Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2019.24430

159 (2019) 40–52
August

Rhizomicrobial-augmented mature vetiver root system rapidly degrades phenol in illegally dumped industrial wastewater

Pimpawat Teeratitayangkul[a,b], Chalita Phutthasimma[c], Siriwan Wichai[b,c], Tanapon Phenrat[a,b,*]

[a]*Research Unit for Integrated Natural Resources Remediation and Reclamation (IN3R) Department of Civil Engineering, Faculty of Engineering, Naresuan University, Phitsanulok, Thailand, emails: pomphenrat@gmail.com (T. Phenrat), pimpawatt@gmail.com (P. Teeratitayangkul)*
[b]*Center of Excellence for Sustainability of Health, Environment and Industry (SHEI), Faculty of Engineering, Naresuan University, Phitsanulok, Thailand, email: siriwanwichai@nu.ac.th (S. Wichai)*
[c]*Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand, email: chali.zimma@gmail.com (C. Phutthasimma)*

Received 20 October 2018; Accepted 18 May 2019

ABSTRACT

Illegal dumping of phenol-concentrated industrial wastewater in a residential area poses serious health risks to the community in Thailand. Thus, a low-cost and easily implementable treatment technique that the affected community can perform is greatly beneficial. Here, we evaluated the enhanced phenol-degradation kinetics using a rhizomicrobial-augmented mature vetiver root system on a floating platform (with and without aeration) in comparison to a previously published study of a young vetiver root system. The mature vetiver root was covered with a biofilm of phenol-degrading rhizomicrobes including bacteria (*Enterobacter* spp., *Pseudomonas* spp., *Rhodococcus* spp., and *Acinetobacter* spp.) and fungi (*Candida* spp., *Rhizopus* spp., *Aspergillus* spp., and *Fusarium* spp.). Phenol (500 mg L^{-1}) was degraded to 1 mg L^{-1} in 249 h using rhizomicrobial-augmented mature vetiver with aeration. Using young vetiver plantlets with aeration, this occurred in 675 h, and using rhizomicrobial-augmented mature vetiver without aeration, this occurred in 766 h. The findings suggest that, in addition to augmented rhizomicrobes, vetiver maturity and aeration substantially contribute to the enhancement. The vetiver maturity increased the root biomass, which releases more peroxidase for accelerating phenol transformation, and enhanced the superoxide dismutase activity, which decreases the side effects of phenol detoxification. Aeration enhanced the peroxidase and superoxide dismutase activity in the vetiver and augmented rhizomicrobes to accelerate the phenol polymerization to non-toxic by-products.

Keywords: Phenol degradation; Vetiver; Phytoremediation; Rhizomicrobial augmentation; Aeration; Illegally dumped wastewater

1. Introduction

Phenol is a raw material for manufacturing surfactants, explosives, paints, fertilizers, textiles, and rubber [1]. Phenol is a toxic substance causing irritation and kidney inflammation. Disinfection of phenol-contaminated water using chlorine yields carcinogenic pentachlorophenol, which is toxic to the human reproductive system [2]. Given the concern about the hazards of phenol to the environment and to public health, the maximum contaminant level (MCL) of phenol in industrial effluent is legally restricted to 1 mg L^{-1}.

* Corresponding author.

Presented at the 6th International Conference on Sustainable Solid Waste Management (NAXOS 2018), 13-16 June 2018, Naxos Island, Greece.

While numerous microorganisms, including bacteria and fungi, are capable of degrading phenol at low and moderate concentration ranges [3], at a high concentration (>100 mg L^{-1}), phenol is known to inhibit microbial activity due to its irritation [4]. This leads to the failure of conventional biological wastewater treatment to detoxify wastewater contaminated with an elevated phenol concentration [5,6]. Phenol-concentrated wastewater (maximum phenol concentration of 500 mg L^{-1}) was illegally dumped at Nong Nae village, Panomsarakam District, Chachoengsao Province, Thailand. According to the World Health Organization, while the MCL of pentachlorophenol in drinking water is only 1 μg L^{-1}, the dumping caused the contamination of shallow groundwater with as much as 225 μg L^{-1} of phenol [7].

For several reasons, vetiver grass (*Vetiveria zizanioides* (L.) Nash), which was introduced to Thailand by King Bhumibol Adulyadej in 1991, is the plant of choice for phytoremediation of heavy metals and toxic organic contaminants, including phenol [8]. Vetiver grass can tolerate harsh environments, various toxic metals [9], and relatively high soil salinity. Vetiver grass is known to yield a high amount of 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 [8,10,11]. The capability of vetiver plantlets to detoxify phenol in Murashige and Skoog's liquid medium under aseptic conditions was previously reported [12]. In addition, for the Nong Nae illegal dumping case, Phenrat et al. (2017) successfully applied phytoremediation using vetiver plantlets on a floating platform to treat illegally dumped wastewater contaminated with phenol in concentrations as high as 500 mg L^{-1}.

The phenol treatment mechanism in wastewater by young vetiver plantlets involves two phases [7]. In Phase I, the vetiver roots produce the inherent phytochemicals in the root exudates including the enzyme peroxidase (POD) and hydrogen peroxide (H_2O_2) as a catalyst and an oxidizing agent in the self-protection mechanism against xenobiotic stress [8,12,13]. This self-protection mechanism chemically transforms phenol to particulate polyphenols or particulate organic matter via phyto-polymerization and phyto-oxidation, which is why the phenol decreased to around 145 mg L^{-1} in the first 360–400 h [7]. This same phenomenon was observed for various kinds of plants. For example, common vetch (*Vicia sativa* L.) removed almost 100% of the phenol (100 mg L^{-1}) within 6 d by increasing the level of POD and ascorbate POD and maintaining superoxide dismutase activity and the malondialdehyde and H_2O_2 levels [14]. 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-dichlorophenol (25 mg L^{-1}) in 72 h without the addition of H_2O_2, while the wild type could degrade 84% of it [15]. Phase I was induced by oxidative stress [7,12]. During this time, the vetiver plantlets were severely deteriorated due to phenol toxicity and irritation. The weight of the vetiver plantlets decreased [7,12], and the same deterioration was observed on the leaves [7]. If the irritation becomes too severe, the vetiver plantlets may not survive [7].

In Phase II, rhizomicrobial growth on and inside the roots of the vetiver plantlets was ~10-fold greater than in the wastewater in the initial conditions [7,16]. The rhizomicrobes participated in the microbial degradation of phenol at this lower phenol concentration (145 mg L^{-1}), increasing the phenol-degradation rate by more than three-fold in Phase I [7]. The combination of phytochemical and rhizomicrobiological processes eliminated phenol in the wastewater in less than 766 h (32 d), while without the vetiver plantlets, phenol degradation by aerated microbial degradation alone may require 235 d [7].

The presence of rhizospheres substantially favors microbial vitality and phenol-degradation activity through a special relationship between the endophyte and host [8,17,18]. Microbes attached to plant roots will form a biofilm, increasing the biomass. Each biofilm reacts to the environment fundamentally differently from a planktonic cell of the same species because the complex microbial biofilm community has a primitive homeostasis, a primitive circulatory system, and metabolic cooperativity [19]. Besides providing physical attachment surfaces to microbes, plant roots also provide beneficial exudates, such as amino acids, simple sugars, complex carbohydrates, and oxygen [19–22]. All of these may cause rhizomicrobes to tolerate phenol and participate in phenol degradation more effectively [20,23–27].

In this study, new important insight into the role of vetiver maturity, rhizomicrobes, and aeration on rapid phenol degradation is revealed. We examined rhizomicrobial-augmented mature vetiver root systems as an enhanced phytoremediation technique for phenol treatment. This should theoretically accelerate phenol-degradation kinetics because the longer, denser, and healthier vetiver root system in this mature vetiver in comparison to the young vetiver plantlets previously studied [7] should tolerate phenol toxicity better and excrete phytochemical exudates that overcome the phenol toxicity more rapidly. Furthermore, we augmented indigenous phenol-degrading microbes onto the mature vetiver root to benefit from the synergy of the vetiver root and rhizomicrobes in phenol degradation. The unexpected role of aeration was also revealed by comparing the mature vetiver performance with and without aeration. Although, plant-microbe phytoremediation systems have been studied for various kinds of plants and contaminants [25,27–30], this study is the first demonstration that the mature vetiver root system augmented with phenol-degrading microbes can be an alternative for enhanced phytoremediation for phenol-contaminated wastewater with the help of aeration.

2. Material and methods

2.1. Vetiver grass, phenol, and wastewater

All experiments were conducted in a greenhouse at room temperature (25ºC–30ºC). Vetiver grass (*Vetiveria zizanioides* (L.) Nash, Songkhla-3 ecotype) was used in this study. The vetiver grass was mature at the age of 7 weeks including 3 weeks of rhizomicrobial augmentation in a hydroponic environment. The rhizomicrobial-augmented root of the vetiver grass was approximately 15–20 cm in length, which was 2–3 times longer than that of the vetiver plantlet used in the previous study [7]. Most of their leaves were greenish.

Phenol (analytical reagent grade, 99% purified) was purchased from Panreac (Barcelona, Spain).

2.2. Rhizomicrobial augmentation

For rhizomicrobial augmentation, the vetiver grasses were carefully removed from the soil (at the age of 4 weeks). The roots were washed with tap water and then planted on the floating platforms hydroponically in the treated wastewater from the illegal dumping pond (i.e., no phenol was left, but phenol-degrading microbes remained). The vetiver plants were grown in a greenhouse under natural light at a temperature of 25°C–30°C with and without aeration, respectively. Since vetiver root acts as a bio-stimulant for the growth of the rhizomicrobes due to its rich nutritional content and proper habitat, the vetiver root systems of each experiment were augmented with indigenous microbes that existed in the wastewater. These microbes were identified as phenol-degrading microbes including bacteria, such as *Enterobacter* spp., *Pseudomonas* spp., *Rhodococcus* spp., and *Acinetobacter* spp., and fungi, such as *Candida* spp., *Rhizopus* spp., *Aspergillus* spp., and *Fusarium* spp. [7]. Vetiver grass was grown for 3 weeks prior to measurements for augmented rhizomicrobes and was used for phenol-degradation experiments.

