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Degradation of nitroesters by plant tissue cultures

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ABSTRACT

Nitrate esters are widely used as effective explosives, important components of explosive ranges, and energetic plasticizers. The environmental problem arising from the production and use of these compounds can be solved using biotechnology. Phytoremediation appears as an efficient technology for this purpose. The uptake and transformation of nitroglycerine (NG) and ethylene glycol dinitrate (EGDN) from wastewater by plants using *in vitro* regenerants of *Juncus inflexus* and *Phragmites australis* were investigated. The plants were exposed to the NG, (600 mg l^{-1}), the parent compound disappeared during 20 days and degradation products as dinitroglycerine (DNG) and mononitroglycerine (MNG) were identified in the medium. During 20 days the starting concentration of 100 mg l^{-1} EGDN disappeared in the case of *J. inflexus* or decreased to 5% in the case of *P. australis*. Ethylene glycol mononitrate as the degradation product was identified.

Using this approach directly to the wastewater from production of explosives, the starting concentration of nitroesters mixture (total concentration 270 mg l^{-1}) was decreased by *in vitro* regenerants of reed (*P. australis)* during 6 weeks to the water contained only MNG (48 mg l^{-1}).

1. Introduction

Nitrate esters of aliphatic alcohols are industrially used as highly efficient explosives as well as vasodilators in the treatment of heart diseases, such as angina pectoris.

Glycerol trinitrate (nitroglycerin, NG, propane-1,2,3-triyl trinitrate) and ethylene glycol dinitrate (EGDN) are used in a mixture of sodium nitrate and a suitable absorbent (often wood pulp) to produce dynamite. EGDN is added to the mixture to lower its freezing point. The EGDN/NG ratio is usually about 8/2 or 9/1. Explosives represent the only commercial use for EGDN. Because EGDN is more volatile than NG, the amount of EGDN airborne from the dynamite mixture is higher than that of NG. A mixture containing NG and EGDN, either in the sheet form or mixed with Royal Demolition Explosive (RDX: hexahydro-1,3,5-trinitro-1,3,5-triazine), can be used to make Semtex, a plastic explosive. Both compounds are produced by the direct nitration of the parent alcohol with nitric acid, usually in the presence of sulphuric acid as a catalyst [1].

As the solubility of nitroesters in water is rather low (EGDN 0.5 g l^{-}, NG 1.8 g l^{-1} at 25 °C), extensive washing with water is used in the production process to remove the residual mineral acids and ions. The water used for washing of NG and/or EGDN then contains suspended droplets of these xenobiotics. Release of the wastewaters to lagoons or soakaways thus represents serious contamination, which can be spread into surrounding soils [2]. As nitrate esters are not formed in plants, they need to be metabolized by common reactions for xenobiotic detoxification [3]. The elucidation of mechanisms for degradation of nitrate esters is of both theoretical and practical significance. It would contribute to effective decontamination of wastewaters and land sites, where nitrate esters had accumulated during their production, storage and utilization [4–6]. Physical and chemical treatments used for the decontamination of xenobiotic pollutants may be disadvantageous in terms of cost efficiency when the pollutant levels are low in large volumes. When microorganisms are used for remediation of xenobiotics, their cultivation at adequate levels over a long period requires both suitable inoculation protocols and continuous nutrient supply. Bacterial metabolism of NG in mixed cultures involves sequential denitration to glycerol dinitrates (DNGs), glycerol mononitrates (MNGs), and eventually glycerol [7]. NG was reported to be metabolized also by fungi (e.g., *Phanerochaete chrysosporium* and *Geotrichum candidum*) [8–10]. It should be taken into account that microorganisms showing highly efficient biodegradation capabilities under laboratory conditions need not perform equally well at actual contaminated sites [11].

Recently, phytoremediation has gained attention as an ecofriendly approach to environmental cleanup [12–16]. Relatively wide use of various phytoremediation techniques has showed their

Abbreviations: EGDN, ethylene glycol dinitrate; NG, nitroglycerine (trinitroglycerine); DNG, dinitroglycerine; MNG, mononitroglycerine.

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advantage as effective and relatively cheap methods for pollutant elimination from the environment. However, until now, a relatively limited knowledge of the mechanisms involved has been acquired. Therefore, more systematic approaches concerning the selection of plant species, genetic manipulation, and optimization of remediation processes are urgently required [17–19]. Phytoremediation has been studied for the treatment of high explosives. Uptake and transformation of trinitrotoluene (TNT), RDX, and other explosives by different plant species has been repeatedly reported [20–27]. In spite of the fact that mechanisms of catabolic processes have not been fully elucidated [28,29], it appears that plants have a substantial capacity to remove and effectively transform organic compounds containing nitro groups. These processes may include both stimulation of plant detoxification pathways and microbial biodegradation of the contaminants [30].

