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REVIEW ARTICLE

Tween 80 surfactant-enhanced bioremediation: toward a solution to the soil contamination by hydrophobic organic compounds

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ABSTRACT

The occurrence of hydrophobic organic compounds (HOCs) in the soil has become a highly significant environmental issue. This problem has been exacerbated by the strong sorption of HOCs to the soils, which makes them unavailable for most remediation processes. More and more works show that surfactant-enhanced biological technologies offer a great potential to clear up HOCs-contaminated soils. This article is a critical review of HOCs removal from soils using Tween 80 (one of the mostly used nonionic surfactants) aided biological remediation technologies. The review begins with a discussion of the fundamentals of Tween 80-enhanced desorption of HOCs from contaminated soils, with special emphasis on the biotoxicity of Tween 80. Successful results obtained by Tween 80-enhanced microbial degradation and phytoremediation are documented and discussed in section 3 and section 4, respectively. Results show Tween 80-enhanced biotechnologies are promising for treating HOCs-contaminated soils. However, considering the fact that most of these scientific studies have only been conducted at the laboratory-scale, many improvements are required before these technologies can be scaled up to the full-scale level. Moreover, further research on mechanisms related to the interaction of Tween 80 with degrading microorganisms and the plants is in high demand.

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Introduction

The ubiquitous occurrence of toxic and/or hazardous hydrophobic organic compounds (HOCs) in soil is a widespread environmental problem due to their potential toxicity, carcinogenicity, mutagenicity and ability to be bioaccumulated in the food chain [1]. HOCs such as polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs) and some pesticides are characterized by a high organic carbon/water partition coefficient (K_{oc}). Because of the low water solubility, HOCs tend to be adsorbed onto solid particles and then pose a long-term threat to ecological safety and human health [2].

The treatment of HOCs-contaminated soil using efficient and environmentally friendly remediation technologies is a major challenge. Traditional techniques like landfilling, chemical oxidation and thermal treatments are costly and damaging to the environment [3,4]. In fact, currently there is no remediation technology that has been tested on the full scale. Bioremediations are the cheapest technologies and are consequently considered to be a good alternative to treating a soil that is polluted with HOCs [5]. However, the strong sorption of HOCs onto the soil matrix represents a significant limitation to bioremediation technologies [6,7]. Surfactants are able to enhance the apparent solubility of hydrophobic pollutants in water. Therefore, researchers often use surfactants to desorb HOCs from the soil, thus enhance the bioremediation efficiency.

Nonionic surfactants have been proved more suitable for soil remediation than either cationic or anionic surfactants [8], since cationic surfactants could be sorbed onto soil particles [9], while anionic surfactants can be precipitated with divalent cations in soil [10]. Among these nonionic surfactants, polyoxyethylene-(20)-sorbitanmonooleate (Tween 80, $C_{64}H_{124}O_{26}$) has gained particular interest. Tween 80 is of low cost and low toxicity on soil microorganisms as compared to most of other nonionic surfactants [11]. Due to these characteristics, numerous studies have been conducted to investigate the application of Tween 80-enhanced bioremediation of HOCs-contaminated soils. The interest of researchers for this technology continues since

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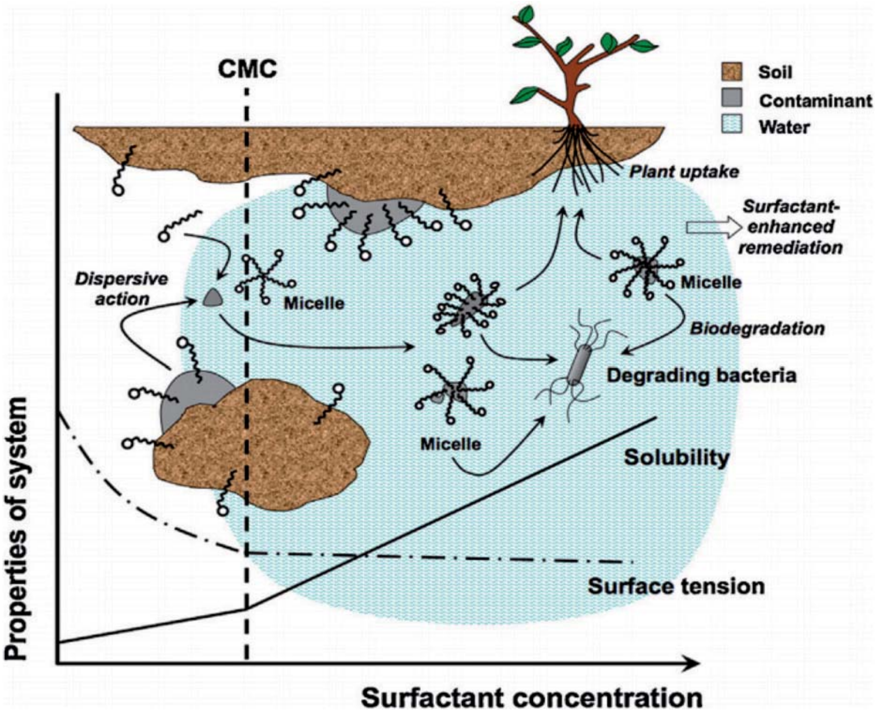

Figure 1. Schematic of Tween 80-enhanced remediation of contaminated soils. Adapted with permission from references [13]. Copyright 2015 Elsevier.

the number of relevant publications is still rising considerably. Several papers [2,12–14] and few book chapters [15,16] have summarized data on the performance of surfactant-enhanced bioremediation technologies. However, to date, a sole evaluation of the studies on Tween 80-enhanced bioremediation of HOCs-contaminated soils has not been reported. Some of the newly developed Tween 80-enhanced bioremediation technologies are not included in these reviews.