The physiological characteristics of the rhizomicrobial-augmented vetiver both with and without aeration, including the number of roots, leaves, and microorganisms, were monitored prior to phenol exposure. The growth of the vetiver grass during the rhizomicrobial augmentation with and without aeration was determined in terms of the weekly fresh weight. The growth rate constants were statistically determined using exponential growth fitting.

2.3. Experimental setup and phenol removal kinetics using vetiver grass

The phenol treatment experiments using rhizomicrobial-augmented vetiver grass on a floating platform were conducted in glass tanks (40 × 20 × 60 cm) filled with 35 L of phenol-contaminated wastewater for two different systems with and without aeration. The phenol-contaminated wastewater for the experiments was prepared by adding phenol to the phenol-contaminated wastewater from the illegal dumping until the phenol concentration reached 500 mg L^{-1}, the highest level of phenol concentration found at the beginning of the illegal dumping incident. For aerated experiments, the air was supplied by pumping air at the rate of 15 L min^{-1} via ceramic diffusers. The aerated and non-aerated experiments were codified as PFA and PF, respectively. Each system was conducted using five different numbers of mature vetiver plants on the platforms at 20, 40, 60, 80, and 100 plants, codified as PFA20, PFA40, PFA60, PFA80, and PFA100, respectively, for aerated experiments and codified as PF20, PF40, PF60, PF80, and PF100, respectively, for non-aerated experiments. Similarly, a platform without vetiver grass was also prepared as a negative control experiment. Each experiment was done in duplicate. Moreover, the performance of vetiver plantlets with aeration, which was studied in previous research [7], was compared with the mature vetiver in this study. The experiments using vetiver plantlets were codified as PB.

The experiments were conducted until the phenol concentration was below the MCL of 1 mg L^{-1}. Phenol, chemical oxygen demand (COD), volatile organic acid, polyphenol (such as lignin and tannin acid), pH, oxidation-reduction potential (ORP), dissolved oxygen (DO), and H_2O_2 were examined as a function of time. The analytical method for each parameter was based on the standard methods for the examination of the water and wastewater [31]. The phenol-degradation rate constants were calculated by assuming pseudo first-order kinetics as follows [7]:

$$C_t = C_0 e^{-kt} \tag{1}$$

where C_t and C_0 are phenol concentrations by time t, and the time when the experiments started, k, is the reaction rate constant (in units of 1/h), and t is the unit of time in hours. The significance of the vetiver numbers on k values was determined using one-way analysis of variance (ANOVA) in Microsoft Excel. The growth rate constants were statistically determined using exponential decay fitting according to the experimental results, which are discussed next.

2.4. Enzyme estimation and assay of activity

During the experiments, the roots of the vetiver grass were randomly sampled at various times for enzyme estimation. Vetiver samples were washed with deionized water to clean the root surface and then were ground in a mortar. A phosphate buffer with a pH of 7.0 was used as an enzyme diluent and then was homogenously mixed and equilibrated with an end-over-end rotator for 10 min. The examination of enzyme activity includes peroxidase (POD; EC.1.11.1.7), superoxide dismutase (SOD; EC.1.15.1.1), and catalase (CAT; EC.1.11.1.16).

The POD is identified as a major contributor to reactive oxygen species (ROS) production in plants and is also involved in callose deposition and the expression of defense genes [32]. The POD activity was determined by spectrometer (Genesys 10S UV-Vis) following the appearance of the colored oxidation products including tetraguaiacol [33,34]. One unit of peroxidase is defined as the capability of catalyzing the oxidation of 1 μmol of guaiacol in 1 min at 25°C.

In addition, SOD is an enzyme against ROS, superoxide (O_2^-), which is produced under stressed conditions to protect living cells and catalyzes the dismutation of superoxide into O_2 and H_2O_2 [35]. The SOD activity is developed using an assay test kit, Bioassay System [36]. One unit of SOD is defined as the concentration of SOD that inhibits the reduction of ferricytochrome. The detection range is 3 to 05 unit mg^{-1} protein for this method.

The CAT is a pervasive enzyme that catalyzes the decomposition of H_2O_2 to water and oxygen. In this study, CAT was quantified using a catalase assay kit from Sigma-Aldrich. One unit of CAT will decompose 1.0 mM of H_2O_2 to O_2 and H_2O in 1 min at pH 7.0 at 25°C at a substrate concentration of 50 mM H_2O_2.

2.5. Microbial analysis

The number of augmented microorganisms was measured using the plate-count technique after they were extracted from the wastewater and vetiver root, as mentioned above. Initially, the samples were placed separately in sterile tubes containing 10 mL of saline solution (0.15 mol L^{-1}) and were shaken vigorously on a vortex mixer for 30 s at room temperature. Serial dilutions were made in duplicate, and 0.1 mL of each dilution was spread onto the tryptic soy agar plates for the bacteria and onto sabouraud dextrose agar plates for the fungi (LABM, UK). The plates were incubated at 31°C for 3 d. Subsequently, the microbial and fungal colonies were counted as CFU mL^{-1}. To identify the strains of the phenol degraders, the biochemistry test and the Analytical Profile Index 20E (API 20E) were used [37].

3. Results and discussion

3.1. Physiological change of mature vetiver grass due to phenol exposure

This section presents the discussion of the general findings for PFA, PF, and PB using the results from PFA100, PF100, and PB100 [7] as representatives. Fig. 1 illustrates the vetiver characteristics, including the number of roots, leaves, and microorganisms from the PFA100 and PF100 experiments prior to phenol exposure. As noted previously, the vetiver grasses for PFA and PF were more mature (7 weeks) than those for PB (4 weeks) [7]. Moreover, PFA and PF were grown in the wastewater containing phenol-degrading microbes with and without aeration, respectively. Thus, their roots should be pre-colonized by phenol-degrading microbes that prefer aeration or no aeration, respectively.

As expected, before phenol exposure, PFA100 had the highest number of roots, leaves, and total microorganisms, followed by PF100 and PB100 [7], respectively. Interestingly, although the augmentation protocol started with a similar number of roots in each root length, after 3 weeks of aeration, PFA100 gained substantially more roots with lengths ranging from 5 to 10 cm, 15 to 20 cm, and 20 to 25 cm compared with PF100 (see Fig. 1(a)). The other root length ranges (0–5 cm and 10–15 cm) appeared to be similar to PF100. Moreover, PFA100 had 30% more green leaves than PF100 (Fig. 1(b); i.e., by randomly sampling 10% of the leaves, PF100 and PFA100 had 33 and 44 green leaves, respectively).

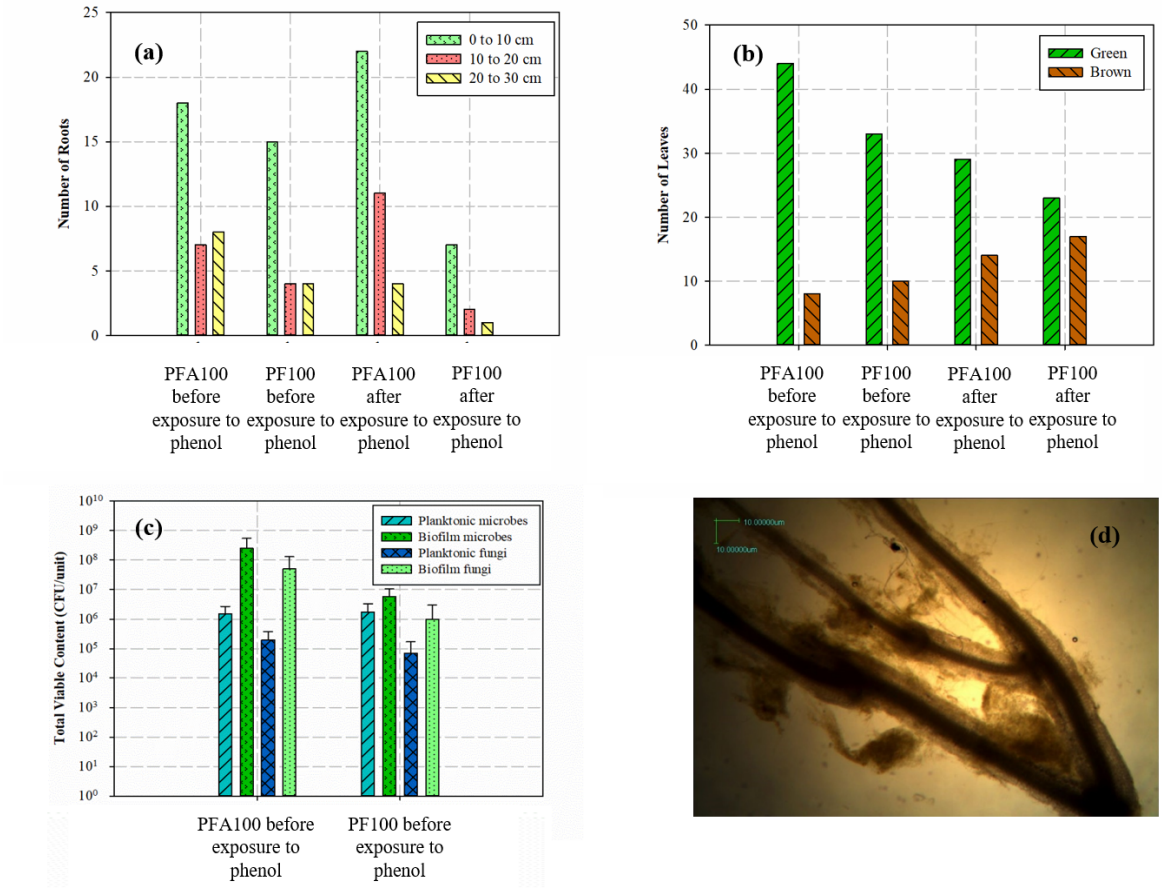

Fig. 1. Characteristics of vetiver grass using PFA100 and PF100 as representatives of PFA and PF experimental conditions prior to phenol exposure; (a) number of vetiver plant roots, (b) number of green and brown leaves, (c) total viable count as planktonic microorganisms and rhizomicrobes, and (d) micrograph of biofilm of rhizomicrobes on a mature vetiver root (PFA100) after 3-week augmentation.