The aim of this study is the optimization of the phytoremediation process in a constructed wetland. We compared the *in vitro* regenerants of *Phragmites australis* and *Juncus inflexus* under laboratory conditions to investigate the possibility of effectively cleaning the wastewaters contaminated during production of nitroesters.

2. Materials and methods

2.1. Cultivation of in vitro regenerants of wetland species

The regenerants of *J. inflexus* and *P. australis* were obtained from BIOPLANTA (Germany). They were cultivated aseptically under 16 h light period at 24 °C in magenta boxes on Sangwan-Gorenflot medium [31] with 30 g l^{-1} sucrose, supplemented with 5 mg l^{-1} 6-benzylaminopurine (BAP), with the pH adjusted to 5.8 with 1 N NaOH. Subculturing was carried out for a period of 4 weeks. A relatively high concentration of BAP suppressed the development of the root system and, therefore, a month before starting the experiments, the cultures were transferred onto a medium without growth regulators. EGDN was added in three different concentrations (100, 500, and 1000 mg l^{-1}); corresponding amounts of ethanol were added to the control plants.

2.2. Sample preparation and detection of nitroesters

The medium from the suspension culture was filtered out using a Büchner funnel, and the cells (approximately 20–30 g fresh mass) were resuspended and extracted by orbital shaker (50 rpm) in 50 ml of acetone for 24 h. The filtered acetone extract was evaporated on a vacuum evaporator, and the solid residue was dissolved in 2 ml of methanol.

The medium was filtered through a SEP-PAK^{TM}-C^{18} cartridge for rapid sample preparation (Waters, USA) and washed out with 10 ml of distilled water to remove sugars and inorganic salts. Explosives and their degradation products were washed out with 10 ml of methanol. This solution was evaporated on a vacuum evaporator at 35 °C. The solid residue was dissolved in 1 ml of methanol.

The presence of NG and degradation products was determined by high-performance liquid chromatography on a reverse-phase SiC^{18}-Biospher-packed stainless-steel column (250 mm × 4 mm). A linear gradient of methanol (10–100%) was applied for 40 min at a flow rate of 1 ml min^{-1}. Substances were detected by comparing the spectra and retention times of the samples and standards using a photo diode array detector Jasco MD 1510 (Essex, UK). The quantity of products was calculated from the chromatograms at a wavelength of 203 nm using the standard calibration curve.

2.3. Ecotoxicity test

Phytotoxicity was assessed by the root growth-inhibition assay using 3-day-old white mustard (*Sinapis alba*, Brassicaceae) seedlings [32]. Mustard seeds were germinated on a filter paper in glass petridishes of diameter 11 cm (25 seeds in each dish) containing 5 ml of growth medium (Table 1), supplemented with 50, 100, 200, and 400 mg l^{-1} of NG, a mixture of the two DNG isomers, and a mixture of the MNG isomers. The seeds were germinated for 72 h in the dark at 24 °C. After this period, the lengths of the primary roots of the seedlings were measured and compared with that of the control. All experiments were carried out in triplicate.

Table 1
Composition of nutrient medium using for mustard seeds germination.

	[mg l^{-1}]
$CaCl_2 \cdot H_2O$	588.0
$MgSO_4 \cdot 7H_2O$	246.5
$NaHCO_3$	129.5
KCl	11.5
pH	7.6–8.0

2.4. Statistical analysis

The results are presented as the means of three independent experiments. Each concentration was tested in triplicate as minimum. Data were analyzed by one-way analysis of variance (ANOVA) using the STATISTICA 0.8 (StatSoft, Inc., USA) software. Tukey's posthoc analyses were used for the evaluation of the significance of differences among particular treatments. The level of significance was established as $p \leq 0.05$.

3. Results and discussion

There are only a few reports concerning degradation of nitroesters in plants. Cultures of plant cells or organs can serve as suitable models to elucidate the mechanisms of uptake or/and transformation of different xenobiotics. Goel et al. [33] showed that sugar beet (*Beta vulgaris*) could effectively degrade NG. Denitration of both NG to glycerol and DNG to MNG was enhanced by the expression of pentaerythritol tetranitrate (PETN) reductase in transgenic seedlings. French et al. [34] followed the same approach and developed transgenic tobacco plants (*Nicotiana tabacum*) that expressed PETN reductase for the enhanced degradation of nitrate ester explosives and TNT. The transgenic seeds were able to germinate and grow in media containing glyceryl trinitrate (NG) and TNT, at levels toxic to the wild-type tobacco. The toxic effect of nitroesters in model systems of plant tissue cultures was studied by Podlipna et al. [35]