In this review paper, we present a general and rigorous review on this topic. First, an overview of the application of Tween 80 to the soil, with special emphasis on the biotoxicity of Tween 80, is described to discuss the fundamentals of the Tween 80-related bioremediation technologies. Tween 80-enhanced microbial degradation and phytoremediation processes are also detailed to clarify the development and state of the art of these technologies. Challenges and outlook of all these technologies are offered in order to inspire more exciting future developments in this very promising field.

Application of Tween 80 to the soil–water system

Desorption and solubilization of the sorbed HOCs

In a soil–water system, the hydrophilic group of Tween 80 tends to be inserted into the aqueous phase and the lipophilic group tends to combine with hydrophobic pollutants or soil particles, as shown in Figure 1 [13]. At low concentrations, Tween 80 exists solely as monomers, and mainly accumulates at the solid–liquid interface. Under this condition, repulsion between the hydrophilic group of the Tween 80 molecule and the pollutants can promote the desorption of HOCs from the soil particle [17]. When the Tween 80 concentration in the aqueous phase further increases above a critical value, known as the critical micelle concentration (CMC, 13.0 mgL^{-1}), Tween 80 micelles emerge [18]. In the soil–water system, the core of the Tween 80 micelle can strongly compete with soil particles for the partitioning of HOCs. The Tween 80 micelles solubilized HOCs have better mobility, thus exhibiting better availability to subsequent biological treatment (e.g. microbial degradation and plant uptake shown in Figure 1 [13]).

The biotoxicity of Tween 80

Considerable studies have evaluated the potential toxicological impact on soil microorganisms [19–22]. In a previous study, Garon et al. [23] investigated the tolerance of the anionic surfactant sodium dodecyl benzene sulfonate (SDBS) and the nonionic surfactant Tween 80 by 18 fungal strains. The results showed that fungal growth was significantly inhibited by SDBS, while Tween 80 were well tolerated by most of the tested strains at concentrations up to $20 \times$ CMC. The low tolerance of the anionic surfactant was likely due to physicochemical interactions between the anionic surfactants and fungal structures such as walls and membranes. On the contrary, it was reported that both the Tween 80 molecule and micelles have no negative effect towards fungal structures, even when the Tween 80 concentration reached to 5000 mg L^{-1} [24].

In most cases, Tween 80 showed no obvious toxicity to the pollutant-degrading microorganisms, while some other nonionic surfactants (e.g. Triton X-100, Brij-35 and Brij-30) resulted in significant microorganism growth inhibition under similar conditions [25]. Some strains could utilize hydrophobic fractions of Tween 80 as a carbon source [26]. However, there are exceptions. A recent study has shown that Tween 80 can solubilize protein and lipid components of cellular membranes of some bacteria, destroying their structure and thereby causing the bacteria's death [27]. In another study, Kumari et al. [28] found that the protein content and enzyme activity in soil with the addition of Tween 80 was almost reduced to half the value without Tween 80 addition. This inhibitory effect is likely due to surfactant toxicity and/or increased toxicity of the pollutants (due to the application of Tween 80) to the pollutant-depredating microorganisms.

Tween 80 treatments appeared to have no inhibition on the plants' growth. Greenhouse microcosm studies show in the range of tested concentrations (500–2500 $mgkg^{-1}$), Tween 80 has no apparent toxicity to *Trifolium pretense* L. growth [29]. Similar results have recently been found by Agnello et al. [30], who reported that Tween 80 (3 × CMC) did not have significant effects on plant (*Medicago sativa* L.) germination and chlorophyl levels. In some cases, plant biomass yields with addition Tween 80 were even higher than without Tween 80 [7]. It was suggested that Tween 80 can increase root permeability, leading to more efficient uptake of nutrients from the soil [31]. Besides, Tween 80 is likely to provide a suitable carbon source for some plants [29].

The feasibility of Tween 80 treatment is also enhanced by the fact that it will not bring a long-term impact on soils. It was found that Tween 80 proved to be extremely biodegradable under aerobic and anaerobic conditions also by indigenous microorganisms [32,33]. Franzetti et al. [32] assessed the solid-phase biodegradability of Tween 80 by the indigenous microorganisms and found that the biodegradation kinetics for Tween 80 in the soil is quite similar to a first-order kinetics, which allows calculation of a corresponding half-life of 35 ± 2 d. Gennaro et al. [33] conducted slurry phase experiments in order to determine the biodegradation kinetics of Tween 80 by landfill soil microorganisms and also observed a first-order kinetics with a kinetic constant equal to 0.029 d^{-1}.

Bioremediation using microorganisms

Due to its cost-effectiveness, bioremediation using microorganisms (fungi and bacteria mainly) has been recognized as the most used process for degrading various organic pollutants in wastewater [34]. However, these technologies could be inefficient for soil remediation since HOCs in soil usually bind tightly onto soil particles, making them inaccessible to microorganism degraders [35]. According to the literature, Tween 80 can enhance the bioavailability of HOCs in model and real soils with different success. Besides, Tween 80 also plays an important role in microbial growth and degradation of HOCs.