Noticeably, most microbes in the three experimental conditions were attached on the roots as biofilm (Figs. 1(c) and (d)). While the planktonic microbes were not substantially different between PFA100 and PF100, the rhizomicrobes were different (by 1–2 orders of magnitude), suggesting the key role of aeration. Full details of species and strains of microbes are discussed in the section 3.5. The ratios of rhizomicrobes over planktonic microbes were 165.56 (for PFA100) to 51.50 (for PB100) [7] for the aerated conditions and 3.29 (for PF100) for the non-aerated conditions. This may be because these phenol-degrading microbes are aerobes and because the air supply also promotes the growth of the vetiver grass to develop a healthy root system [38]. This subsequently provides beneficial exudates, such as amino acids, simple sugars, complex carbohydrates, and oxygen, which are favorable for the attached microbial growth [19–22].

For aerated and non-aerated control experiments (with mature vetiver grass without phenol), the average fresh weights of the vetiver platforms with and without aeration were exponentially increased at the rate of 19 × 10^{-4} h^{-1} (over 216 h) and 7 × 10^{-4} h^{-1} (873 h), respectively. During the phenol-degradation experiments, the average fresh weights of the vetiver platforms were exponentially decreased for all the experiments, suggesting an adverse effect from phenol toxicity. The biomass reduction rates were 5 to 20 × 10^{-4} h^{-1} for PFA, 6 to 10 × 10^{-4} h^{-1} for PF, and 7 to 54 × 10^{-4} h^{-1} for PB [7]. As hypothesized in this study, the phenol toxicity obviously had a greater harmful effect on the vetiver plantlets (PB; 2.7 times greater biomass reduction rates) than the mature vetiver grass (PFA and PF). A potential explanation of how maturity and rhizomicrobial augmentation of vetiver grass decreases the adverse effects from phenol exposure is based on the difference in the number of catalytic biomolecules released by mature and young vetiver plants, which will be discussed later in this study. In addition, this stress was the driving force for the self-protection mechanism of vetiver, which led to phenol degradation, as discussed next.

3.2. Kinetics of phenol degradation

Fig. 2(a) exhibits the phenol-degradation kinetics of PFA100, PF100, and PB100 [7] as examples of PFA, PF, and

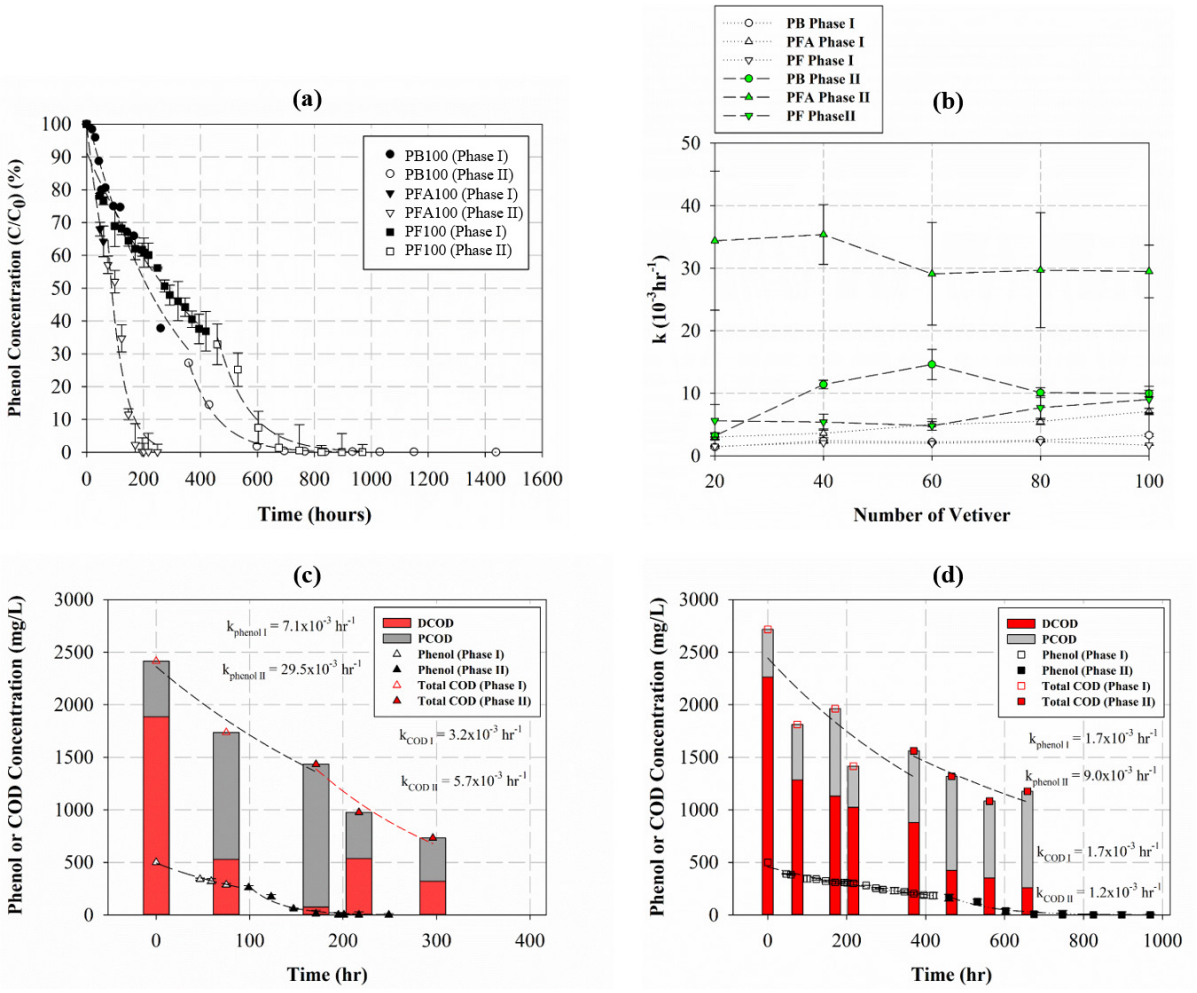

Fig. 2. (a) Comparative phenol-degradation kinetics of 100 vetiver plantlets (PB100) and 100 mature vetiver plants with and without aeration (PFA100 and PF100) based on the two-phase mechanism. (b) Phenol-degradation rate constants for Phases I and II as a function of the number of vetiver plants in the experiments. Detailed transformation kinetics of phenol and chemical oxygen demand (COD), both dissolved COD (DCOD) and particulate COD (PCOD) for (c) PFA100 and (d) PF100.

PB experiments, respectively. Phenol was degraded to 1 mg L^{-1} (MCL) in 249, 675, and 766 h by PFA100, PF100, and PB100, respectively. The general condition of the phenol-degradation kinetics in all experiments with vetiver is an exponential decay in the first portion of the kinetics followed by a faster exponential decay in the second portion, which is in good agreement with the two-phase mechanism [7]. Thus, to quantitatively evaluate the enhanced phenol degradation by the rhizomicrobial-augmented mature vetiver platforms, the pseudo first-order reaction (Eq. (1)) was applied to extract the pseudo first-order rate constants from the experimental results as summarized in Table 1 (for PB from the previous study [7] and the PFA and PF experiments).

Also shown in Table 1, the two-phase mechanism demonstrated very good fit ($R^2 \geq 0.83$) for the data of the mature vetiver platforms with and without aeration. A couple of interesting trends can be observed from the kinetics results. First, for Phase I, the number of vetiver plants appeared to govern the phenol-degradation rate constants of PFA (i.e., the greater the number of vetiver plants in the platform, the faster the degradation rate constant, from $3.00 \pm 0.50 \times 10^{-3}$ h^{-1} of PFA20 to $7.10 \pm 0.50 \times 10^{-3}$ h^{-1} of PFA100; also confirmed by the p-value at 0.004 ($p < 0.05$) using one-way ANOVA in Table 1). For Phase II, the phenol-degradation rate constants (ranging from $34.40 \pm 11.12 \times 10^{-3}$ h^{-1} of PFA20 to $29.50 \pm 4.20 \times 10^{-3}$ h^{-1} of PFA100) were not sensitive to the number of vetiver plants (as confirmed by the p-value at 0.081 ($p > 0.05$) using one-way ANOVA).

A similar trend is also observed for PB [7] but is not as pronounced as for PFA. For Phases I and II of PF (without aeration), the phenol-degradation rate constants were both sensitive to the number of vetiver plants (as confirmed by the p-value of 0.003 ($p < 0.05$) for Phase I and by the p-value of 0.015 ($p < 0.05$) for Phase II using one-way ANOVA in Table 1). These findings imply the significant role of aeration in phenol degradation using vetiver in Phase I. This is unexpected since the phenol degradation in Phase I is governed by phytochemicals released from the vetiver root as a response to phenol toxicity. The role of aeration on the activity of reactive biomolecules for phenol degradation will be further discussed in the section on enzymes.

For Phase II, the aeration also had a significant role in increasing the phenol-degradation rate constants (by 4.15–11.41 times in comparison to Phase I). Nevertheless, the degradation rate constants became insensitive to the number of vetiver plants. This suggests that even 20 plants of rhizomicrobial-augmented mature vetiver with aeration (PFA20) were sufficient for degrading phenol in Phase II, presumably because of highly active rhizomicrobial activity. For the system without aeration, the rhizomicrobes may not be highly active, so the larger number of vetiver plants appeared to help in enhancing phenol degradation.