3.1. Test of toxicity

The wastewater originating from the manufacture of dynamite and other explosives contained both starting compounds — NG and EGDN (Fig. 1). According to the information of ammunition plants the concentration of NG in wastewater generally ranges from 100 to 500 mg l^{-1} and concentration of EGDN from 500 to 2000 mg l^{-1}. The latter one represented the major constituent. Initially, evaluation of the phytotoxicity of nitrate esters was undertaken. Until now, only relatively low attention has been paid to this topic, probably due to the relatively lower toxicity of NG and EGDN to humans, in comparison with other explosives, such as TNT. The test was based on measurement of the inhibition of growth of the primary root of the mustard seedlings after a 3-day exposure to different concentrations of the compounds of interest. NG or EGDN were applied in the following concentrations: 0, 50, 100, 200, and 400 mg l^{-1}. According to ANOVA analysis the statistical significant differences between toxicity of NG and EGDN were found for concentration 200 and 400 mg l^{-1} (Fig. 2). NG concentration of 400 mg l^{-1} completely

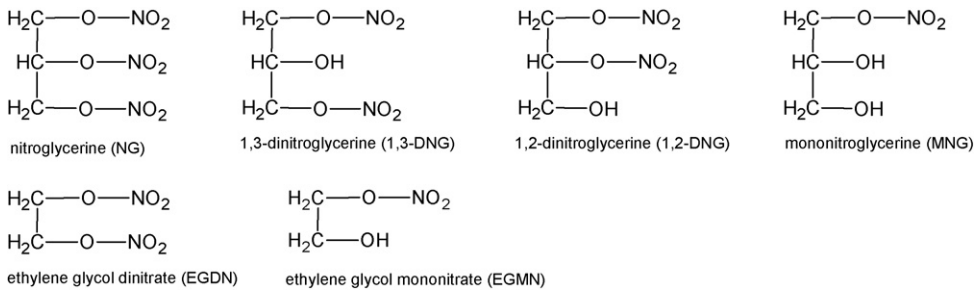

Fig. 1. Structure of parent compounds nitroglycerine (NG) and ethylene glycol dinitrate (EGDN) and their degradation products – 1,2 and 1,3-dinitroglycerine (DNG), mononitroglycerine (MNG) and ethylene glycol mononitrate (EGMN).

inhibited the germination of mustard seeds, whereas EGDN, at the same concentration, decreased the root length only by 40%.

This is, according to the authors' knowledge, the first report on the toxic effect of EGDN in plants using the standard ecotest which enable to compare the toxicity of EGDN with another compounds. The current data concerning NG are in accordance with the results of French et al. [34], who described severe stunting of both root and shoot of tobacco in the presence of NG (227 mg l^{-1}) [34].

3.2. Degradation of NG

Uptake and transformation of NG in rush (*J. inflexus*) were evaluated using a medium containing 600 mg l^{-1} NG. Within 20 days, the NG content decreased under the detection limit. The degradation products detected in the medium were 1,3-DNG, 1,2-DNG, and MNG. The contents of both DNGs increased within 12 days, reaching the maximum of 50 and 40 mg l^{-1}, respectively, on the 13th day. MNG concentration started increasing after 10 days. At the end of the experiment, the concentration of MNG was about 40 mg l^{-1}. The total quantity of degradation products found in the medium was approximately 5-fold lower than the starting concentration of NG (Fig. 3). The low amount of degradation products in the medium was probably caused by plant uptake and accumulation of these compounds in either vacuoles or other cell compartments.

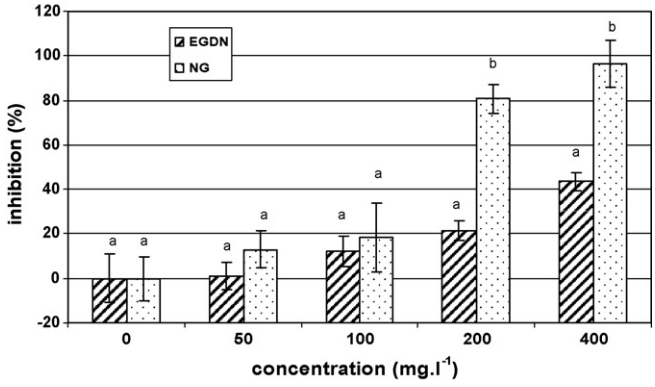

Fig. 2. Inhibition of growth of primary roots of 3-day-old white mustard seedlings in the presence of nitroglycerine (NG) or ethylene glycol dinitrate (EGDN) in comparison to the control (100%). The results are the means of three replicate measurements ± standard deviations. The treatments with statistically significant differences (according to ANOVA, $p \geq 0.05$) are marked by different letters (a, b).