Tween 80-enhanced fungal biodegradation

The addition of Tween 80 to enhance the bioavailability of HOCs has received considerable attention in soil mycoremediation. Zheng et al. [36,37] performed studies in which Tween 80 was used to promote the white rot fungus *P. chrysosporium* degradation of PAHs in the contaminated soil, producing successful results with high PAHs removals. Tween 80 has enhanced the solubility of PAHs and did not affect the growth of *P. chrysosporium*. Further studies also showed that Tween 80 did not affect the production of ligninolytic enzymes that are responsible for the degradation of PAHs [38–40]. Particularly, Tween 80 was able to promote the oxidation ability of manganese peroxidase (MnP), one of the most important white rot fungi's extracellular enzymes [41,42]. In this process, Tween 80 provides the carbon–carbon double bond (C = C) and an unsaturated fatty acid chain may be readily oxidized by MnP to form a peroxide (H_2O_2) and subsequently turned it into a peroxyl radical (HOO•), which is the actual oxidant for the degradation of the nonphenolic model compound [43].

Apart from *P. chrysosporium*, many other fungal strains (e.g. *Pleurotus ostreatus* [44], *Polyporus* sp. S133 [45], *Trametes versicolor* [24], *Pestalotiopsis sp.* NG007 [46], *Lasiodiplodia theobromae* [47]) have been applied together with Tween 80 to degrade HOCs in the soil. A good example can be found in a study by Wang et al. [47] in which the total removal rate of PAHs by a novel fungus (*Lasiodiplodia theobromae*) reached 53.3%, which is 13.1% higher than that achieved by *P. chrysosporium*. It was reported that Tween 80 can not only enhance PAHs bioavailability but also increase the population of *Lasiodiplodia theobromae* and enzymes secretion from *Lasiodiplodia theobromae*. In this experiment, Tween 80 enhanced the activity of all the four enzymes tested, including laccase, which is considered to be more responsible for the degradation of PAHs compared to the other enzymes [48]. Indeed, Tween 80 has been previously determined to be effective mediators for laccase, and the effects were due to the transient formation of particularly reactive thiyl radicals (RS·) [48]. On the other hand, several researchers have suggested further that Tween 80 could provide a suitable carbon source for fungi [49].

Tween 80-enhanced bacterial biodegradation

In Tween 80-enhanced soil bioremediation, Tween 80 also posed biological aspects to change the bacterial cell surface properties [50]. Some researchers speculated that the effect of Tween 80 on bacterial biodegradation is related to cell surface hydrophobicity (CSH) [50]. On the one hand, CSH is strictly correlated with the contact between HOCs molecules and bacteria. The contact of HOCs-molecules and the cells is extremely important for biodegradation to take place outside the bacterial cells. On the other hand, CSH is a critical factor affecting the uptake of HOCs through transmembrane transport, which is a crucial step for biodegradation to occur inside the bacterial cells [51]. Recently, several studies have conclusively demonstrated the ability of Tween 80 to alter CSH [52,53]. It was believed that the surfactant changes bacterial CSH mainly by releasing of lipopolysaccharide from bacterial cell surface or absorbing surfactant molecules on the cell surface [54]. It is worth noting that a higher concentration of Tween 80 not always corresponded to a higher CSH. Zhang and Zhu [50] showed that CSH in *Klebsiella oxytoca* PYR-1 increased initially and then slightly decreased with increasing Tween 80 concentration from $0.2 \times$ CMC to $8 \times$ CMC. Zhao et al. [53] also found that CSH of *Bacillus licheniformis* B-1 decreased when the concentrations of Tween 80 in the soil–water system were higher than 1200 mgL^{-1}. The possible explanation for this phenomenon is that the adsorption of surfactant molecules or micelles onto the cell surface decreased CSH [54].

In a recent study, the effects of two nonionic surfactants (Brij-35 and Tween-80) and β-cyclodextrin (β-CD) on the CSH of *Bacillus licheniformis* and the degradation of beta-cypermethrin (β-CY) were studied [53]. Results show the strain can only degrade β-CY that was dissolved in the aqueous phase in the absence of β-CD and surfactants, which led to the lowest β-CY removal among the tests. The enhancement on CSH and β-CD solubility consequently caused the increase in β-CY degradation in the soil–water system. The most efficient chemical agent for promoting β-CY biodegradation was identified as Brij-35, despite that the highest enhancements on CSH and β-CD solubility were found in the Tween 80 group. Two mechanisms can be responsible for this phenomenon [53]. Firstly, the utilization of β-CY by *Bacillus licheniformis* cells would be decreased since this strain preferentially utilized Tween 80 during degradation. Secondly, the contact between the cells and β-CY was reducing when Tween 80 concentration in the system decreased as the time was prolonged.

