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Combined phytoremediation of metal-working fluids with maize plants inoculated with different microorganisms and toxicity assessment of the phytoremediated waste

Jose A. Lucas García [a,*,1], Lucia Grijalbo [a,1], Beatriz Ramos [a,1], Francisca Fernández-Piñas [b,1], Ismael Rodea-Palomares [b,1], Francisco J. Gutierrez-Mañero [a,1]

[a] Departamento de Biología, Facultad de Farmacia, Universidad San Pablo CEU, Urb. Monteprincipe, Boadilla del Monte, 28668 Madrid, Spain
[b] Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, 2 Darwin Street, Cantoblanco, 28049 Madrid, Spain

HIGHLIGHTS

- ▶ Successful metal-working fluid (MWF) phytoremediation combining maize and microorganisms is proposed.
- ▶ Phytoremediation of MWF was achieved by rhizofiltration rather than by inoculated microorganisms.
- ▶ Toxicity tests confirmed effectiveness of phytoremediation.
- ▶ Photosynthetic parameters revealed a decrease in phytoremediated MWF toxicity.
- ▶ Cyanobacterial bioreporter revealed that some bacteria-plant phytoremediated MWF were less toxic than others.

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ABSTRACT

The aim of this study was to validate the effectiveness of a phytoremediation procedure for metal-working fluids (MWFs) with maize plants growing in hydroponic culture in which the roots grow on esparto fibre and further improve bioremediation potential of the system with root beneficial bacteria, seeking a synergistic effect of the plant–microorganism combination. Chemical oxygen demand (COD), pH, total and type of hydrocarbons measured after phytoremediation indicated that the process with maize plants was successful, as demonstrated by the significant decrease in the parameters measured. This effect was mainly due to the plant although inoculated microorganisms had a relevant effect on the type of remaining hydrocarbons. The success of the phytoremediation process was further confirmed by two toxicity tests, one of them based on chlorophyll fluorescence measurements on maize plants and another one based on cyanobacteria, using a bioluminescent toxicity bioassay; both tests demonstrated that the phytoremediated waste was significantly less toxic than the initial non-phytoremediated MWFs.

1. Introduction

Metal working fluids (MWFs) are employed in different manufacturing facilities (including automotive engine, transmission and stamping plants); in machining processes such as turning, grinding, boring, tapping, threading, gear shaping, reaming, milling, broaching, drilling, hobbing, and band and hacksawing. They serve for cooling of work pieces and tools, lubricating the process, and flushing away chips, fines, swarf, and residues (Moscoso et al., 2012). Annual Worldwide production reaches 22.4×10^9 L (Great Britain, 2000). Environmental concerns and health safety have favoured more tightening regulations of MWF disposal. The European Union Water Directives 2000/60/EC and 2000/76/EC have provided a framework that lists and identifies actions to be taken in order to minimize the impact on the environment.

There is a diversity of methods of MWF wastewater treatment, which can be classified as chemical, physical and biological (Kim et al., 1994; Portela et al., 2001; Ji et al., 2004; Muszyński et al., 2007; Kobya et al., 2008; Anderson et al., 2009). However, one difficulty faced in the treatment of a MWF containing wastewater is that the exact composition of the oils cannot be determined

* Corresponding author. Tel.: +34 913724733; fax: +34 913510496.
E-mail address: alucgar@ceu.es (J.A. Lucas García).
[1] These authors contributed equally to this work.

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because substances of 85–95% purity are used (Rabenstein et al., 2009). Furthermore, one single MWF can contain up to 60 different components, including emulsifiers as fatty alcohols or amino alcohols, corrosion inhibitors (fatty acids, amines and borates), extreme pressure additives, foaming inhibitors and biocides, and the percentages of each compound are usually trade secrets of the MWF manufacturers.

Phytoremediation is an emerging technology that utilizes plants and then the associated rhizosphere microorganisms to remove, transform, or contain toxic chemicals located in soils, sediments, ground water, surface water, and even the atmosphere. Currently, phytoremediation is used for treating many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides, and landfill leachates (Susarla et al., 2002; Wenzel, 2009).

MWF handling in the area where this work was made (city of Madrid, Spain) is regulated by the regional law10/1993 on industrial waste discharges into urban sanitary sewer system. This law details the features that MWF should have to be released into the environment. Chemical oxygen demand (COD) and pH values are normally, of all parameters regulated by the law, the ones which are above the allowed values, and therefore must be reduced prior to release to the environment. However, the decrease in COD and the pH does not necessarily imply that the MWF does not have biotoxic effects. The law does not regulate this aspect and it is very important to know if the waste potentially has toxic effects on the biota. There are some works in literature which show that after a bioremediation process, the produced residue is more toxic than the residue before bioremediation (Zuzana et al., 2008; Tang et al., 2011).