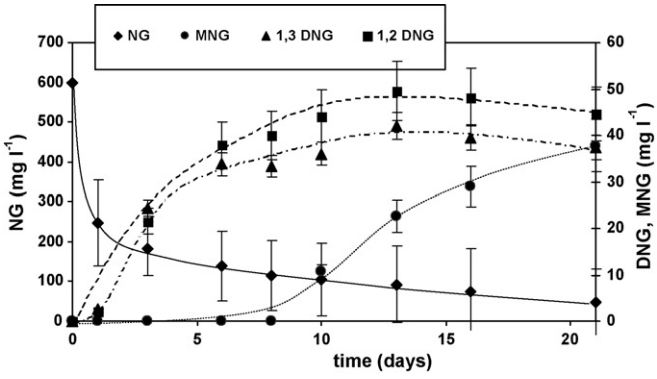

Fig. 3. Degradation of nitroglycerine (NG) by regenerants of *Juncus inflexus*, initial concentration 600 mg l^{-1}, degradation products 1,2-dinitroglycerine (1,2-DNG), 1,3-dinitroglycerine (1,3-DNG) and mononitroglycerine (MNG) were identified in the medium.

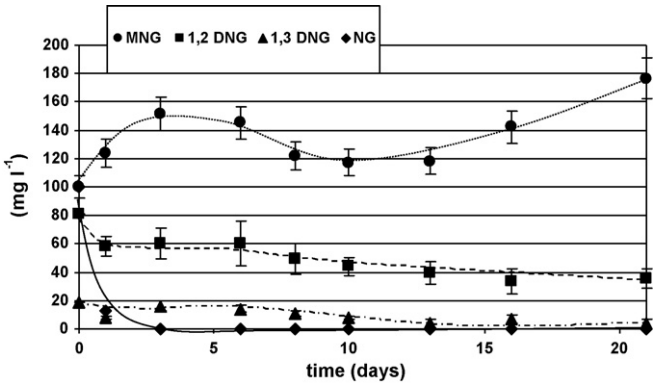

Fig. 4. Degradation of mixture of nitroglycerine (NG), initially concentration 100 mg l^{-1}; 1,2-dinitroglycerine (1,2-DNG), initially concentration 80 mg l^{-1}; 1,3-dinitroglycerine (1,3-DNG) initial concentration 20 mg l^{-1} and mononitroglycerine (MNG), initial concentration 100 mg l^{-1} by regenerants of *Juncus inflexus*, concentration in the medium.

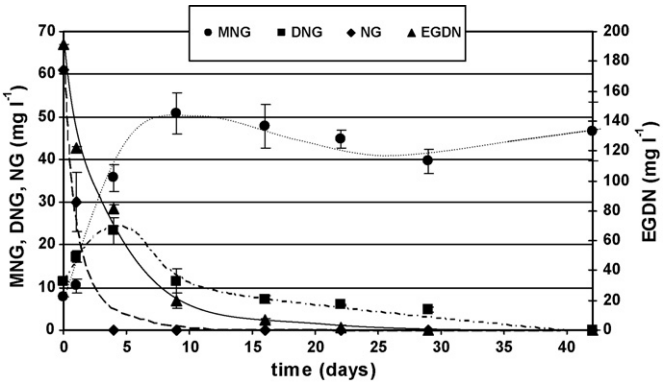

Fig. 5. Remediation of wastewater (diluted by cultivating medium in ratio 1:5) using the *in vitro* regenerants of reed (*Phragmites australis*).

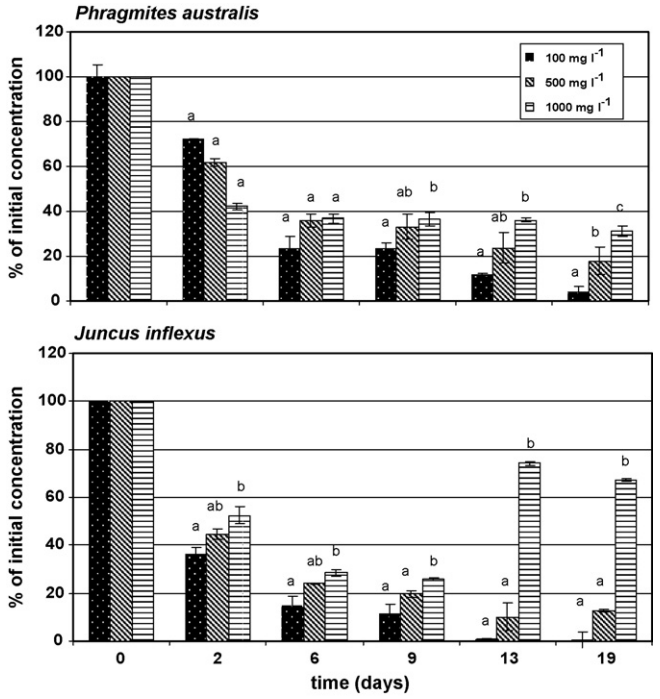

Fig. 6. Degradation of ethylene glycol dinitrate (EGDN) using reed and rush *in vitro* regenerants in the course of cultivation on medium supplemented by different initial concentration (100, 500 and 1000 mg l^{-1}), content in the medium. The results are the means of three replicate measurements ± standard deviations. The treatments with statistically significant differences (according to ANOVA, $p \geq 0.05$) are marked by different letters (a, b, c).