Negative effects of Tween 80 on the biodegradation

It is typically accepted that the solubilization of pollutants in the soil–water system occurs at Tween 80 doses much higher than the clean water CMC value. For instance, Piskonen and Itävaara [55] found that using four types of surfactant (Tween 80, Brij 35, Triton X-100 and Tergitol NP-10) at lower concentrations of CMC cannot significantly improve the PAHs bioremediation in contaminated soils. In most cases, excess Tween 80 doses were used to acquire high solubilization of the pollutants. However, it should be noted that Tween 80 at higher concentrations might inhibit the biodegradation of pollutants [46,49,50]. For example, the degradation of asphalt by *Pestalotiopsis* sp. NG007 dropped from 65.8% to 43.0% when increasing Tween 80 doses from 1.0% to 1.5% [46]. This concentration effect was firstly noted by Guha and Jaffe [56], who showed that the bioavailability of phenanthrene decreased with increasing the concentrations of the four different surfactants. This phenomenon can be due to the inhibition of mass transfer from Tween 80 micelles to the cells or enzymes. The mass transfer process is proportional to the concentration gradient, which depends on the concentration of the pollutants in the micellar phase [57]. Therefore, as the pollutants are diluted in a large micellar mass, the transfer of pollutants to the cells or enzymes decreased [58]. The other reason for this phenomenon might be that Tween 80 has toxic effects at higher concentrations [46]. As reported by Yanto and Tachibana [46], the biomass of *Pestalotiopsis* sp. NG007 was reduced from 0.486 g to 0.394 g when increasing Tween 80 doses from 1.0% to 1.5%.

In some cases, Tween 80 exhibited totally inhibitory effects on biodegradation [28,59–60]. For example, in a recent study, even though the desorption of dichlorodiphenyltricgloroethane (DDT) from the soil was promoted by Tween 80, DDT biodegradation was significantly inhibited [59]. This is in accord with the observations by some other researchers when studying the bioremediation of PAHs contaminated soils [60]. The same behavior has also been observed in pure

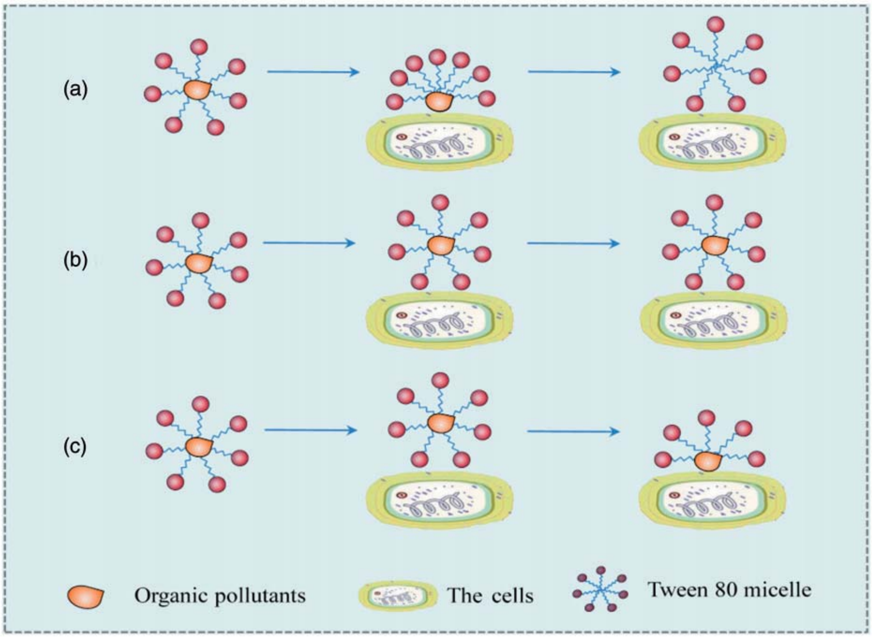

Figure 2. Mechanism of bioavailability of the hydrophobic organic contaminant, partitioned in the micellar phase of Tween 80. (a) The micellar-phase contaminant is directly available to the cell. (b) The micellar-phase contaminant is not available to the cell. Tween 80 micelle is toxic to the cell and prevents the contact between the cell and contaminant molecule. (c) The micellar-phase contaminant is not directly available to the cell. The cell uptakes of Tween 80 preferentially.

liquid phase. For instance, Xiao et al. [61] showed that biodegradation of phenanthrene by *Sphingomonas* decreased from 83.6% to 33.5% with the addition of Tween 80. The possible explanations for this inhibition include: (i) application of Tween 80 to the soil may decrease the microbial populations responsible for degradation, as mentioned earlier, Tween 80 is known to exhibit negative effects on the growth of some strains due to the surfactant toxicity [46,58], (ii) Tween 80 is a preferred carbon source for some degrading microorganisms in comparison to pollutants such as pyrene and phenanthrene [61], consequently, a preferred degradation of Tween 80 would inhibit the direct cell-pollutants contact and (iii) the micellar-solubilized HOCs show lower availability to the microorganisms [59]. Different types of models have been proposed to describe the biodegradation of micellar-phase HOCs. As shown in Figure 2(a), in some cases, HOCs partitioned into the micellar phase of Tween 80 can be directly delivered into the cell and biodegraded [62]. Therefore, Tween 80 solubilization of sorbed HOCs from soil can enhance HOC biodegradation effectiveness. However, for some types of microorganisms, the micellar-phase HOCs are not bioavailable (Figure 2(b)) or directly bioavailable (Figure 2(c)) to them. The potential mechanisms for the inhibition include toxicity of Tween 80 to microorganisms and preferential microbial uptake of Tween 80 as the substrate [63]. In these cases, Tween 80 solubilization of HOCs may not be beneficial for the biodegradation.

Biodegradation of HOCs in Tween 80-enhanced soil-washing effluent

Over the last two decades, the applications of surfactants-enhanced soil washing or flushing technology have increased significantly [64]. Many researchers [65–68] have pointed out the preeminent role of Tween 80 for the extraction of HOCs from soils among the tested nonionic surfactants. This is believed to be due to Tween 80's lower CMC and higher hydrophile–lipophile balance compared to the other surfactants [65].