Second, for Phases I and II, at the same number of vetiver plants, the rate constants of phenol degradation generally follow the order of PFA>>PB>PF (Fig. 2(b)). The role of vetiver maturity with rhizomicrobial augmentation and aeration was analyzed by comparing the rate constants

Table 1
Phenol-degradation kinetics for PB [7], PFA, and PF experiments based on the two-phase mechanism

Experiment	Phase 1			Phase 2			Enhance degradation rate (time)
	R^2	Concentration range (mg L^{-1})	k ($\times 10^{-3}$ h^{-1})	R^2	Concentration range (mg L^{-1})	k ($\times 10^{-3}$ h^{-1})	
PB0	0.9180	565–0.1	1.10 ± 0.10	–	–	–	
PB20	0.8685	550–120	1.40 ± 0.20	0.9711	120–0.1	3.20 ± 0.50	2.29
PB40	0.9855	530–90	2.40 ± 0.20	0.9976	90–0.1	11.4 ± 0.70	4.75
PB60	0.9745	533–117	2.20 ± 0.10	0.9912	117–0.1	14.60 ± 2.40	6.64
PB80	0.9462	537–104	2.50 ± 0.20	0.995	104–0.1	10.10 ± 0.80	4.04
PB100	0.9656	553–83	3.30 ± 0.20	0.997	83–0.1	9.97 ± 0.50	3.02
PFA0	0.9571	520–385	2.00 ± 0.60	–	–	–	–
PFA20	0.8831	540–354	3.60 ± 0.70	0.9862	354–0.7	35.40 ± 4.80	9.83
PFA40	0.8729	562–265	5.00 ± 0.90	0.8552	265–0.7	29.10 ± 8.20	5.82
PFA60	0.9654	567–167	5.50 ± 0.50	0.8346	167–0.7	29.70 ± 9.20	5.40
PFA80	0.9858	588–246	7.10 ± 0.50	0.9616	246–0.1	29.50 ± 4.20	4.15
PFA100	0.8364	540–443	3.00 ± 0.50	0.8213	443–0.7	34.40 ± 11.12	11.47
PF0	0.9689	576–65.6	2.00 ± 0.50	–	–	–	–
PF20	0.9441	585–105	2.10 ± 0.10	0.9400	105–0.9	5.40 ± 1.3	2.57
PF40	0.9693	544–261	2.00 ± 0.01	0.9533	261–0.75	4.80 ± 0.70	2.40
PF60	0.9705	593–142	2.30 ± 0.10	0.9738	142–0.85	7.70 ± 1.90	3.35
PF80	0.9674	543–136	1.70 ± 0.01	0.9259	136–0.45	9.00 ± 2.10	5.29
PF100	0.9858	519–166	1.50 ± 0.01	0.9175	166–0.3	5.60 ± 2.6	3.73

among the three experimental conditions. By calculating the ratio between the rate constants for PFA100 and PB100 (k_{PFA100}/k_{PB100}), we obtained the k_{PFA100}/k_{PB100} values of 2.15 and 2.96 for Phases I and II, respectively. These ratios can be viewed as enhancement factors (Eq. (2)):

$$EF_O = EF_R \times EF_M \times EF_A \quad (2)$$

where EF_O is the overall enhancement factor, which is contributed from the enhancement factor due to the longer root length of the mature vetiver (EF_R), the augmented rhizomicrobes (EF_M), and the aeration (EF_A).

With this equation, we can distinguish the contribution from the three factors. For example, the sum of the average length of the vetiver root of PFA100 was 388 cm (i.e., 2.38 times longer than that of PB100, which was 162 cm based on Fig. 1(a)). If the enhanced phenol degradation was affected solely by the root length of the mature vetiver, the value of k_{PFA100}/k_{PB100} should be around 2.38 (EF_R) for both phases. For this reason, by solving Eq. (2) (given that $EF_A = 1$ because both PFA and PB were conducted with aeration), the contribution from the rhizomicrobial augmentation (EF_M) of PFA100 on the phenol degradation was 0.90 and 1.24 times for Phases I and II, respectively. Similarly, by calculating the ratio between the kinetic rate constants of PFA100 and PF100 (k_{PFA100}/k_{PF100}), we obtained the k_{PFA100}/k_{PF100} values of 4.18 and 3.28 for Phases I and II, respectively. These values can be separated into the contribution of EF_R at 1.74 (the total root length of PFA100 was 388 cm, while that of PF100 was 222 cm) and ER_A at 2.40 and 1.88 for Phases I and II (by solving Eq. 2, given that $EF_M = 1$ because both PFA and PF were augmented), respectively. Thus, in summary, in this study, the EF_R values were from 1.74 to 2.38, the EF_M values were from 0.90 (Phase I) to 1.24 (Phase II), and the EF_A values were from 2.40 (Phase I) to 1.88 (Phase II) for 100 vetiver plants.

3.3. Phenol-degradation mechanism: role of vetiver maturity, aeration, and rhizomicrobial augmentation

Phenol degradation using a rhizomicrobial-augmented mature vetiver root system still follows the two-phase mechanism (i.e., the combination of phytochemical transformation and rhizomicrobial degradation) proposed in the previous study [7]. Nevertheless, vetiver maturity, rhizomicrobial augmentation, and aeration play substantial roles in the relative contribution of phyto-polymerization and rhizomicrobial degradation, which can be mechanistically observed by a comparative analysis of phenol and COD degradation kinetics of PB100 [7], PFA100, and PF100.

For PFA100 (Fig. 2(c)), in Phase I, the phenol-degradation rate constant was 7.1×10^{-3} h^{-1} and the COD removal rate constant was 3.2×10^{-3} h^{-1}. Although, both phenol and the COD removal rate constants of PFA100 were greater than those of PB100 by around 2.15 and 1.68 times, respectively [7], due to vetiver maturity and rhizomicrobial augmentation, the COD removal rate constant was only 45.07% of the phenol-degradation rate constant in Phase I. Similarly, for Phase II of PFA100, the phenol-degradation rate constant was 29.5×10^{-3} h^{-1}, while the COD removal rate constant was 5.7×10^{-3} h^{-1} (i.e., 19.32% of the phenol-degradation rate constant in Phase II). The substantially lower percentage of the COD removal rates with respect to the phenol removal rates of PFA100 in comparison to PB100 [7] implies that vetiver maturity and rhizomicrobial augmentation of PFA100 promote phenol transformation (i.e., PCOD formation as seen from the accumulation of PCOD in the bar charts in Fig. 2(c)) more than COD removal. This is partly because rhizomicrobes augmented on the PFA100 were phenol-degrading microbes that favor phenol transformation via catalytic (POD) oxidation [39–46].

In contrast, for PF100 (without aeration) in Phase I (Fig. 2(d)), the phenol-degradation rate constant and the COD removal rate constant were both 1.7×10^{-3} h^{-1}. This is the lowest rate among all three treatment conditions. Interestingly, the COD removal rate is equal to the phenol removal rate, suggesting that phenol was degraded via phyto-oxidation or microbial oxidation rather than phyto-polymerization. Noticeably, PCOD was not formed as observed in Fig. 2(d). In Phase II, when the phenol concentration decreased to 146 mg L^{-1}, the phenol-degradation rate constant increased to 9.0×10^{-3} h^{-1}, but the COD removal rate constant was still 1.2×10^{-3} h^{-1}, suggesting that phenol-degrading rhizomicrobes may participate in phenol transformation to PCOD but did not completely mineralize the phenol. Obviously, PCOD was accumulated in Phase II of PF100. The discrepancy between phenol treatment with and without aeration reveals the essential roles of aeration on the phenol-degradation pathway using vetiver with both planktonic microbes and rhizomicrobes.

3.4. Enzyme activity: role of vetiver maturity, aeration, and rhizomicrobial augmentation

Phenol degradation using rhizomicrobial-augmented mature vetiver is partly governed by catalytic oxidation by POD and H_2O_2 released from the root as reported in the case of the vetiver plantlets in the previous studies [7,12]. Nevertheless, vetiver maturity, aeration, and rhizomicrobial augmentation play substantial roles on enzymatic activity of the vetiver root, which can be systematically observed by comparing the kinetics of the released POD and SOD and the change in ORP and DO over time for PB100 [7], PFA100, and PF100 (Figs. 3 and 4). For PB100, once exposed to a high phenol concentration (500 mg L^{-1}), the vetiver plantlets with aeration were stressed by the phenol phytotoxicity and irritation [7]. Oxidative stress was unavoidable. In fact, the deterioration of the PB100 root and leaf systems was so severe that it could be visually seen [7]. Moreover, as shown in Fig. 4(a), within 19 h after phenol exposure, the ORP of PB100 dropped from 208 to –13 mV and stayed negative for 600 h prior to gradually reversing to a positive value [7]. The decrease of ORP suggested the efflux of reduced biomolecules, such as glutathione (GSH) and β-nicotinamide adenine dinucleotide (NADH) from the vetiver root, which is a sign of cell death [47,48].

Nevertheless, as a vetiver self-protection mechanism, after exposure to a high phenol concentration for 3 h, the POD in the root (from 50.25 U mg^{-1} of protein prior to phenol exposure) increased to 201 U mg^{-1} protein (in Phase I) before gradually decreasing to 31.50 U mg^{-1} protein at 484 h when the phenol degradation passed to Phase II in which rhizomicrobes dominantly degraded the phenol and COD.