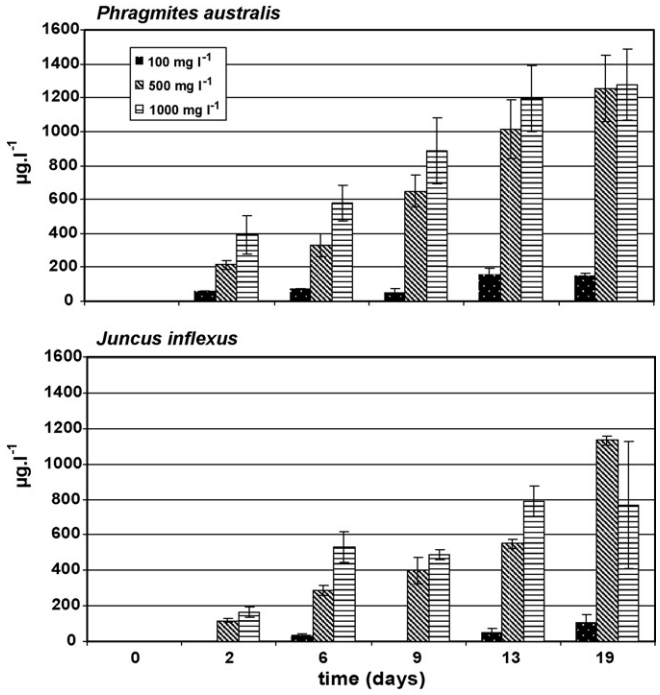

Fig. 7. Content of ethylene glycol mononitrate (EGMN) in the medium during degradation of ethylene glycol dinitrate (EGDN) by reed and rush *in vitro* regenerants, the initial concentration of EGDN was 100, 500 and 1000 mg l^{-1}.

3.3. Cleaning of wastewaters containing nitroesters

Phytoremediation of wastewater, which contained both NG and its degradation products, was followed using aseptically cultivated regenerants of rush. The medium was supplemented with a cocktail containing NG (100 mg l^{-1}), a mixture of DNGs (100 mg l^{-1}, 1,2- and 1,3-DNG in the ratio 4:1), and MNG (100 mg l^{-1}). NG was depleted from the medium very quickly (within 3 days). Both dinitroglycerins were very slowly degraded to MNG, the content of which consequently increased. After twenty days (end of experiment), the total contents of NG and its metabolites were lower then the initial NG concentration (Fig. 4). These two experiments confirm the ability of rush and probably other wetland plant species to degrade and accumulate NG. Denitration of NG was also followed in pure bacterial cultures of *Bacillus thuringiensis* plus *B. cereus* and *Enterobacter agglomerans.* These cultures seem to have active hydrolytic pathway, although formation of nitrate was not established. In contrast, White et al. showed unequivocally that assimilation of nitrogen from NG in pure cultures of a *Pseudomonas* sp. and *Agrobacterium radiobacter* occurred through nitrite (not nitrate) [7,36]. The concomitant formation of mainly glycerol-1,3-dinitrate (and small amounts of the corresponding 1,2-isomer) was found. Cells were able to denitrate both dinitrates to mononitrates, but not further. They also converted PETN to its tri- and dinitrates. The enzyme involved in denitration was identified in the crude cell extracts as NADH-dependent NG reductase [37].

The real wastewaters arising from the washing out steps of production of explosives (such as dynamite) were tested in cooperation with the ammunition factory (Explosia a.s., Czech Republic). These wastewaters contained residues of NG, DNG, MNG, and predominantly EGDN. The total amount of nitroesters in this wastewater was in the range of 1–2 g l^{-}. This high concentration could be toxic for plants and therefore, the wastewater was diluted with the nutrient medium in the ratio 1:5 for the next experiments. The initial concentrations used were EGDN – 190 mg l^{-1}, NG – 60 mg l^{-1}, and both DNG and MNG about 10 mg l^{-1}. The metabolism of nitroesters in wastewater by *in vitro* regenerants of reed (*P. australis*) was followed. During the 40 days of the study, the contents of all compounds except MNG decreased below the detection limit. Rapid degradation of NG to DNG caused a transient elevation of DNG in the first 5 days. Simultaneously, further degradation to MNG took place, resulting in the rapid accumulation of MNG after 10 days, in contrast to other metabolites (Fig. 5). The potential further degradation of MNG cannot be excluded, but it appears to be very slow. These results represent the first step in the practical application of phytoremediation to clean up the wastewaters polluted during nitroester production.