The Tween 80 enhanced soil-washing/soil-flushing process is generally efficient to extract the pollutants but not to destroy them, thus a combined treatment is needed. In the literature, various studies have reported the use of biodegradation to remove HOCs from soil-washing/soil-flushing effluents [69–74]. In the solid- or slurry-phase biodegradation systems, in some cases, as Tween 80 concentration decreases due to the biodegradation, the pseudosolubilized HOCs within Tween 80 micelles might rebind to soil organic matter (SOM) and once again have limited bioavailability [74]. Under such circumstance, biodegradation after the soil-washing/soil-flushing process would offer two advantages: (i) the effluent has a negligible soil content, and therefore, the pollutants would not rebind to SOM, and (ii) the microbial degradation of Tween 80, the pollutants would bind to the cells and be degraded. The Tween 80-enhanced soil washing has been shown to be effective in the extraction of HOCs from soil, and biodegradation has been successfully tested for removal of PAHs [72] and endosulfan [69] from the effluent. In the first study, the joint washing and microbial degradation gave total PAH removal of 92.6%. In the second study, the 15 days biologic treatment removed 92.91% of endosulfan from the washing effluent.

A critical element of this combined technology is the influence of soil properties. Previous studies have reported high HOCs removals from the soil by washing, but these results were mostly obtained from spiked soils [55,65,75]. The results in some studies show that it is hard to achieve effective decontamination of real-polluted soils with relative high SOM concentrations using Tween 80-enhanced washing. In a recent study, only 9.1% of total PAHs were removed from the real-polluted soil (organic carbon content =11.8%) using 2.5% Tween 80 solution [72]. It was suggested that the HOCs can be strongly sorbed to SOM, and as a result, their desorption rates from the soil matrix to the aqueous phase is decreased. According to the latest study, this problem could be solved by reusing Tween 80 solution in the washing effluent. Gharibzadeh et al. [76] studied the reuse possibility of biologically treated washing effluent for the next cycles of soil washing process and obtained very promising results. It was observed that the seventh cycle of extraction increased removal efficiency of phenanthrene to >97% in a real-contaminated soil.

Remarks and future prospects

In summary, numerous applications of Tween 80-enhanced biodegradation processes have been reported in the literature. Table 1 summarizes some of the most significant studies on Tween 80-enhanced soil biodegradation processes and includes the experimental conditions and the most important results obtained.

A previous review shows biodegradation technologies are economically feasible soil remediation but are not currently robust, and much attention should be paid to parameters such as the bioavailability of the pollutants [77]. Over the last two decades, the application of Tween 80 to enhance the bioavailability of soil organic pollutants has received significant attention. In most cases, Tween 80 shows no negative effects on the growth of degrading microorganisms, in some cases, it even brings about positive effects. Many studies have demonstrated that Tween 80 could improve bacterial cell surface properties and promote the oxidation ability of fungi's extracellular enzymes. Note, however, that the application of Tween 80 to biodegradation does not always yield positive results. In several experiments described in the literature, Tween 80 resulted in the inhibition of the biodegradation [55,59,60]. The inhibitory effect is likely due to the toxicity of Tween 80 on the pollutants-degrading microorganisms or the preferential microbial uptake of Tween 80 as substrate.

Recently, there has been growing interest in using biodegradation to remove HOCs in the washing effluent. Under certain conditions, the combined use of Tween 80-enhanced soil washing and biodegradation may present advantages over Tween 80-enhanced soil–water biodegradation. However, the efficiency of this combined technology depends largely on SOM content. Recently, the combination of a sequential soil washing followed by bioremediation of the washing effluent was developed to overcome this limitation. The results show this technique is promising for large-scale applications. However, important challenges remain to be overcome including the soil adsorption of Tween 80 and nonselective biodegradation of Tween 80 in the washing effluent. Significant effort should also be devoted to consider important aspects of this technology such as energy consumptions and operation problems.

Tween 80-enhanced biodegradation is suitable for remediating HOCs-contaminated soils but many factors affect the results. Despite the good results that have been obtained in the laboratory, many improvements are required before these technologies can be scaled up to pilot level. In most cases, spiked real or synthetic soils other than the real-polluted soil occurring in the natural environment were used. However, huge differences have been obtained between HOCs removal efficiencies in a spiked soil and an actual polluted soil. A good example can be found in the studies of Wang et al. [47] in which the same Tween 80-enhanced bioremediation method using *Lasiodiplodia theobromae* yielded 92.1% removal in spiked clay loam, compared to 53.3% removal for the actual polluted sandy loam. Therefore, in the near future, significant effort should be devoted to studying the differences between treating real polluted and spiked soils. In addition, many issues still need to be investigated at the laboratory scale. For example, the mechanism underlying the inhibitory effect of Tween 80 to biodegradation needs further research, especially on Tween 80's toxicity to some types of PAHs-degrading bacteria. Besides, a comprehensive understanding of the effects of Tween 80 concentration on the biodegradation performance is of crucial importance.

Table 1. Overview of studies conducted on the remediation of HOCs contaminated soils by Tween 80-enhanced microbial degradation.