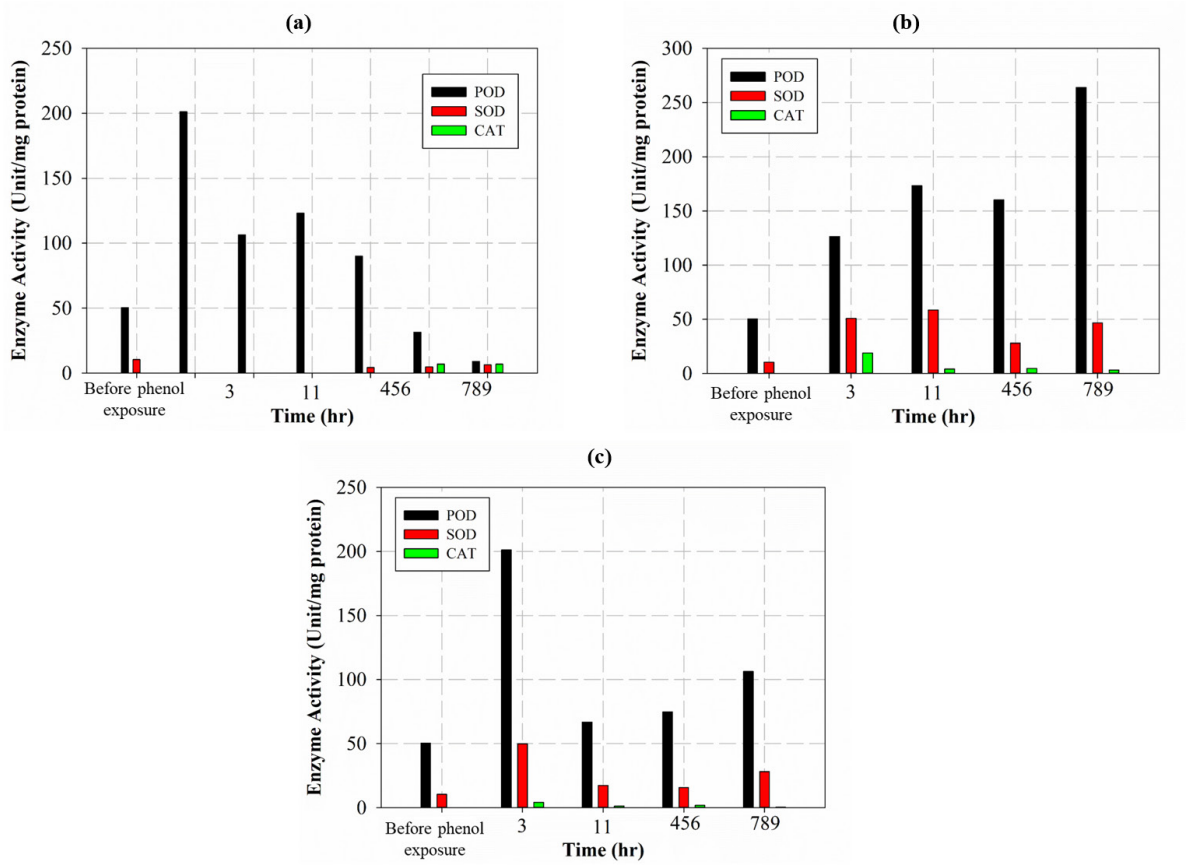

Fig. 3. Dynamics of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) activity as a function of time (Phases I and II) for (a) PB100, (b) PFA100, and (c) PF100.

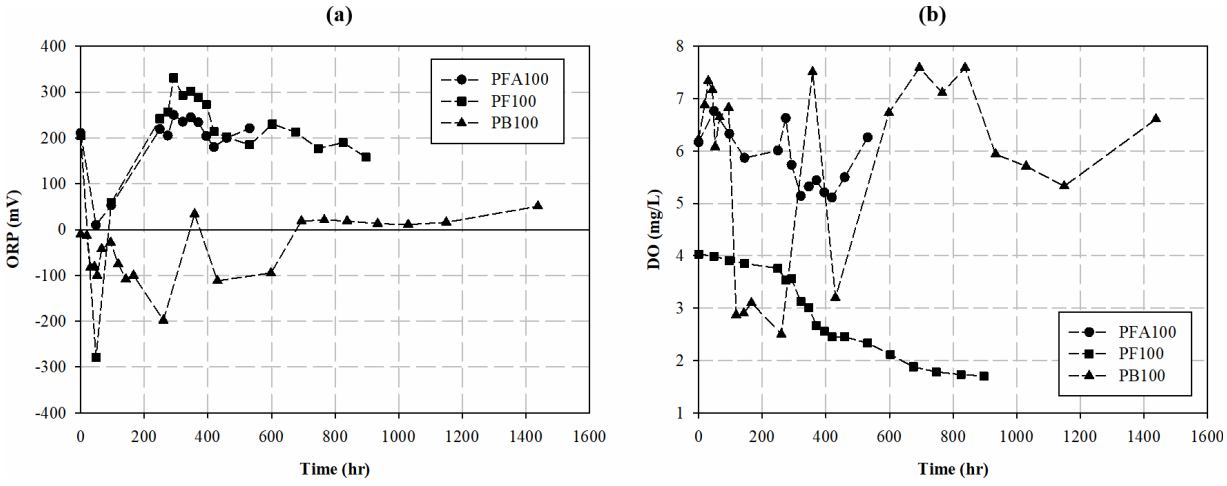

Fig. 4. Dynamics of (a) oxidation-reduction potential (ORP) and (b) dissolved oxygen (DO) for PB100, PFA100 and PF100.

In the solution, H_2O_2 remained at 10 mg L^{-1} in Phase I and gradually increased to 15.58 mg L^{-1} at the end of Phase II. As a main phenol-detoxification mechanism, both H_2O_2 and POD catalytically oxidized phenol to phenoxy radicals, which can subsequently undergo self-polymerization or complexation with natural organic matter to form non-toxic polyphenols or particulate polyphenols (Fig. 5) [7,12,33].

Nonetheless, some phenoxy radicals may subsequently cooxidize NADH and GSH in the vetiver cells [49] to form β-nicotinamide adenine dinucleotide (cation radical) (NAD•) and glutathione disulfide radical anion (GSSG•), respectively [50]. These radicals further oxidized O_2 to yield superoxide ($O_2^{\bullet-}$), a ROS that can cause stress to the vetiver. This formation of $O_2^{\bullet-}$ by the O_2– consuming chain

reaction of phenoxy radicals is likely in PB100 since, even with aeration, the DO of PB100 dropped from 7 to 2.8 mg L^{-1} in 118 h prior to increasing to 6 to 7 mg L^{-1} after 361 h (the shift from Phase I to II). This means that the phenol detoxification via POD and H_2O_2 can cause a side effect by $O_2\bullet^-$ formation, especially in Phase I. If SOD is sufficiently released by the vetiver, it can transform $O_2\bullet^-$ to H_2O_2 to reduce the stress [35]. Moreover, the H_2O_2 can then be further utilized for phenol transformation.

However, as shown in Fig. 3(a), PB100 produced a very small amount of SOD (4.24 U mg^{-1} of protein in Phase I and 6.32 U mg^{-1} of protein in Phase II) [7] in comparison to the mature vetiver (PFA100; Fig. 3(b)). This may result in the accumulation of $O_2\bullet^-$ in the vetiver cell of PB100, which promoted chlorosis, necrotization, and hypersensitive response [51], as experimentally observed. This observation agrees with the literature reporting the clear role of SOD on the difference between a salt-tolerant cultivar and a salt-sensitive cultivar of tomato under salinity stress [52].

Similarly, for PFA100, the mature vetivers with aeration were stressed by phenol exposure (500 mg L^{-1}). However, the deterioration of the PFA100 root and leaf systems was much less than that for PB100. Moreover, as shown in Fig. 4(a), within 48 h after phenol exposure, the ORP of PFA100 dropped from 211 to 10 mV prior, promptly recovering to 219 mV in 249 h. Although, the decrease of ORP suggested the efflux of reduced biomolecules from the vetiver root, it was rapidly recovered, confirming that the adverse effect of phenol on the mature vetiver was not as severe as the case of PB100. This is presumably due to the stronger and healthier root system of the mature vetiver.

As a vetiver self-protection mechanism, after being exposed to a high phenol concentration for 3 and 11 h, the POD in the root (from 50.25 U mg^{-1} protein prior to phenol exposure) increased to 126 and 173.29 U mg^{-1} protein, respectively, (in Phase I) and gradually increased from 160.26 to 263.85 U mg^{-1} protein at 456 to 879 h when the phenol degradation passed to Phase II. Although, the maximum POD activity of PFA100 in Phase I was 1.16 times less than that of PB100, the total root length (and mass) of PFA100 was 2.38 times greater than PB100. Thus, overall, the phenol degradation by catalytic oxidation was supposed to be faster in PFA100 than in PB100, as experimentally observed.

While H_2O_2 and POD catalytically oxidized phenol to phenoxy radicals, which may subsequently form non-toxic polyphenols and particulate polyphenols or form toxic $O_2\bullet^-$, the formation of $O_2\bullet^-$ by the O_2– consuming chain reaction of phenoxy radicals was supposed to be minor for PFA100.

