 (suspension)	0.9	111	et
PP4003, 1:5 diluted, pH = 7.5, *Geomyces pannorum* inoculum (suspension), 40 days treated	0.5	200	et
PP4003, 1:5 diluted, pH = 7.5, *Bacteria* spp. inoculum (suspension), 40 days treated	0.7	143	et
Geomyces pannorum, on synthetic yeast medium	2.4	42	vt
Bacteria spp., on synthetic yeast medium	2.0	50	vt
PP4003, 1:10 diluted, pH = 7.5, with SG bacteria inoculum (suspension), 40 days treated	20.8	4.8	t
PP4003, 1:5 diluted, pH = 7.5, with SG bacteria inoculum (suspension), 40 days treated	2.1	47.6	vt
Septic Gobbler in distilled water	>100	0	nt

[a] nt: non toxic; t: toxic; vt: very toxic; et: extremely toxic.

Table 6
SG bacteria-treated 1:10 diluted waste suspension (pH = 7.5) etheral extracts chromatographic peaks area

Treatment time (days)	Retention time									Peaks area, ∑	Reduction (%)
	8.3	12.6	28.6	28.9	29.7	30.7	31.2	31.5	31.6		
0	684	132	2392	138	871	10525	16727	5565	5572	42606	–
18	–	–	1101	–	322	3488	5591	2138	2760	15400	63.8
40	–	–	1082	–	–	777	746	272	1093	3970	90.7

t_R 8.3, 12.6, 28.9, 29.7 and considerable decomposition of other compounds (Fig. 3). The 40 day total reduction of the identified organic compounds amounted 90.7% (Table 6). In contrast to *G. pannorum* and *Bacteria* spp., a control of SG bacteria in distilled water (Table 4) did not show any toxicity, and biodegradation by using SG bacteria diminished toxicity to algae cells significantly; however, it still remained toxic (∼5 T.U., dilution 1:10, Table 4).

Forty-eight-hours crustacean *D. magna* test data were overall analogous to that established by algal Charatox test, thus confirming the high toxicity of PP4003. The dose–response curves of daphnia mortality of untreated PP4003 (1:10) suspension and that of treated by SG bacteria for 40 days are shown in Fig. 4. Since 20% effect level was used as the lowest percentage effect considered to have a significant toxic impact [20,21], SG-treated PP4003 (1:10) solution should be ca. 50-fold diluted to get innocuous waste (LC_{50} < 2%, Fig. 4). Meanwhile, untreated PP4003 (1:10) solution should be respectively more than 330-fold diluted.

As revealed by GC/MS data, all of the microorganisms used caused decomposition of the initially identified organic compounds, especially those possessing aldehyde and keto groups. The compounds with aldehyde group (numbers 1 and 2; Table 1) were totally destroyed by the activity of all the microorganisms employed. Compounds with a keto group (numbers 4, 7 and 8; Table 1) were partially fragmented and were more sensitive to the activity of SG bacteria. After treatment by using SG bacteria, the compounds with fragmentation ions *m*/*z* 55, 57, 67, 69, 81, 83, 95, 97, 109, 111, 123, 125 and 139 (Fig. 3) were found in waste suspension, that are inherent to the unsaturated aliphatic hydrocarbons and

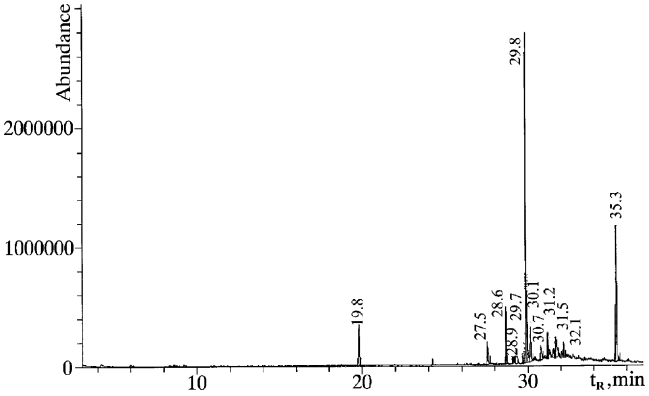


Fig. 3. GC/MS chromatogram of etheral extract of PP4003 suspension (1:10 dilution with distilled water, pH = 7.5) treated by SG bacteria for 40 days.

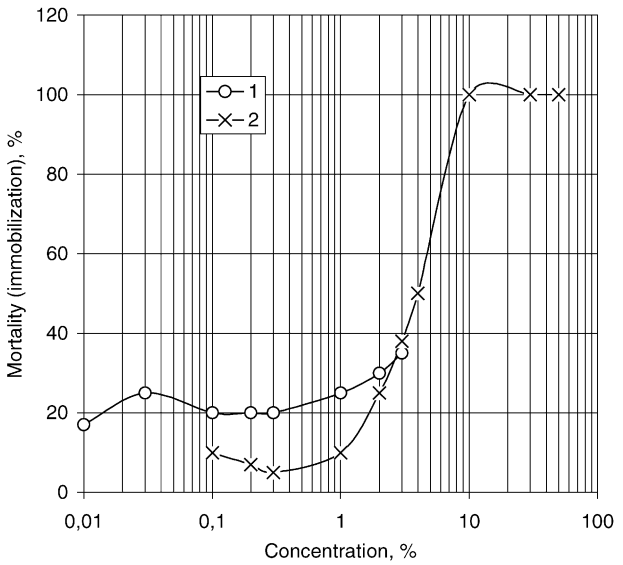


Fig. 4. Forty-eight-hours *Daphnia magna* mortality test: (1) untreated PP4003 (1:10) suspension (pH = 7.5), (2) 40 days SG bacteria treated PP4003 (1:10) suspension (pH = 7.5).

alcohols. Obviously, the activity of the microorganisms used caused the cleavage of a benzene ring.

As the results show, both separated from soil and commercial microorganisms do diminish photowaste's COD to some extent, in proportion to the amount of degraded organic compounds. Waste toxicity decreased significantly when SG bacteria were applied. At the same time, COD reduction was 2.5–3.5 times higher than that achieved by using microorganisms separated from soil and adapted to PP4003 solution. In latter case, the toxins secreted by microorganisms might contribute a high remaining toxicity. SG microbes are designated to eliminate the wide range of organic wastes including gasoline, hydrocarbons, phenols, brake fluid, kerosene, so it is hardly surprising that SG do destroy some of PP4003 waste's constituents.

As a general remark, SG bacteria seem to be a promising culture for partial decomposition and detoxification of spent Polychrome 4003 developer. It should also be emphasized the importance of accompanying toxicity data next to chemical, in order to characterise the harmfulness of photoeffluents.

4. Conclusions

Biodegradation of the organic compounds present in spent offset-printing developer Polychrome 4003 was achieved by

laboratory selected microorganism cultures *G. pannorum*, *Bacteria* spp. and commercial Septic Gobbler bacteria. The 40-day treatment by the selected cultures enabled to reduce the waste COD as well as the amount of identified organic compounds by 30%. The treatment with SG bacteria for the same period reduced COD by 81% and the total amount of identified compounds by 91%. According to a rapid electrophysiological test with macrophytic algae *N. obtusa*, the toxicity of a photoprocessed Polychrome 4003 solution was classified as extremely toxic, and it remained at the same toxicity rank after the treatment with *G. pannorum* and *Bacteria* spp. More effective biodegradation with SG bacteria diminished the toxicity substantially.

Generally, SG bacteria seem to be a promising culture for the pretreatment of Polychrome 4003 developer waste. Toxicity tests are essential for the characterisation of wastewater decontamination.

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