Microorganism	Pollutant/concentration/ type of soil	Operation conditions	Remarks	References
Indigenous microorganisms	TPHs/15 gkg^{-1}/real polluted sandy loam	1% (v/v) of Tween 80, 50 g of soil, moisture content = 60%, room temperature, 98 d.	Tween 80 promoted the growth of soil microorganisms. Tween 80 increased ^{14}C-hexadecane degradation from 9% to 36%.	[19]
Pseudomonas stutzeri BP10	Pyrene/100 $mgkg^{-1}$/spiked garden soil	200 $mgkg^{-1}$ of Tween 80, 100 g of soil, 37 °C, 28 d.	Tween 80 reduced dioxygenase enzyme activity to about 38%. Tween 80 reduced the degradation of pyrene by 35.	[28]
Heterotrophic bacteria	PAHs/70.38 $mgkg^{-1}$/real-polluted landfill soil	0, 0.5, 1, 2, 4 gL^{-1} of Tween 80, 4 g of soil, 30 °C, 5 d.	Significant increases in the PAH apparent solubility were observed only in experiments with Tween 80 concentration above 2 gL^{-1}.	[32]
Pestalotiopsis sp. NG007	Asphalt/1000–30,000 $mgkg^{-1}$/ spiked sandy soil	1–3% (v/v) of Tween 80, 30 g of soil, 30% (w/w) distilled water, 25 °C, 120 d.	Tween 80 increase ligninolytic enzymes activities. High concentration of Tween 80 inhibited the biodegradation of asphalt due to its negative effects on fungal growth.	[46]
Bacillus licheniformis B-1	Beta-cypermethrin (β-CY)/ 20 mg kg^{-1}/spiked farmland soil	0, 0.3, 0.6, 1.2, 2.4 gL^{-1} of Tween 80, Brij-35 or β-cyclodextrin, 1 kg of soil, 26 °C, 22 d.	Tween 80 could be used as a carbon source by the strain to stimulate its growth. The highest CSH value (69.89%) occurred at 1.2 gL^{-1} of Tween 80.	[53]
Lasiodiplodia theobromae	Benzo[a]pyrene/50 mg kg^{-1}/ spiked clay loam	0, 0.5, 1, 2, 3 and 5 gkg^{-1} of Tween 80, 200 g of soil, 28 °C, 18–55 d.	Tween 80 could increase the population of *L. theobromae* and enzyme secretion from *L. theobromae*. Addiction of 5 gkg^{-1} of Tween 80 increased BaP removal rates from 32.1% to 92.1%.	[47]
Indigenous microflora	DDT/99.46 $mgkg^{-1}$/real-polluted sandy soil	6.55 $mgkg^{-1}$ of Tween 80, 2 kg of dry soil, 56 d.	Tween 80 addition showed an inhibitory effect on the degradation of DDT. Addiction of Tween 80 decreased DDT removal rates from 94.3% to 79%.	[59]
Ochrobactrum sp.	α-endosulfan/18.9 $mgkg^{-1}$/ spiked soil	Effluent: 1500 mgL^{-1} of Tween 80, 30 mgL^{-1} of endosulfan; Biodegradation: 1 gL^{-1} of glucose, 35 °C, 15 d.	86.83 ~ 92.91% of α-endosulfan in the eluents was removed after 15 days biodegradation. The microbial degradation process can be well described by the first-order kinetic model.	[69]
Mycobacterium sp.	PAHs/total 258.0 ± 21.8 $mgkg^{-1}$/real-polluted sandy soil	Washing: 5 kg of soil, 50 L of 2.5% (w/v) Tween 80. Biodegradation: 28 °C, 30 d.	The joint application of Tween 80 and biodiesel showed higher desorption capacity for PAHs. The combined washing and biodegradation gave a total PAH removal of 92.6%.	[72]
Bacterial consortium	Phenanthrene/500 $mgkg^{-1}$/ spiked sandy soil	Washing: 0.5–10% (w/w) of Tween 80, 180 rpm, 2h. Biodegradation: 180 rpm, 25 ± 1 °C, 7d.	A removal efficiency of 74.4%±3.5% was obtained by washing. The reusability of the biologically recycled solution was maintained up to 7th cycle, reaching a removal efficiency of >99%.	[76]

Phytoremediation

Another type of bioprocess (phytoremediation) in which plants are used to treat the contamination also appears to be a promising technology that is ecologically sound and cost-effective [78]. Following the pioneering work of Bacci and Gaggi [79] in 1985, when plants were shown to the translocation of PCBs from soil, many studies have been published on using phytoremediation technology to deplete HOCs in soil [80,81]. Phytoremediation utilizes plants to stabilize, transform or remove contaminants located in soils, sediments or water [82]. The different phytoremediation processes are shown in Figure 3 [83]. In brief, HOCs in soil can either be adsorbed to the roots and taken up inside the