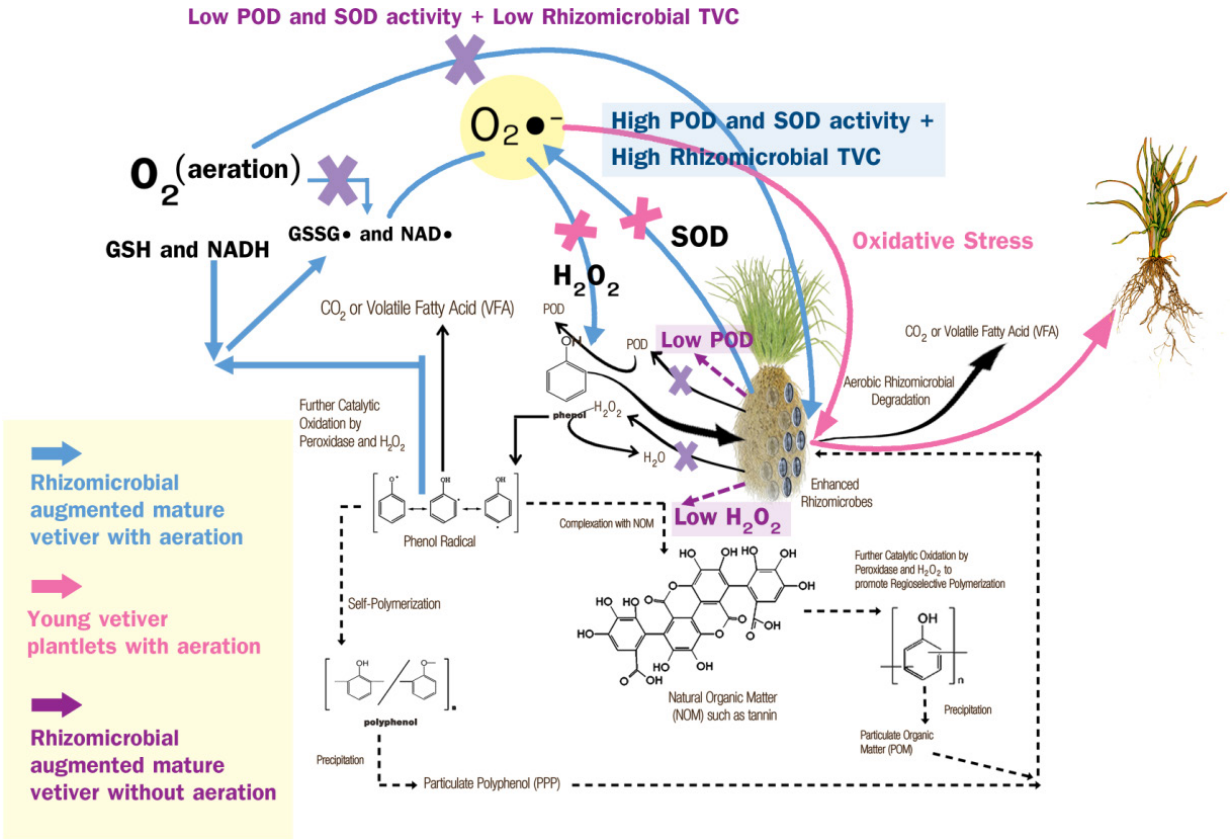

Fig. 5. Conceptual model of the roles of vetiver maturity, rhizomicrobial augmentation, and aeration on phenol transformation. Black arrows and black-dashed arrows illustrate phenol transformation. Blue arrows illustrate the reactive oxygen species (ROS) and roles of enzymes in phenol detoxification using aerated rhizomicrobial-augmented mature vetiver. Pink arrows illustrate the ROS and roles of enzymes in young aerated vetiver plantlets. Pink crosses illustrate the missing pathways in young aerated vetiver plantlets. Purple arrows illustrate the ROS and roles of enzymes in unaerated rhizomicrobial-augmented mature vetiver. Purple crosses illustrate the missing pathways in unaerated rhizomicrobial-augmented mature vetiver.

This is because the DO of PFA100 only slightly dropped from 6.7 to 5.1 mg L^{-1}, suggesting a small amount of DO was consumed for $O_2\bullet^-$ formation in comparison with PB100. Moreover, PFA100 appeared to produce a substantial amount of SOD (up to 58.52 U mg^{-1} protein in Phase I and 46.69 U mg^{-1} protein in Phase II (i.e., 10 times greater than the SOD produced by PB100), which should rapidly transform $O_2\bullet^-$ to H_2O_2 to reduce the stress [35]. These findings may be partly responsible for the much lesser deterioration of the mature PFA100 root and leaf systems in comparison to those of the young PB100 (Fig. 5).

Interestingly, phenol exposure caused less stress to the mature vetivers without aeration (PF100) than those with aeration (PFA100). The biomass reduction rate of PF100 was just 10 x 10^{-4} h^{-1} (2 times less than PFA100). As shown in Fig. 4(a), within 48 h after phenol exposure, the ORP of PF100 dropped from 205 to –279 mV prior to promptly recovering to 242 mV in 249 h, confirming that the adverse effect of phenol on the mature vetiver without aeration was not as severe as in the case of PB100. This is presumably due to the stronger and healthier root system of the mature vetiver. Moreover, rhizomicrobes that formed biofilm (Fig. 1(d)) on the root of the vetiver may help decrease phenol exposure to the vetiver root.

Nevertheless, after being exposed to a high phenol concentration for 3 h, the POD activity in the root (from 50.25 U mg^{-1} protein prior to phenol exposure) briefly increased to 201 U mg^{-1} protein (in Phase I) but then rapidly decreased and remained relatively stable from 66.98 to 74.79 U mg^{-1} from 11 to 456 h after phenol exposure (Phase I). The POD in the root increased again to 106.39 U mg^{-1} at 879 h (toward the end of Phase II). Noticeably, in Phase I, the POD concentration of PF100 was, on average, 2.35 times lower than that of PFA100. This is due to the absence of aeration in PF100, which is in good agreement with a recent study that reported that aeration increased the POD and CAT activity in the root tissue of *Nymphaea tetragona* Georgi [53]. In this study, we also observed the lower SOD and CAT activities of PF100 in comparison with PFA100, confirming that aeration has an important role over POD, CAT, and SOD activity. Moreover, the root length (and mass) of PFA100 was 1.74 times greater than that of PF100. Thus, the overall phenol degradation by catalytic oxidation of PFA100 was supposed to be much faster than that of PF100, as experimentally observed.

Finally, the role of rhizomicrobial augmentation can be noticed when comparing the enzyme activity of PB100 [7] and PFA100 in Phase II. For PB100 in Phase II, the POD activity decreased to 31.5 U mg^{-1} protein at 484 h and to 9 U mg^{-1} protein at 504 h because enzymatic polymerization in Phase I detoxified the phenol to yield benign polyphenols. Moreover, the phenol concentration was decreased to a much less irritating level. For PFA100 with rhizomicrobial augmentation in Phase II, the POD activity increased to 160 U mg^{-1} protein at 456 h and to 263.85 U mg^{-1} protein at 879 h, although the phenol concentration was decreased to lower than an irritating level. This finding suggests that, for PFA100, the catalytic phenol transformation by H_2O_2 and POD was still highly active even in Phase II where the phenol concentration became much lower. This partly explains the greater phenol degradation rate constants by PFA100 in comparison with PB100 in Phase II.

The released POD may also form rhizomicrobes augmented on the root of the mature vetiver. As discussed next, the rhizomicrobes consist of *Candida* spp. [42,44], *Fusarium* spp. [39], *Rhizopus* spp. [40], *Aspergillus* spp. [41,42], *Enterobacter* spp. [43], and *Pseudomonas* spp. [45,46], all of which are capable of releasing POD for phenol transformation. Nevertheless, microbes and rhizomicrobes also participated in phenol mineralization through meta cleavage and ortho cleavage pathways, as in the case of PB100. As shown in Figs. 3(b) and (c), the SOD activities of both PFA100 and PF100 decreased, while the rate constants of phenol degradation increased in Phase II. This means that, although the phenol degradation became faster, the formation of $O_2\bullet^-$ via the oxidation of NADH became lower in volume. It implies that a substantial part of the greater phenol degradation is attributed to microbial-assisted phenol polymerization or mineralization, which does not form much $O_2\bullet^-$.

3.5. Dynamics of augmented microbes during phenol degradation

Fig. 6 illustrates the dynamics of augmented microbes (total viable count (TVC) and viable genus) in aerated (PFA100) and non-aerated (PF100) systems during phenol degradation. Several interesting trends can be noted and linked to the enzyme activity and phenol degradation. First, prior to phenol exposure (t = 0 h), a greater variety of bacteria and fungi were available in the aerated system in comparison to the non-aerated system. As shown in Figs. 6(a) and (b), three strains of bacteria, including *Enterobacter* spp., *Pseudomonas* spp., and *Rhodococcus* spp., together with a strain of fungi, *Candida* spp., were present in both planktonic and attached forms (biofilm on the vetiver roots) in PFA100, while *Acinetobacter* spp. only presented as an attached form. Nevertheless, for the same strains of microbes in the aerated system, the attached forms (TVC = 10^7 to 10^8 CFU mg^{-1}) were more abundant than the planktonic forms (10^4–10^6 CFU mg^{-1}) for 2–3 orders of magnitude. For the non-aerated system, only two strains of bacteria, *Enterobacter* spp. and *Pseudomonas* spp., and a strain of fungi, *Candida* spp., were viable in both planktonic and attached forms in PF100 (Figs. 6(c) and (d)). Two more strains of bacteria, *Rhodococcus* spp. and *Micrococcus* spp., were also available as planktonic forms but were not attached on the vetiver root. Similarly, for the same strains of microbes in the non-aerated system, the attached forms (10^4 to 10^7 CFU mg^{-1}) were more abundant than the planktonic forms (10^4–10^6 CFU mg^{-1}) but only for an order of magnitude.

The lesser variety and lower TVC of microbes and rhizomicrobes in the non-aerated system was presumably because these phenol-degrading microbes are aerobes. Moreover, the air supply will promote the growth of vetiver grass to develop a healthy root system [38], which subsequently provides beneficial exudates, such as amino acids, simple sugars, complex carbohydrates, and oxygen, that are favorable for the attached microbial growth [19–22].

