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Vetiver plantlets in aerated system degrade phenol in illegally dumped industrial wastewater by phytochemical and rhizomicrobial degradation

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Abstract This research evaluated the feasibility of using vetiver plantlets (*Vetiveria zizanioides* (L.) Nash) on a floating platform with aeration to degrade phenol (500 mg/L) in illegally dumped industrial wastewater (IDIWW). The IDIWW sample was from the most infamous illegal dumping site at Nong Nae subdistrict, Phanom Sarakham district, Chachoengsao province, Thailand. Laboratory results suggested that phenol degradation by vetiver involves two phases: Phase I, phytopolymerization and phyto-oxidation assisted by root-produced peroxide (H_2O_2) and peroxidase (POD), followed by phase II, a combination of phase I with enhanced rhizomicrobial degradation. The first 360–400 h of phenol degradation were dominated by phytopolymerization and phyto-oxidation yielding particulate polyphenols (PPP) or particulate organic matter (POM) as by-products, while phenol decreased to around 145 mg/L. In Phase II, synergistically, rhizomicrobial growth was ~100-folds greater on the roots of the vetiver plantlets than in the IDIWW and participated in the microbial degradation of phenol at this lower phenol concentration, increasing the phenol degradation rate by more than three folds. This combination of phytochemical and rhizomicrobiological processes eliminated phenol in IDIWW in less than 766 h (32 days), while without the vetiver plantlets, phenol degradation by aerated microbial degradation alone may require 235 days. To our knowledge, this is the first that systematically reveals the complete phenol degradation mechanism by vetiver plantlets in real aerated wastewater.

Keywords Phenol · Root-produced peroxide and peroxidase · Rhizomicrobial degradation · Phytoremediation · Illegal dumping · Wastewater

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Introduction

Illegal dumping of industrial waste and wastewater is a vexing environmental problem globally affecting environmental and public health (Ichinose and Yamamoto 2011; Massari and Monzini 2004; Triassi et al. 2015). Similarly, over the last few years, illegal dumping of industrial waste and wastewater has become a major environmental problem in Thailand. More than 50 cases of illegal industrial waste dumping were found in remote areas close to residential zones throughout the country, but most (40 cases) are in the eastern part of Thailand (Fig. S1a in supporting information (SI)) (Bootkote 2013; Soonsuk 2013). Nevertheless, the most infamous illegal dumping case in Thailand is probably the dumping of phenol (C_6H_6O)-contaminated wastewater in a 15-rai pond (1 rai = 1600 m^2) (Fig. S1b in SI) in the Nong Nae subdistrict, Phanom Sarakham district, Chachoengsao province (see more details about Nong Nae incident in SI). As a result of the illegal dumping, shallow groundwater was contaminated with as much as 225 μg/L of phenol, while the acceptable maximum contamination level (MCL) of pentachlorophenol in drinking water is only 1 μg/L (USEPA 2010). Phenol is a toxic substance causing irritation and kidney inflammation, and disinfection of phenol-contaminated water using chlorine yields carcinogenic pentachlorophenols, which can also affect the human reproductive system (EPA 2002; Lee and Morris 1962, Veeramachaneni et al. 2001).

Undoubtedly, a sustainable remediation measure to treat phenol in illegally dumped industrial wastewater (IDIWW) is needed. Phytoremediation is a green technology, gaining more and more popularity these days (Bahraminia et al. 2016; Brandt et al. 2006; Cook and Hesterberg 2013; Danh et al. 2009; Gopalakrishnan et al. 2009a, b; Huang et al. 1997; Ondo Zue Abaga et al. 2014; Rentz et al. 2005; Roongtanakiat 2009; Roongtanakiat et al. 2007; Salt et al. 1995; Singh et al. 2006, 2008; Truong and Hart 2001; Truong and Baker 1998). In addition to its cost effectiveness (Danh et al. 2010) and non-environmental disruption, phytoremediation is a great remedial action candidate as its simplicity allows affected villagers to participate in the implementation of the technology.