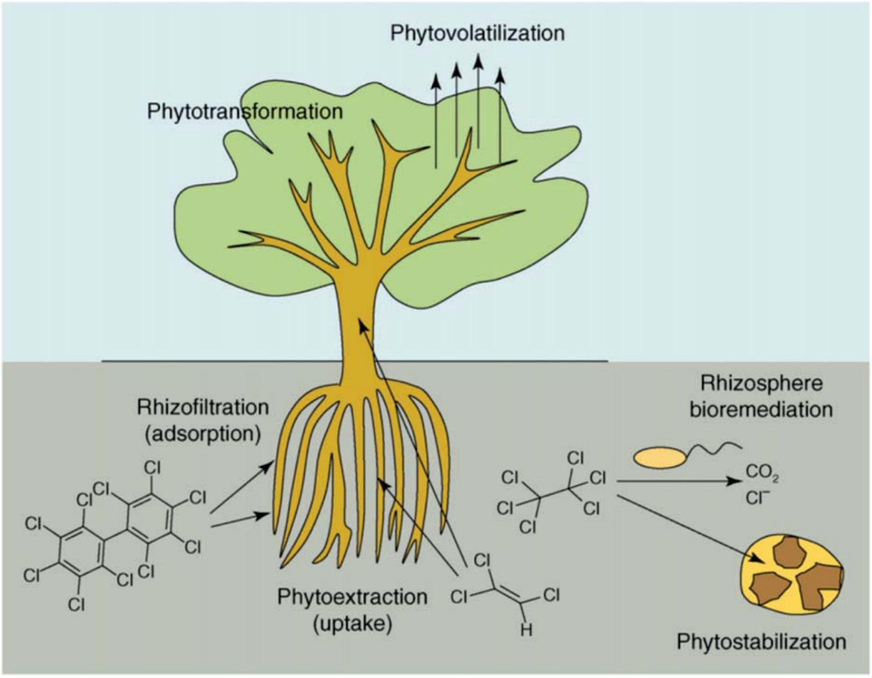

Figure 3. The processes involved in phytoremediation. Phytoextraction: plant uptake of pollutants from soil inside the plant tissues. Rhizofiltration: the adsorption of pollutants to the roots. Phytotransformation: the transformation of pollutants inside plant tissues by plant enzymes. Phytovolatilization: the volatilization of pollutants inside plant tissues into the atmosphere. Rhizosphere bioremediation (rhizodegradation): the degradation of pollutants in soil by microbes in the root zone. Phytostabilization: the incorporation of pollutants into soil materials. Adapted with permission from references [83]; Copyright 2008 Elsevier.

plant tissues (phytoextraction) or degraded by the microorganisms in the root zone (rhizoremediation).

Phytoremediation only requires modest nutrient input, but it offers many potential beneficial side effects, such as carbon sequestration and feedstock for biofuel production [84]. More importantly, plants can also supply nutrients for the rhizosphere microorganisms. It was reported that about 40% of a plant's photosynthate could be exuded by roots into the soil as sugars, aromatic compounds and organic acids, which are rich in energy and carbon for microorganisms' growth [85].

Tween 80-enhanced phytoextraction

Mass transfer and the bioavailability of HOCs for plant uptake and microbial biodegradation are the primary limitations on phytoremediation of soils contaminated with HOCs. HOCs in the contaminated soils are mostly bound to soil particles, which are believed to be inaccessible to plant roots. For example, Cheng et al. [86] observed that *Agropyron elongatum* uptake of the phenanthrene and pyrene was insignificant when studying the removal of PAHs from soil by pig manure compost-enhanced phytoremediation. To predict plant uptake of organic contaminants, several researchers developed experimental relationships based on log K_{ow} [87]. Based on their theory, only "moderately hydrophobic" organic compounds ($0.5 < \log K_{ow} < 4.5$) could be significantly taken up by plant roots. Also, according to this theory, plant uptake of organic compounds with log $K_{ow} > 4.5$ will rapidly decrease with the increase of hydrophobicity.

In some greenhouse studies, the presence of Tween 80, even at very low concentrations, resulted in significant positive effects on plants uptake of HOCs. A good example can be found in a study by Romeh [88] in which $0.5 \times$ CMC of Tween 80 solution produced a remarkable promotion of azoxystrobin uptake. The uptake ratio of the Tween 80-enhanced phytoremediation was about 1.25 times higher than those of *Plantago major* treatment alone. Similar results have been found by other researchers [29,89,90], for instance, Yan et al. [29] demonstrated that the maximal plant uptake of phenanthrene in Tween 80 treatment ($1 \times$ CMC) was 2.16 times higher than in the Tween 80-free control group. There are observations that co-addition of carboxylic acids (citric and oxalic acids) can further increase a plant's uptake of HOCs [91]. In such conditions, carboxylic acids can cause release of organic carbon from the soil, which, in turn, leads to higher pollutant availability in the soil solution.

Tween 80 was also used together with plant growth stimulating substances (e.g. gibberellic acid (GA_3) [90], silicon dioxide [89] and pig manure compost [86,92,93]) to enhance the phytoremediation of HOCs contaminants. The combined treatment is expected to improve both plant growth and the bioavailability of pollutants. In a promising study, conducted by Sun et al. [90], the combination of Tween 80 (5 $mmolkg^{-1}$) and GA_3 (plant hormone, 1 $mmolkg^{-1}$) increased plant growth by 55% (17% caused by the function of Tween 80) in terms of shoot biomass, compared with the control group. Consequently, the corresponding Benzo[a]pyrene accumulation in the roots, stems, leaves and shoots increased by 4.14–5.86, 1.86–2.31, 0.67–1.22 and 1.33–1.55 times, respectively, compared with the control plant.

Effect of Tween 80 on rhizodegradation

Considerable research has demonstrated that rhizodegradation plays an important role in the remediation of soils contaminated with organic compounds [94,95]. Ramamurthy and Memarian [96] conducted greenhouse phytoremediation experiments on engine oil-contaminated soil following different treatments, including addition of nonionic surfactants Triton X-100 and Tween 80 at various concentrations. The authors observed that Tween 80 concentrations are positively correlated with rhizodegradation rates of engine oil. However, opposite trend between surfactant concentrations and rhizodegradation rates and were found in Triton X-100 groups. To further investigate the effects of surfactants on rhizodegradation, the authors [97] studied the changes of basal soil respiration (BSR) rate. Results show the addition of Tween 80 resulted in an enhanced activity of microbe in terms of BSR rate, while an increase in Triton X-100 concentration resulted in a significant decrease in BSR rate. In the experiments, Tween 80 presented no significant impact on plant growth; however, it seems to have the positive effect on the population of soil microorganisms [96,97]. It is most likely that Tween 80 could provide a suitable carbon source for the microorganisms [84].