Second, once exposed to a high phenol concentration (500 mg L^{-1}), planktonic microbes in the aerated system decreased their TVC and diversity. After 11 h of phenol exposure, *Rhodococcus* spp. disappeared. Similarly, the TVC values of *Enterobacter* spp., *Pseudomonas* spp.,

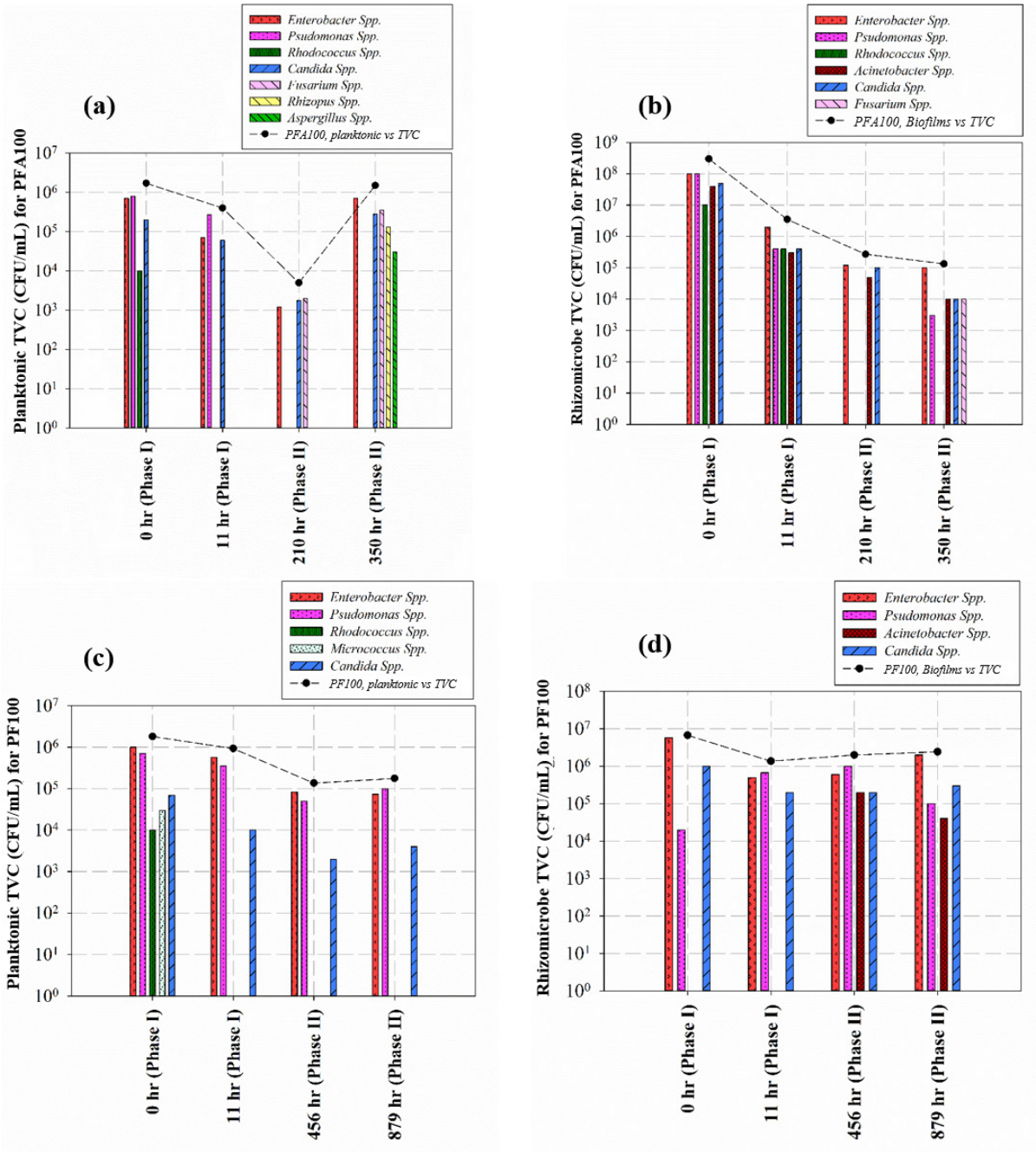

Fig. 6. Dynamics of augmented microbes (total viable count (TVC) and viable strains) in both planktonic form (a) for PFA100 and (c) for PF100 and the attached form (b) for PFA100 and (d) for PF100 for the aerated and unaerated systems.

and *Candida* spp. decreased by an order of magnitude (5×10^4 – 5×10^5 CFU mg^{-1}). After 210 h of exposure (toward the end of Phase II), there was still a sign of the adverse effect of phenol on microbes since the TVC was decreased by 2 orders of magnitude more (10^3 CFU mg^{-1}). Nevertheless, there was also a recovery sign since *Fusarium* spp. appeared at an increasing *TVC*. After 350 h of exposure, *Enterobacter* spp. grew back to the same TVC as before phenol exposure. Similarly, various strains of fungi, including *Candida* spp, *Rhizopus* spp., *Aspergillus* spp., and *Fusarium* spp., appeared and grew back to the same TVC as before the phenol exposure, confirming the recovery of planktonic microbes in the system after complete phenol degradation in the system of the aerated mature vetiver. Similarly, for rhizomicrobes in the aerated mature vetiver, after exposed to a high phenol concentration (500 mg L^{-1}) for 11 h, their TVC decreased by 2 orders of magnitude (5×10^5 – 5×10^6 CFU mg^{-1}). After 210 h of phenol exposure, the TVC of the existing microbes was decreased by an order of magnitude more, while *Pseudomonas* spp. and *Rhodococcus* spp. disappeared. After 350 h (toward the end of Phase II), rhizomicrobes was recovered as evident

from the present of *Pseudomonas* spp. and *Fusarium* spp., although the TVC (10^4–10^5 CFU mg^{-1}) was lower than in the conditions prior to phenol exposure.

Third, for mature vetiver in the non-aerated system, planktonic microbes gradually decreased their TVC. After 11 h of phenol exposure, the TVC decreased just by half an order of magnitude. After 456 h (Phase II), the TVC decreased by an order of magnitude. Slight recovery was also observed after 879 h (the end of Phase II). Noticeably, planktonic microbes were much less sensitive to phenol exposure in the PF100 in comparison with PFA100. Similarly, for rhizomicrobes of PF100, after phenol exposure from 11 h to 879 h, the TVC of most rhizomicrobes, including *Enterobacter* spp. and *Candida* spp., decreased just by an order of magnitude at most, while the TVC of *Pseudomonas* spp. increased by 1–1.5 orders of magnitude. Moreover, *Acinetobacter* spp. appeared after 456 h (Phase II). Obviously, rhizomicrobes of PF100 were much less sensitive to phenol exposure than PFA100.

Noticeably, the differences in the dynamics of the TVC values and the survival of rhizomicrobes of PF100 and PFA100 are inversely related to the POD activity and the phenol-degradation rate constants of PF100 and PFA100 discussed in section 3.2 and 3.4. While the rhizomicrobes of PFA100 were severely damaged by phenol exposure, the POD activity in Phase II of PFA100 was 2.48 times greater than PF100 and the phenol-degradation kinetics of PFA100 were 3.43 times higher than PF100. This finding implies that aeration promoted the oxidative stress of PFA100 in both the vetiver and augmented phenol-degrading rhizomicrobes by forming POD and H_2O_2, which subsequently accelerated the phenol degradation via enzymatic oxidation. This oxidative stress has positive and negative effects, which not only transformed the phenol but also harmed the vetiver and microbes.

4. Conclusion

Vetiver maturity increased the root mass, which releases more POD for phenol transformation. The vetiver maturity enhanced SOD activity, decreasing the accumulation of toxic superoxide, which can cause stress to vetiver during the phenol-detoxification process. Unexpectedly, aeration also played a crucial role since it enhanced the POD and SOD activity in the vetiver and rhizomicrobes in Phases I and II. Finally, augmented rhizomicrobes enhanced the POD activity in Phase II, which speeds up the phenol polymerization. To our knowledge, the roles of these important factors have never been reported in previous studies [7,12].

Acknowledgments

This research was funded by the Office of the Royal Development Projects Board through Naresuan University (Grant Nos. R2558A092, R2557A057, and R2555B098). We are deeply thankful for the excellent advice and continuous support from our expert consultants, including Dr. Weerachai Nanakorn, Dr. Narong Chomchalow, Ms. Suwanna Pasiri, and Dr. Pittayakorn Limthong. We deeply appreciate the trust from the Nong Nae community who welcomed our research team like a family. We appreciate the Chachoengsao Development Station, Land Development Department, and Ministry of Agriculture and Cooperatives, Thailand, for supporting vetiver grass for our research. Finally, we value the King of Thailand Vetiver Award (2015) and the Vetiver Network International (TVNI (Award) 2015) granted to this research by the Chaipattana Foundation (Thailand) and the TVNI.

References

[1] G. Busca, S. Berardinelli, C. Resini, L. Arrighi, Technologies for the removal of phenol from fluid streams: short review of recent developments, J Hazard Mater., 160 (2008) 265–288.
[2] D.N. Veeramachaneni, J.S. Palmer, R.P. Amann, Long-term effects on male reproduction of early exposure to common chemical contaminants in drinking water, Hum. Reprod., 16 (2001) 979–987.
[3] T. Al-Khalid, M. El-Naas, Aerobic Biodegradation of Phenols: A Comprehensive Review, Crit. Rev. Environ. Sci. Technol., 42 (2012) 1631–1690.
[4] G.A. Hill, C.W. Robinson, Substrate inhibition kinetics: phenol degradation by Pseudomonas putida, Biotechnol. Bioeng., 17 (1975) 1599–1615.
[5] W. Wang, H. Han, Recovery strategies for tackling the impact of phenolic compounds in a UASB reactor treating coal gasification wastewate, Bioresour. Technol., 103 (2012) 95–100.
[6] M. Gao, M.H. Diao, S. Yuan, Y.K. Wang, H. Xu, X.H. Wang, Effects of phenol on physicochemical properties and treatment performances of partial nitrifying granules in sequencing batch reactors, Biotechnol. Rep., 13 (2017) 13–18.
[7] T. Phenrat, P. Teeratitayangkul, I. Prasertsung, R. Parichatprecha, P. Jitsangiam, N. Chomchalow, S. Wichai, Vetiver plantlets in aerated system degrade phenol in illegally dumped industrial wastewater by phytochemical and rhizomicrobial degradation, Environ. Sci. Pollut. Res., 24 (2017) 13235–13246.
[8] L.T. Danh, P. Truong, R. Mammucari, T. Tran, N. Foster, Vetiver grass, Vetiveria zizanioides: a choice plant for phytoremediation of heavy metals and organic wastes, Int. J. Phytorem., 11 (2009) 664–691.
[9] N. Aibibu, Y. Liu, G. Zeng, X. Wang, B. Chen, H. Song, L. Xu, Cadmium accumulation in vetiveria zizanioides and its effects on growth, physiological and biochemical characters, Bioresour. Technol., 101 (2010) 6297–6303.
[10] L.T. Danh, P. Truong, R. Mammucari, N. Foster, Economic Incentive for Applying Vetiver Grass to Remediate Lead, Copper and Zinc Contaminated Soils, Int. J. Phytorem., 13 (2010) 47–60.
[11] P. Truong, T.T. Van, E. Pinners, Vetiver System Applications: Technical Reference Manual, in, The Vetiver Network International, San Antonio, Texas, 2007, pp. 126.
[12] S. Singh, J.S. Melo, S. Eapen, S.F. D'Souza, Potential of vetiver (Vetiveria zizanoides L. Nash) for phytoremediation of phenol, Ecotoxicol Environ. Saf., 71 (2008) 671–676.
[13] K. Das, A. Roychoudhury, Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants, Front Environ Sci., 2 (2014) 53.
[14] S.G. Ibáñez, L.G.S. Alderete, M.I. Medina, E. Agostini, Phytoremediation of phenol using Vicia sativa L. plants and its antioxidative response, Environ. Sci. Pollut. Res., 19 (2012) 1555–1562.
[15] M.A. Talano, D.C. Busso, C.E. Paisio, P.S. González, S.A. Purro, M.I. Medina, E. Agostini, Phytoremediation of 2,4-dichlorophenol using wild type and transgenic tobacco plants, Environ. Sci. Pollut. Res., 19 (2012) 2202–2211.
[16] Y. Osem, Y. Chen, D. Levinson, Y. Hadar, The effects of plant roots on microbial community structure in aerated wastewater-treatment reactors, Ecol. Eng., 29 (2007) 133–142.
[17] M.A. Kamran, J.H. Syed, S.A. Eqani, M.F. Munis, H.J. Chaudhary, Effect of plant growth-promoting rhizobacteria inoculation on cadmium (Cd) uptake by Eruca sativa, Environ. Sci. Pollut. Res., 22 (2015) 9275–9283.
[18] L. Zhang, J. Zhao, N. Cui, Y. Dai, L. Kong, J. Wu, S. Cheng, Enhancing the water purification efficiency of a floating