3.4. Degradation of EGDN

EGDN is the major component of the explosive dynamite. In the consequence, it is therefore, the major xenobiotic in contaminated wastewaters, too. The ability of two wetland plant species, rush and reed, to degrade this compound was compared. Three different initial concentrations were tested, the highest one being 1 g l^{-1}. This latter concentration caused welting and browning of the plants after 3-day incubation [35]. EGDN was taken up by the plants. The speed of depletion of the parent compounds from the medium was dependent on the initial EGDN concentration (Fig. 6). The toxic effect of EGDN on the degradation process was evident. In the case of rush, the plants died after 10 days. Then the accumulated xenobiotics were released back into the medium. During degradation, one metabolite was found, which was identified by mass spectrometry as ethylene glycol mononitrate (Fig. 7). The content of EGMN in the medium was higher in the case of reed for all treatments;

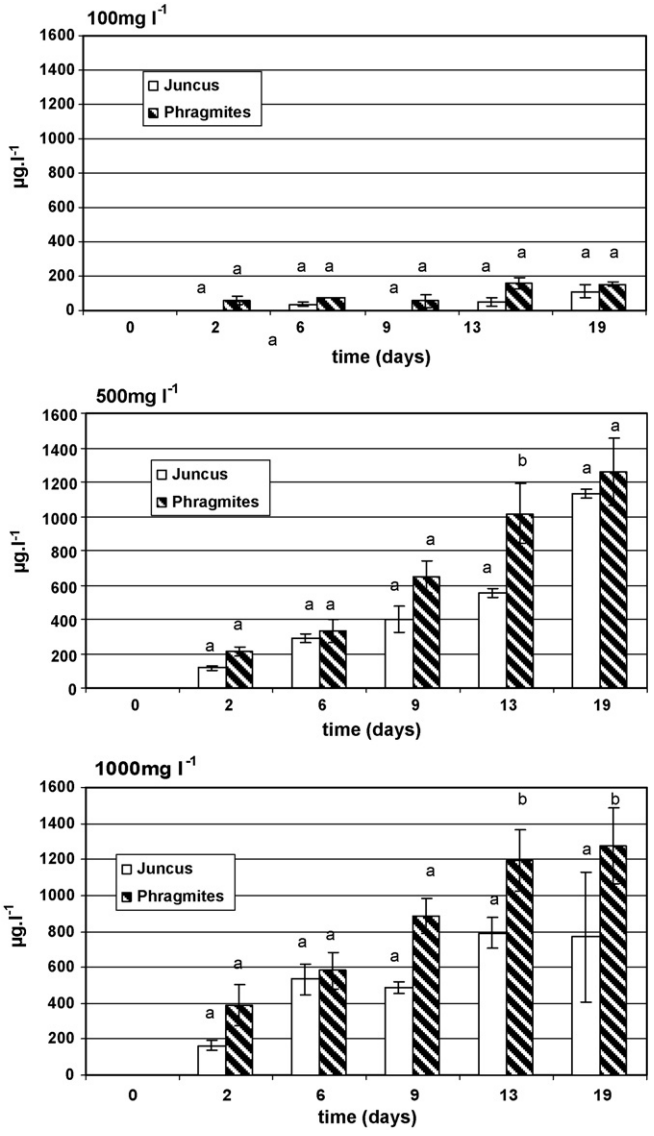

Fig. 8. Content of ethylene glycol mononitrate (EGMN) in the medium during degradation of ethylene glycol dinitrate (EGDN), the initial concentration of EGDN was 100, 500 and 1000 mg l^{-1}; comparison between reed (*Phragmites australis*) and rush (*Juncus inflexus*), the treatments with statistically significant differences (according to ANOVA, $p \geq 0.05$) are marked by different letters (a, b). The results are the means of three replicate measurements ± standard deviations.

the statistically significant differences were established by Tukey's posthoc analyses (Fig. 8).

4. Conclusions

The results of this study proved that water plants are suitable for phytoremediation of wastewaters that contain nitroesters, similar to the removal of contamination with TNT [22]. For nitroesters, this approach appears to be even more safe and efficient, because nitroesters (at least NG and PETN) can be fully degraded [38]. The glycerol produced can be used in plant metabolisms as a carbon source [39]. Thus, the phytoremediation process yields no potentially toxic degradation products. In addition, phytoremediation using water plants had been patented recently [40]. If we compare the phytoremediation efficiency of rush and reed, our results indicate the more rapid degradation by rush plants under low concentration of EGDN, but this plant species is more sensitive to high concentration of EGDN (Fig. 6).

