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ACTION OF PLANT ROOT EXUDATES IN BIOREMEDIATIONS: A REVIEW

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Abstract

DUNDEK, P., HOLÍK, L., HROMÁDKO, L., ROHLÍK, T., VRANOVÁ, V., REJŠEK, K., FORMÁNEK, P.: *Action of plant root exudates in bioremediations: a review.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 1, pp. 303–308

This work presents a summary of literature dealing with the use of plant root exudates in bioremediations. Bioremediation using plants (phytoremediation or rhizoremediation) and associate rhizosphere to decontaminate polluted soil is a method based on the catabolic potential of root-associated microorganisms, which are supported by the organic substrates released from roots. These substrates are called "root exudates". Root exudates support metabolism of pollutants-decomposing microorganisms in the rhizosphere, and affect sorption / desorption of pollutants. Awareness of exudation rates is necessary for testing soil decontamination. Commonly, water-soluble root exudates of different plants are studied for their qualitative composition which should be related to total carbon of exuded water-soluble compounds. This paper presents the determined rate of plant root exudation and the amount of root exudates carbon used to form artificial rhizosphere.

root exudate, plant, pollutant, soil, metabolism, rhizosphere

Microbial metabolism in the rhizoplane (soil-free root surface) and the rhizosphere (zone in close proximity to the roots) is probably not limited by available carbon, and this limitation appears only when soil is separated from root (Helal and Sauerbeck, 1986; Cheng *et al.*, 1996). Compounds released from roots into rhizosphere are selectively utilized by soil microorganisms. For example, Shepherd and Davies (1994) reported the highest ratio between microbial consumption and release from roots of rape plants for some of studied amino acids: Asparagine (ASN), Arginine (ARG), Glutamic acid (GLU), Glutamine (GLN), Lysine (LYS). Except fast microbial utilization and CO_2 production, water-soluble exudates are fixed into more resistant microbial metabolites or incorporation into less soluble soil organic matter will occur as well as introduction into soil clay matrix which physically protects them from microbial degradation (Hütsch *et al.*, 2002).

Degradation of organic pollutants – the role of plant root exudates

Bioremediation using plants (phytoremediation or rhizoremediation) and associate rhizosphere to decontaminate polluted soil is a method based on the catabolic potential of root-associated microorganisms, which are supported by the organic substrates in root exudates and by a favorable microenvironment in the rhizosphere. Degradation of pollutants in soil is affected by numerous interacting factors such as moisture, pH, temperature, O_2 levels, bioavailability (sorption, bacterial dispersion, concentration and solubility), and bacterial nutrient requirements (Providenti *et al.*, 1993). According to the authors, temperature and moisture are often inversely related to sorption of contaminants in soil. The enhanced microbial activity in the rhizosphere was linked to decontamination of soils polluted by e.g. polycyclic aromatic hydrocarbons (PAHs) and 2,4,5-trichlorophenoxyacetic acid (Boyle and Shann, 1998; Banks *et al.*, 1999; Joner *et al.*, 2004). The impact of root exudates on degradation of organic pollutant is not only in supporting growth of degrading microorganisms, but it also seems that changes in structure and function of microbial community (co-metabolism) in the rhizosphere may be of significance (Alexander, 1994; Yoshitomi and Shann, 2001). Root-derived C in exudates can be an excellent primary substrate for co-metabolic degradation of pollutants (Banks *et al.*, 1999; Cunningham *et al.*, 1997; Cutright and Lee, 1994; Wilson and Jones, 1993). As reported in review article by Mohan *et al.* (2006), plants used for phytoremediation enhance degradation of organic contaminants in soil by a) stimulation of microbial activity, b) increasing the number of sites in the organic matrix available for PAH adsorption, c) with the contribution of root matter to the soil organic matter, d) exuding compounds that increase the bioavailability of the contaminant and e) enhance desorption of pollutants from the soil matrix. Microbial biofilm is developed in the rhizoplane and roots alter soil environment (e.g. reduction/oxidation reactions, moisture and aeration) to favour degradation of pollutants (Pearce *et al.*, 1995; Anderson *et al.*, 1993). As reported in the review by Mohan *et al.* (2006), bioremediation of PAH is influenced by many factors, including pH, temperature, microflora, electron acceptors, microenvironment, nutrients, co-substrate, soil properties and contaminants characteristics. Water-soluble root exudates are probably mobile and dispersed in the soil but are concentrated mostly in the rhizosphere (Yoshitomi and Shann, 2001). The combustion of root exudates of different plants was studied using different approaches (see below) to measure the efficiency of degradation of organic pollutants.