treatment wetland using a biofilm carrier, Environ. Sci. Pollut. Res., 23 (2016) 7437–7443.
[19] A. Valipour, Y.-H. Ahn, Constructed wetlands as sustainable ecotechnologies in decentralization practices: a review, Environ. Sci. Pollut. Res., 23 (2016) 180–197.
[20] E. Kurzbaum, F. Kirzhner, S. Sela, Y. Zimmels, R. Armon, Efficiency of phenol biodegradation by planktonic Pseudomonas pseudoalcaligenes (a constructed wetland isolate) vs. root and gravel biofilm, Water Res., 44 (2010) 5021–5031.
[21] U. Stottmeister, A. Wiessner, P. Kuschk, U. Kappelmeyer, M. Kästner, O. Bederski, R.A. Müller, H. Moormann, Effects of plants and microorganisms in constructed wetlands for wastewater treatment, Biotechnol. Adv., 22 (2003) 93–117.
[22] M.L. Da Silva, R. Kamath, P.J. Alvarez, Effect of simulated rhizodeposition on the relative abundance of polynuclear aromatic hydrocarbon catabolic genes in a contaminated soil, Environ. Toxicol. Chem., 25 (2006) 386–391.
[23] E. Kurzbaum, Y. Zimmels, F. Kirzhner, R. Armon, Removal of phenol in a constructed wetland system and the relative contribution of plant roots, microbial activity and porous bed, Water Sci. Technol., 62 (2010) 1327–1334.
[24] H. Moormann, P. Kuschk, U. Stottmeister, The effect of rhizodeposition from helophytes on bacterial degradation of phenolic compounds, Acta Biotechnol, 22 (2002) 107–112.
[25] H. Saleem, M. Arslan, K. Rehman, R. Tahseen, M. Afzal, Phragmites australis — a helophytic grass — can establish successful partnership with phenol-degrading bacteria in a floating treatment wetland, Saudi J. Biol. Sci., In Press (2018).
[26] M. Afzal, S. Khan, S.I.M.S. Mirza, Q.M. Khan, Inoculation method affects colonization and activity of Burkholderia phytofirmans PsJN during phytoremediation of diesel-contaminated soil, Int. Biodeterior. Biodegradation, 85 (2013) 331–336.
[27] O.M. Ontañon, P.S. González, L.F. Ambrosio, C.E. Paisio, E. Agostini, Rhizoremediation of phenol and chromium by the synergistic combination of a native bacterial strain and Brassica napus hairy roots, Int. Biodeterior. Biodegrad., 88 (2014) 192–198.
[28] H. Saleem, K. Rehman, M. Arslan, M. Afzal, Enhanced degradation of phenol in floating treatment wetlands by plant-bacterial synergism, Int. J. Phytorem., 20 (2018) 692–698.
[29] Y.N. Ho, J.L. Hsieh, C.C. Huang, Construction of a plant-microbe phytoremediation system: combination of vetiver grass with a functional endophytic bacterium, Achromobacter xylosoxidans F3B, for aromatic pollutants removal, Bioresour. Technol., 145 (2013) 43–47.
[30] A. Ullah, S. Heng, M.F. Hussain Munis, S. Fahad, X. Yang, Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review, Environ. Exp. Bot., 117 (2015) 28–40.
[31] R.B. Baird, A.D. Eaton, E.W. Rice, Standard Methods for the Examination of Water and Wastewater, 23rd ed., American Water Works Association/American Public Works Association/Water Environment Federation, New York, 2017.
[32] A. Baxter, R. Mittler, N. Suzuki, ROS as key players in plant stress signalling, J. Exp. Bot., 65 (2014) 1229–1240.
[33] S. Singh, J.S. Melo, S. Eapen, S.F. D'Souza, Phenol removal using Brassica juncea hairy roots: role of inherent peroxidase and H_2O_2, J. Biotechnol., 123 (2006) 43–49.
[34] B. Chance, A.C. Maehly, Assay of catalases and peroxidases, Methods Enzymol., 2 (1955) 764–775.
[35] R.G. Alscher, N. Erturk, L.S. Heath, Role of superoxide dismutases (SODs) in controlling oxidative stress in plants, J. Exp. Bot., 53 (2002) 1331–1341.
[36] H. Kuthan, H.-J. Haussmann, J. Werringloer, A spectrophotometric assay for superoxide dismutase activities in crude tissue fractions, Biochem. J., 237 (1986) 175–180.
[37] A.L. Truant, Manual of Commercial Methods in Clinical Microbiology International Edition, John Wiley & Sons, 2016.
[38] M. Chérif, Y. Tirilly, R.R. Bélanger, Effect of oxygen concentration on plant growth, lipidperoxidation, and receptivity of tomato roots to Pythium F under hydroponic conditions, Eur J Plant Pathol., 103 (1997) 255–264.
[39] M.C.N. Saparrat, M.J. Martínez, H.A. Tournier, M.N. Cabello, A.M. Arambarri, Production of ligninolytic enzymes by Fusarium solani strains isolated from different substrata, World J Microbiol Biotechnol., 16 (2000) 799–803.
[40] H. León-Santiesteban, R. Bernal, F.J. Fernández, A. Tomasini, Tyrosinase and peroxidase production by Rhizopus oryzae strain ENHE obtained from pentachlorophenol-contaminated soil, J. Chem. Technol. Biotechnol., 83 (2008) 1394–1400.
[41] A. Conesa, C.A.M.J.J. van den Hondel, P.J. Punt, Studies on the production of fungal peroxidases in Aspergillus niger, Appl. Environ. Microbiol., 66 (2000) 3016–3023.
[42] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile-dyecontaining effluents: a review, Bioresour Technol., 58 (1996) 217–227.
[43] V. Kumar, R. Chandra, Characterisation of manganese peroxidase and laccase producing bacteria capable for degradation of sucrose glutamic acid-Maillard reaction products at different nutritional and environmental conditions, World J Microbiol Biotechnol., 34 (2018) 32.
[44] J. Lee, Biological conversion of lignocellulosic biomass to ethanol, J. Biotechnol., 56 (1997) 1–24.
[45] D.C. Kalyani, S.S. Phugare, U.U. Shedbalkar, J.P. Jadhav, Purification and characterization of a bacterial peroxidase from the isolated strain Pseudomonas sp. SUK1 and its application for textile dye decolorization, Ann. Microbiol., 61 (2011) 483–491.
[46] D.C. Kalyani, P.S. Patil, J.P. Jadhav, S.P. Govindwar, Biodegradation of reactive textile dye Red BLI by an isolated bacterium Pseudomonas sp. SUK1, Bioresour. Technol., 99 (2008) 4635–4641.
[47] R. Franco, J.A. Cidlowski, Glutathione efflux and cell death, Antioxid. Redox. Signaling., 17 (2012) 1694–1713.
[48] M.A. Kohanski, D.J. Dwyer, B. Hayete, C.A. Lawrence, J.J. Collins, A common mechanism of cellular death induced by bactericidal antibiotics, Cell, 130 (2007) 797–810.
[49] I.M. Moller, Plant mitochondria and oxidative stress: Electron Transport, NADPH Turnover, and Metabolism of Reactive Oxygen Specie, Annu. Rev. Plant Physiol. Plant Mol. Biol., 52 (2001) 561–591.
[50] G. Galati, O. Sabzevari, J.X. Wilson, J.P. O'Brian, Prooxidant activity and cellular effects of phenoxyl radicals of dietary flavonoides and other polyphenolics, Toxicol., 177 (2002) 91–104.
[51] R.K. Tewari, F. Hadacek, S. Sassmann, I. Lang, Iron deprivation-induced reactive oxygen species generation leads to non-autolytic PCD in Brassica napus leaves, Environ. Exp. Bot., 91 (2013) 74–83.
[52] V. Mittova, M. Tal, M. Volokita, M. Guy, Up−regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt−induced oxidative stress in the wild salt−tolerant tomato species Lycopersicon pennellii., Plant Cell Environ., 26 (2003) 845–856.
[53] X.M. Lu, P.Z. Lu, M.S. Huang, L.P. Dai, Seasonal variations and aeration effects on water quality improvements and physiological responses of Nymphaea tetragona Georgi., Int. J. Phytorem., 15 (2013) 522–535.