Although pollutants and their concentration in the environment can be characterized rapidly by chemical methods, the overall environmental quality cannot be reflected by only chemical analysis. Concerning the ecotoxicity of pollutants, biological methods are more suitable in determining possible environmental hazard of pollutants on the ecology and environment. However, efforts to conduct site specific risk assessments using ecotoxicity tests and correlation to contaminant concentration is limited and relatively unsuccessful for hydrocarbon contamination (Dorn and Salanitro, 2000). Toxicity assessment of the contaminated environments with bioassays could provide meaningful information regarding a characterization procedure in ecological risk assessment (Al-Mutairi et al., 2008). The ecological toxicity diagnosis includes different methods by using microorganisms, animals and plants as testing approaches. Among these, two toxicity bioassays one for plants based on the photosynthetic efficiency of plants by use of pulse amplitude modulated (PAM) chlorophyll fluorescence and another one based on bioluminescence inhibition of a self-luminescent cyanobacterial bioreporter have proved very useful in environmental analysis of aquatic environments (Rodea-Palomares et al., 2009, 2010; Rosal et al., 2010a, 2010b).

Based on the above, a combined phytoremediation process with maize plants inoculated with different microorganisms was designed to decrease COD and pH values of the MWFs to regional regulatory levels. The aim of this study was to validate the effectiveness of a phytoremediation procedure for metal-working fluids (MWFs) with maize plants growing in hydroponic culture in which the roots grow on esparto fibre and further improve bioremediation potential of the system with root beneficial bacteria, seeking a synergistic effect of the plant–microorganism combination. Chemical oxygen demand (COD), pH, total and type of hydrocarbons will be measured as indicators at the end of the phytoremediation process, and two toxicity bioassays will be undertaken to check the toxicity of the MWFs: the photosynthetic efficiency of maize and bioluminescence inhibition of a self-luminescent cyanobacterial bioreporter.

2. Materials and methods

2.1. Metal-working fluid (MWF)

The MWF, used as the model effluent in this study, was an operationally exhausted synthetic fluid (Houghton Iberica S.A., Spain), used as a coolant and lubricant in large-scale continuous metal working processes, to machine tungsten carbide and steel. In brief, the main chemical constituents include a formaldehyde-based biocide; alkyl benzotriazole (metal passivator); C16/C18-fatty alcohol polyglycol ether (corrosion inhibitors); isopropanolamine (lubrication agents), and 3-iodo-2-propynylbutylcarbamate. Fresh MWF is typically supplied as a concentrate, which is diluted with water to form a 2% v/v working fluid prior to use in machining operations. John Deere Company provided the MWF used in this study from its plant of Madrid. These fluids were previously remediated in the company using physicochemical procedures. However, chemical oxygen demand (COD) and pH values of these fluids were over the regulatory values, according to the regional law 10/1993 on industrial waste discharges into urban sanitary sewer system, being allowed to be released into the environment.

2.2. COD and pH determinations

COD was determined by colorimetric analysis using a Merck Photometer SQ 118 with COD cuvette test kits (range 500–10,000 mg L^{-1}). MWF samples were prefiltered through 0.2 mm pore-size filters (Millipore, UK) and analyses performed according to the manufacturer's instructions.

pH was measured directly on MWFs with a CRISON micro pH 2100 pH meter.

2.3. Quantitative and qualitative hydrocarbons analysis

Both quantitative and qualitative analyses were carried out by the SIDI (Interdepartmental Technical Research Facility at the Universidad Autónoma de Madrid, Madrid, Spain). For quantitative analysis, hydrocarbons from 100 mL of MWF were extracted with 50 mL of extracting solution (50 mL water plus 7.5 μL chlorhydric acid plus 2.5 mL petroleum ether). After 15 min shaking, organic phase was dried with sodium sulphate anhydrous. Residue was dissolved in 1 mL of extracting solution. Analysis was carried out by gas chromatography using a Varian 3900 provided with a flame ionization detector (FID), and a column Factor IV (30 m × 0.25 mm × 0.25 μm).

For qualitative analysis, hydrocarbons from 100 mL of MWF were extracted with 50 mL dichloromethane. After 15 min shaking, organic phase was dried with sodium sulphate anhydrous. Residue was dissolved in 1 mL dichloromethane. Analysis was carried out by gas chromatography–mass spectrometry (GC–MS) using a Varian 3800 provided with a triple quadrupole, and a column Factor IV (30 m × 0.25 mm × 0.25 μm). Mass spectra were acquired through spectrum from 50 to 650 atomic mass unit (amu).

2.4. Design of the phytoremediation experiments

Five experiments of phytoremediation were carried out; in all experiments, maize plants were used and in each of the experiments, a different microorganism was inoculated to the growing plants. In all cases, seeds of maize were surface-sterilized with