Root exudates of maize can support growth of soil microorganisms in liquid cultures better than a simple carbon source such as glucose. On the other hand, root exudates contain some more recalcitrant water-soluble compounds which is proved by their lower utilization by soil microorganisms when compared to glucose (Yoshitomi and Shann, 2001). The authors proved stimulation of microbial mineralization of pyrene by application of maize exudates onto unplanted soil, and this stimulation was of the same degree as observed in an actual rhizosphere soil. Chaineau *et al.* (2000) looked at the biodegradation of petroleum hydrocarbons by corn grown in hydroponic culture. The authors speculated that the increase in hydrocarbon degradation was due to root exudates changing hydrocarbon bioavailability in solution or stimulation degradative microbial populations. Mineralization of the organophosphate parathion was stimulated in soil by the addition of bush bean root exudates, but not to the extent observed in the planted system (Hsu and Bartha, 1979). Root exudates from bush bean solution culture were found to stimulate mineralization more than synthetic exudates. Burken and Schnoor (1996) were successful in enhancing atrazine mineralization with the addition of root exudates from poplar cuttings, with the highest stimulation resulting from the addition of ground root tissue. From C_4 plants, panic grass (*Panicum virgatum*) is a suitable candidate for use in multi-species tree-shrub-grass riparian buffer where bioremediation of herbicides and nitrates is desired. According to Lin *et al.* (2004) sensitivity of this plant to herbicides differs according to the type of preparative, and is influenced by shade. Wenger *et al.* (2005) studied the effect of maize root exudates on degradation of atrazine and its toxic breakdown products (desethylatrazine and desisopropylatrazine) in hydroponic experiments. The authors reported that the disappearance of up to 30% atrazine and desethylatrazine, and the removal of up to 10% of desisopropylatrazine from solution could be explained by formation of hydroxylated metabolites in the solution. Joner *et al.* (2004) planted lucerne (*Medicago sativa* L.) in PAHs contaminated soil which was brought to 75% water-holding capacity using deionized water or nutrient solution. The most efficient decontamination was found in the case of four- and three-ring PHAs while five- and six-ring PHAs concentrations decreased to 62–87% of their control (Joner *et al.*, 2004). The advantage of fertilized plants is better growth and it is use in the experiment was advantageous due to increased volume of rhizosphere soil resulting in an increased total bacterial, fungal and PAHs degrader numbers and most likely increased biodegradation (White *et al.*, 2006). Rentz *et al.* (2005) found that *Sphingomonas yanoikuyae* JAR02 removed 15–20% of benzo[a]pyrene over 24 h during growth on root exudates. Kamath *et al.* (2004) or Rentz *et al.* (2004) reported repression of PAH degradation for bacteria exposed to root extracts, exudates, and root derived substrates. Phillips *et al.* (2000) found that temperature, moisture and nutrients amendment (phosphorus) have affected degradation of petroleum hydrocarbons and PAHs in soil. Corgié *et al.* (2004) demonstrated that biodegradation rate of phenanthrene showed a strong gradient with higher values in the rhizosphere and with no degradation far from the roots, and concluded that phenanthrene and root exudates induced modifications of bacterial communities and spatially modified the activity of degrading bacteria. According to Reilley *et al.