Various kinds of plants and their hairy root cultures (HRC) that are capable of production of peroxidases (PODs) were reported to effectively detoxify phenols via enzyme-catalyzed oxidation and polymerization using either external hydrogen peroxide (H_2O_2) or inherent H_2O_2 as the oxidizing agent (Harvey et al. 2002). In fact, H_2O_2 and horseradish POD or soybean POD were known to treat phenols via polymerization and precipitation years before the phytotreatment of phenols (Klibanov et al. 1983; Kurnik et al. 2015; Tong et al. 1998; Wright and Nicell 1999). Nevertheless, the prohibitive cost of the enzyme makes it economically unfavorable. As a result, relatively low cost of phytoremediation makes this phytodegradation technique promising. For example, the POD extracts from *Daucus carota* and *Solanum aviculare* with the addition of H_2O_2 accelerate the transformation of phenol, 2-chlorophenol, and 2,6-dichlorophenol (De Araujo et al. 2002, 2004). Similarly, HRC of *Brassica napus* was able to remove 2,4-dichlorophenol (2,4-DCP) (100–1000 mg/L) from aqueous solutions in the presence of H_2O_2 (5 to 10 mM) as effectively as 97–98 % within 1 h. The HRC could be reused to remove 2,4-DCP for six consecutive cycles (Agostini et al. 2003). Singh et al. (2006) reported that even without the addition of H_2O_2, HRC of Indian mustard (*Brassica juncea* L. Czern and Coss), beet root (*Beta vulgaris* L.), white radish (*Raphanus sativus* L.), and neem (*Azadirachta indica* L. Juss) successfully degraded phenol via intercellular production of H_2O_2 and peroxidase, presumably due to the self-protection mechanism of the plants against xenobiotic stress (Singh et al. 2006). In addition, *B. juncea* showed the highest phenol removal (97 %) followed by *B. vulgaris* (70 %), *R. sativus* (54 %), and *A. indica* (51 %) (Singh et al. 2006). Some research groups used transgenic hairy root clones of plants, which overexpress the TPX1 gene to increase POD activity. Transgenic tomato (*Lycopersicon esculentum* Mill. cv. Pera) with the addition of 5 mM H_2O_2 removed 85 % of phenol in comparison to the removal of 70 % by wild type (Oller et al. 2005). Similarly, transgenic tobacco HRC with basic POD genes from tomato (TPX1 and TPX2) removed as much as 90 % of phenol in 1 h with the addition of 5 mM H_2O_2. Furthermore, whole plants were also used for phenol treatment. For example, common vetch (*Vicia sativa* L.) removed almost 100 % of phenol (100 mg/L) within 6 days by increasing the level of POD and ascorbate POD and maintaining superoxide dismutase activity, malondialdehyde, and H_2O_2 levels (Ibáñez et al. 2012). Double transgenic tobacco (*Nicotiana tabacum* cv. Wisconsin) with TPX1 and TPX2 tomato POD genes was reported to degrade as much as 98 % of 2,4-DCP (25 mg/L) in 72 h without the addition of H_2O_2 while the wild type could degrade 84 % of 2,4-DCP (Talano et al. 2012).

For several reasons, vetiver grass (*Vetiveria zizanioides* (L.) Nash), which was introduced to Thailand by his majesty King Bhumibol Adulyadej in 1991, is the plant of choice for phytoremediation of heavy metals and toxic organic contaminants, including phenol (Danh et al. 2009). Vetiver grass can tolerate harsh environments, various toxic metals, and relatively high soil salinity. Vetiver grass is known to yield high root biomass, which is favorable for phytostimulation, phytostabilization, and rhizofiltration. Within a year, the root system can be 3- to 4-m deep and may be as long as 7 m after 3 years, making it an ideal plant for phytoremediation (Danh et al. 2009, 2010; Truong and Hart 2001; Truong et al. 2007; Truong and Baker 1998).

Vetiver was also evaluated for its potential to treat phenol in Murashige and Skoog's liquid medium under aseptic conditions (Singh et al. 2008). Within 4 days, vetiver plantlets