Acknowledgements

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References

[1] T. Urbanski, Chemistry and Technology of Explosives, PWN-Polish Scientific Publishers and Pergamon Press, Warsaw and Oxford, 1965.
[2] S.J. Marshall, G.F. White, Complete denitration of nitroglycerin by bacteria isolated from a washwater soakaway, Appl. Environ. Microbiol. 67 (2001) 2622–2626.
[3] G.F. White, J.R. Snape, Microbial cleavage of nitrate esters: defusing the environment, J. Gen. Microbiol. 139 (9) (1993) 1947–1957.
[4] D.A. Kaplan, Biological degradation of explosives and chemical agents, Curr. Opin. Biotechnol. 3 (1992) 253–260.
[5] M. Meng, W.-Q. Sun, L.A. Geelhaar, G. Kumar, A.R. Patel, G.F. Payne, M.K. Speedie, J.R. Stacy, Denitration of glycerol trinitrate by resting cells and cell extracts of *Bacillus thuringiensis*/*cereus* and *Enterobacter agglomerans*, Appl. Environ. Microbiol. 61 (1995) 2548–2553.
[6] J.E. Walker, D.A. Kaplan, Biological degradation of explosives and chemical agents, Biodegradation 3 (1992) 369–385.
[7] G.F. White, J.R. Snape, S. Nicklin, Bacterial biodegradation of glycerol trinitrate, Int. Biodeterior. Biodegrad. 38 (1996) 77–82.
[8] C. Ducrocq, C. Servy, M. Lenfant, Bioconversion of glyceryl trinitrate into mononitrates by *Geotrichum candidum*, FEMS Microbiol. Lett. 65 (1989) 219–222.
[9] C. Ducrocq, C. Servy, M. Lenfant, Formation of glyceryl 2-mononitrate by regioselective bioconversion of glyceryl trinitrate: efficiency of the filamentous fungus *Phanerochaete chrysosporium*, Biotechnol. Appl. Biochem. 12 (1990) 325–330.
[10] D. Servent, C. Ducrocq, Y. Henry, A. Guissani, M. Lenfant, Nitroglycerin metabolism by *Phanerochaete chrysosporium*: evidence for nitric oxide and nitrite formation, Biochim. Biophys. Acta 1074 (1991) 320–325.
[11] R.M. Goldstein, L.M. Mallory, M. Alexander, Reasons for possible failure of inoculation to enhance biodegradation, Appl. Environ. Microbiol. 50 (4) (1985) 977–983.
[12] E.L. Rylott, N.C. Bruce, Plant disarm soil: engineering plants for the phytoremediation of explosives, Trends Biotechnol. 27 (2) (2008) 73–81.
[13] J.-f. Peng, Y.-h. Song, P. Yuan, X.-y. Cui, G.-l. Qui, The remediation of heavy metals contaminated sediment, J. Hazard. Mater. 161 (2–3) (2009) 633–640.
[14] T.G. Reichenauer, J.J. Germida, Phytoremediation of organic contaminants in soil and groundwater, ChemSusChem. 1 (8–9) (2008) 708–717.
[15] P. Soudek, Š. Petrová, D. Benešová, J. Kotyza, T. Vaněk, Phytoremediation and possibilities of increasing its effectivity, Chem. Listy 102 (5) (2008) 346–352.
[16] T. Vaněk, J.P. Schwitzguébel, Plant biotechnology for the removal of organic pollutants and toxic metals from wastewaters and contaminated sites, in: The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions, Kluwer Academic Publishers, Dordrecht, 2003, ISBN 1-4020-1141-5, pp. 285–293.
[17] J.P. Schwitzguébel, T. Vaněk, Some fundamental advances in phytoremediation for xenobiotic chemicals, in: Steven C. McCutcheon, Jerald L. Schnoo (Eds.), Phytoremediation: Transformation and Control of Contaminants, John Wiley and Sons Inc., 2003.
[18] B. van Aken, Transgenic plants for enhanced phytoremediation of toxic explosives, Curr. Opin. Biotechnol. 20 (2009) 231–236.
[19] P.C. Abhilash, S. Jamil, N. Singh, Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics, Biotechnol. Adv. 27 (2009) 474–488.
[20] A. Nepovím, M. Hubálek, R. Podlipná, S. Zeman, T. Vaněk, In vitro degradation of 2,4,6-trinitrotoluene by plant tissue culture of *Solanum aviculare* and *Rheum palmatum*, Eng. Life Sci. 24 (2004) 46–49.
[21] A. Nepovím, R. Podlipná, P. Soudek, P. Schröder, T. Vaněk, Effects of heavy metals and nitroaromatic compounds on horseradish glutathione S-transferase and peroxidase, Chemosphere 57 (2004) 1007–1015.
[22] A. Nepovim, A. Hebner, P. Soudek, A. Gerth, H. Thomas, S. Smrcek, T. Vanek, Phytoremediation of TNT by selected helophytes, Chemosphere 60 (2005) 1454–1461.
[23] T. Vanek, A. Nepovim, R. Podlipna, A. Hebner, Z. Vavrikova, A. Gerth, H. Thomas, S. Smrcek, Phytoremediation of explosives in toxic wastes, NATO Sci. Ser. Ser. IV Ear. 69 (2006) 455–465.
[24] K.C. Makris, K.M. Shakya, R. Datta, D. Sakar, D. Pachanoor, High uptake of 2,4,6-trinitrotoluene by vetiver grass – potential for phytoremediation? Environ. Pollut. 146 (2007) 1–4.
[25] M. Vila, S. Lober-Pascal, F. Laurent, Fate of RDX and TNT in agronomic plants, Environ. Pollut. 148 (2007) 148–154.
[26] J.M. Yoon, D.J. Oliver, J.V. Shanks, Shanks, phytotoxicity and phytoremediation of 2,6-dinitrotoluene using model plant, *Arabidopsis thaliana*, Chemosphere 68 (2007) 1050–1057.
[27] G. Adamia, M. Ghoghoberidze, D. Graves, G. Khatisashvili, G. Kvesitadze, E. Lomidze, D. Ugrekhelidze, G. Zaalishvili, Absorption, distribution, and transformation of TNT in higher plants, Ecotox. Environ. Saf. 64 (2006) 136–145.
[28] R. Podlipná, A. Nepovím, M. Vágner, T. Vaněk, A novel oxidoreductase participating TNT detoxification in plant, Biol. Plantarum 51 (2007) 367–371.