* (1996) vegetated soil had significantly lower concentration of PAHs then unvegetated soil, ranging from 30 to 44% more degradation in the vegetated soil. Miya and Firestone (2001) found that biodegradation rate of phenanthrene in soil amended with root exudates of slender oat (*Avena barbata* Pott ex Link) was lower than in soil amended with root debris *plus* root exudates in the rhizosphere. Enhanced rates of phenanthrene biodegradation were observed in rhizosphere soil planted with slender oat when compared unplanted soil (Miya and Firestone, 2000). Plant roots provide degrader organisms with a variety of compounds including nutrients that help to select and support microbes able to compete in this environment. Banks *et al.* (1999) found that residual benzo[a]pyrene was lower in soil planted with *Festuca arundinacea* Schreber when compared with unplanted soil. Binet *et al.* (2000) reported higher degradation of 3-6-ring PAHs in the rhizosphere of ryegrass (*Lolium perenne* L.) when compared with non-rhizospheric soil; and lower dissipation of PAHs after soil ageing. The increased dissipation of PAHs in the rhizosphere may also be due to a decreased extractability of the PAHs with the formation of bound residues. Walton *et al.* (1994) suggested that rhizosphere could stabilize pollutants by polymerization reactions such as humification. Kästner *et al.* (1999) reported formation of bound residues during microbial degradation of anthracene in soil. Joner *et al.* (2002) studied C, N and P limitations to dissipation of PAHs in model rhizosphere where every day the soil was percolated with artificial root exudates (ARE; 1 mg $C.mL^{-1}$) containing 50 mM glucose, fructose and saccharose, 25 mM succinic and malic acids, 12.5 mM arginine, serine, cysteine; N and / or P in form of 1 mM NH_4NO_3 and/or NaH_2PO_4; or double or triple combinations of these. The authors found the highest dissipation of 3 and 4 ring PAHs in soil receiving ARE+N, followed by treatment N+P and ARE+N+P. Joner *et al.* (2002) found treatment ARE+N+P to be the best for dissipation of 5-ring PAHs. Root exudates released into soil are decomposed by microorganisms, stimulate decomposition of the native soil organic matter, and some of their components are incorporated into more stable fraction of soil (Vančura, 1988). Haby and Crowley (1996) showed that degradation of 3-chlorobenzoate was increased by addition of a synthetic root exudates made up of glucose, mannitol, benzoate, or starch. Ouvrard *et al.* (2006) tested the effect of model organic compounds, i.e. malic acid, malonic acid and ethylenediaminetetraacetic acid (EDTA) representing root exudates, on phenanthrene sorption on a reference non polluted agricultural soil material. The authors found increased sorption of phenanthrene due to presence of any of the studied organic compounds. This effect of organic acids can be explained by an adsorption of the organic acids on the soil itself and, as result, creation of a new range of adsorption sites. Except for this, Ouvrard *et al.* (2006) found increased sorption of phenanthrene with increasing concentration of malic acid. White *et al.* (2003) measured the highest abiotic desorption of weathered *p,p'*- DDE (by 19–80%) in connection with an application of different organic acids (succinic, tartaric, malic, malonic, oxalic, citric, EDTA) at 0.05 M acid concentration; in case of application of these acids at concentration of 0.001 M desorption of *p, p'*- DDE was not increased. According to Yang *et al.* (2001) citric acid may increase the availability of persistent organic pollutants (POPs) including PAHs and *p, p'*-DDE (White and Kottler, 2002). According to Yang *et al.* (2001) organic acids chelate inorganic ions in soil which results in the release of previously bound humic acids and a subsequent increase in the availability of previously sorbed hydrocarbons. It was observed that after the application of organic acid (c = 0.001mol), the release of ions such as Al, Fe and Mn or P increased at least six times. Nasser and Mawad (1975) found inhibition of bacterial transformation of ammonium salts into nitrites and nitrates when synthetic exudates were applied into soil.