[29] F. Gandia-Herrero, A. Lorenz, t. Larson, I.A. Graham, D.J. Bowles, E.L. Rylott, N.C. Bruce, Detoxication of the explosive 2,4,6-trinitrotoluene in Arabidopsis: discovery of bifunctional *O*- and *C*-glukosyltransferases, Plant J. 56 (2008) 963–974.
[30] K.E. Gerhardt, X.-D. Huang, B.R. Glick, B.M. Greenberg, Phytoremediation and rhizodegradation of organic soil contaminant: Potential and challenges, Plant Sci. 176 (2009) 20–30.
[31] R.S. Sangwan, R. Gorenflot, *In vitro* culture of *Phragmites* tissue. Callus formation, organ differentiation and cell suspension culture, Z. Pflanzenphysiol. 75 (1975) 256–269.
[32] J. Čihalík, H. Čulíková, P. Dusílek, P. Finfrlová, V. Konrád, E. Podlešáková, M. Sedláček, L. Skálová, K. Velek, Metodický návod pro hodnocení rizikovosti tuhého průmyslového odpadu, Acta Hygien. Epidem. Microbiol. 55 (1989) 38–41.
[33] A. Goel, G. Kumar, G.F. Payne, S.K. Dube, Plant cell biodegradation of a xenobiotic nitrate ester, nitroglycerin, Nat. Biotechnol. 15 (1997) 174–177.
[34] C.E. French, S.J. Rosser, G.J. Davies, S. Nicklin, N.C. Bruce, Biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase, Nat. Biotechnol. 17 (1999) 491–494.
[35] R. Podlipna, Z. Fialova, T. Vanek, Toxic effect of nitroesters on plant tissue cultures, Plant Cell Tiss. Org. 94 (3) (2008) 305–311.
[36] G.F. White, J.S. Snape, S. Nicklin, Biodegradation of glycerol trinitrate and pentaerythritol tetranitrate by *Agrobacterium radiobacter*, Appl. Environ. Microbiol. 62 (1996) 637–642.
[37] J.R. Snape, N.A. Walkley, A.P. Morby, C. Nicklin, G.F. White, Purification, properties and sequence of glycerol trinitrate reductase from *Agrobacterium radiobacter*, J. Bacteriol. 179 (24) (1997) 7796–7802.
[38] T. Vaněk, A. Nepovím, R. Podlipná, S. Zeman, M. Vágner, Phytoremediation of selected explosives, Water Air Soil Poll.: Foc. 3 (2003) 259–267.
[39] E. Maestri, F.M. Restivo, M. Gulli, F. Tassi, Glutamate-dehydrogenase regulation in callus-cultures of *Nicotiana plumbaginifolia* – effect of glucose feeding and carbon source starvation on the isoenzymatic pattern, Plant Cell Environ. 14 (1991) 613–618.
[40] A. Nepovím, T. Vaněk, Z. Vavříková, R. Podlipná, Decontamination of explosive containing waste-waters using plants. Czech. Pat. Apll. CZ 296791 (2007).