Root exudation rate – the use of such knowledge

In experiments where unplanted soil is treated by natural or syntetic root exudates, or where microorganisms extracted from soil are treated by natural or syntetic root exudates, the rate of addition, which is commonly not equivalent to natural rates of plant release into rhizosphere, is important. According to Griffiths *et al.* (1999) substrate loading has a dramatic effect on community structure, and changes in microbial community can be the reason of increased degradation of organic pollutants (Yoshitomi and Shann, 2001). Yoshitomi and Shann (2001) collected aseptic maize root exudates and found production of approximately 400–600 µg $C.g^{-1}$ dry root matter over a 24 h period, which falls within the range reported by other authors (Rovira, 1969; Matsumoto *et al.*, 1979; Kraffczyk *et al.*, 1984). Trofymow *et al.* (1987) estimated carbon input into rhizosphere to be 100 µg $C.g^{-1}$ soil over a 24 h period. Griffiths *et al.* (1999) argues that values of 50 µg $C.g^{-1}$ soil over a 24 h period are the best reflection of rhizodeposition rate. A study by Cheng *et al.* (1996) measured values up to 1500 µg of water-soluble $carbon.g^{-1}$ in rhizoplane of maize, and approx. ten times less in the rhizosphere soil under field maize cultivation. Miya and Firestone (2001) amended soil with phenanthrene by root exudates in dose 0.54 mg $C.kg^{-1}$ and 1.7 mg $N.kg^{-1}$ soil every other day i.e. 4.9 mg $C.kg^{-1}$ and 15.2 mg $N.kg^{-1}$ soil over the course of the 20-d experiment. Cheng *et al.* (1996) found rhizosphere: bulk soil ratio of water-soluble carbon in the field maize experiment to be 2.2. Inputs of exudates from plant roots are subjected to daily fluctuations that can occur during plant development. Yoshitomi and Shann (2001) applied 10–20 µg C of root exudates per well. Macrovolume analysis was performed with concentration of root exudates of 0.1 $mg.mL^{-1}$ of cultivation medium. According to Traoré *et al.* (2000), concentration of soluble root exudates in soil is estimated to be < 2 000 µg $C.g^{-1}$. Trofymow *et al.* (1987) estimated that 100 $µg.g^{-1}$ of soil was deposited by oat roots in 24 h near the growing tip. Bremer and Kuikman (1994) suggested that carbon concentrations may increase to 750 µg $C.g^{-1}$ soil in the immediate proximity of root. Yoshitomi and Shann (2001) flushed aseptic root exudates onto unplanted soil in the dose of about 15 µg $C.g^{-1}$ soil over 24 h period, and this flushing was performed every 2 days. Joner *et al.* (2002) percolated daily soil with artificial root exudates in the dose of 100 µg $C.g^{-1}$. Rentz *et al.* (2005) applied root exudates of *Populus* sp. (5.7 mg $C.L^{-1}$), oat (7.6 mg $C.L^{-1}$) and *Morus* sp. (43.1 mg $C.L^{-1}$) and found that growth of *Sphingomonas yanoikuyae* JAR02 on these substrates as a sole carbon and energy source was lower that on succinate. In the experiment, growth of *S. yanoikuyae* JAR02 was combined with degradation of benzo[a]pyrene and *Morus* sp. root exudates which were used in dose

60.1 mg C.L[−1]. Malcová and Gryndler (2003/2004) used 10 mg of lyophilised root exudates per 50 m[3] of media to determine the effect of root exudates on toxicity of heavy metals to arbuscural mycorrhizal fungus (Malcová *et al.*, 2002).

SUMMARY

Plant root exudates are intensively studied for various purposes. One of these purposes is to determine their suitability for decontamination of polluted soils. Various plants have different abilities to release water-soluble compounds with different exudation rate from their roots. Root exudation is of importance in bioremediation using plants (phytoremediation or rhizoremediation) and associate rhizosphere to decontaminate polluted soil is a method based on the catabolic potential of root-associated microorganisms, which are supported by the organic substrates in root excretions, by a favorable microenvironment in the rhizosphere, and by an action of root exudates on bioavailability (sorption / desorption) of pollutants. Knowledge on root exudation of different plants and on qualitative composition of root exudates may be used to synthetically prepare these compounds to be applied to soil to create artificial rhizosphere. Knowledge on root exudation rate is necessary for knowing the dose of root exudates to be artificially supplied into soil for experimental purposes including testing degradation of pollutants. This knowledge is summarized in this review.

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