Recently, there has been greater interest in using phytoremediation to treat heavy metal and organic compounds cocontaminated soils, which was found to yield good efficiencies, especially with the addition of low-molecular-weight (LMW) organic acids and surfactants [98–100]. A detailed study was recently published by Agnello et al. [98], in which pot-culture experiments were designed to assess the phytoremediation potential of *Medicago sativa* in a heavy metal–petroleum hydrocarbon cocontaminated soil. The authors studied the influence of Tween 80 and citric acid applied individually and together on rhizodegradation of petroleum hydrocarbons. The results showed that the single application of Tween 80 had a negative effect on the population of petroleum-degrading microorganisms [98]. The mechanism underlying this inhibitory effect is the increase in root permeability caused by Tween 80 can lead heavy metals to exert plant toxicity. Interestingly, this negative effect on plant growth could be avoided by the joint application of Tween 80 and citric acid [98]. This is in accord with the observation in a previous study, which indicated that chelating properties of LMW organic acid could alleviate heavy metals stress on plants [101]. As a result, the combination of Tween 80 and citric acid significantly improved petroleum-degrading microorganisms (2.4-fold increase) and enzyme activity (5.3-fold increase) in the rhizosphere of plants.

Remarks and future prospects

Extensive research has revealed the presence of Tween 80, even at low concentrations (sub-CMC), can lead to significant positive effects on phytoremediation for HOCs-contaminated soil. Table 2 is a compilation of the studies on phytoremediation and highlights the experimental conditions and the most important results obtained. Laboratory observations proved that the addition of Tween 80 solution to the soil can promote both the plant uptake and microbial degradation of HOCs. Further, the joint application of Tween 80 and plant growth stimulating substances, such as plant hormone, can provide a stronger capability to remove soil contaminants.

Use of Tween 80-enhanced phytoremediation to cleanup heavy metals-HOCs cocontaminated soils has recently become an important research topic. The coexistence of heavy metals in the soil may largely affect the removal of HOCs. Under such condition, the prevention of growth inhibition caused by heavy metals toxicity would be an important issue. According to some pioneering studies, the joint application of Tween 80, together with a heavy metals chelating agent (e.g. LMW organic acids) could be promising for the future phytoremediation of cocontaminated soils. However, to date, only limit information in the literature is available on this process. For instance, the link and correlation between degrading microorganisms enhancement and petroleum hydrocarbon removal in the presence of Tween 80 and citric acid is still unknown [98]. In future studies, more effort should be conducted on this field, since heavy metals-HOCs cocontamination is becoming a worldwide environmental issue.

Table 2. Overview of studies conducted on the remediation of HOCs contaminated soils by Tween 80-enhanced phytoremediation.

Plant	Pollutant/concentration/type of soil	Operation conditions	Remarks	References
Agropyron elongatum	pyrene/300 $mgkg^{-1}$/spiked sandy soil	0, 20 and 100 $mgkg^{-1}$ of Tween 80, 60% of water-holding capacity, 20–28 °C, 60 d.	Tween 80 had no impact on the germination and total biomass yield of the plant. Increase in Tween 80 doses increased the removal of pyrene.	[93]
Plantago major	Azoxystrobin/20 $mgkg^{-1}$/ spiked clay loam soil	9.2 mgL^{-1} of Tween 80, 500 g of air-dried soil, 50 mL of tap water every 4 days, 14 d.	The uptake and translocation ratio was about 1.25 and 1.19 times higher when compared with *Plantago major* alone on day 10, respectively.	[89]
Tagetes patula	Benzo[a]pyrene/5 $mgkg^{-1}$/ spiked agricultural soil	5 $mmolkg^{-1}$ of Tween-80, 1, 3 and 5 $mmolkg^{-1}$ of gibberellic acid, 1 kg of soil, capacity, 92 d.	1 $mmolkg^{-1}$ was the most suitable gibberellic acid concentration. The accumulation of Benzo[a]pyrene in shoot increased by 1.40–2.83 times.	[90]
S. humboldtiana	DDTs/101.3 ± 13.4 $mgkg^{-1}$/ real-polluted aridisol	9.2 mgL^{-1} of Tween 80, 1200 g of soil, 10–26 °C, natural sunlight (light:dark cycle 14:10 h), 40 d.	Some soil pollutant–surfactant interactions might occurred and hindered plant uptake of DDTs.	[92]
Agropyron elongatum	Pyrene/321 ± 12 $mgkg^{-1}$/ spiked sandy soil	100 $mgkg^{-1}$ of Tween 80, pots: 40 cm × 20 cm × 20 cm, 60% of water-holding capacity, 60 d.	Coaddition of Tween 80 and PMC increased the removal of pyrene in the soil from 12.1% to 90.3%. Tween 80 increased soil microbial activity.	[86]
Solanum nigrum	PAHs; 0.569 $mgkg^{-1}$/real-polluted soil	0.5 and 1.0 $mmolkg^{-1}$ of Tween 80, 25 ± 4 °C, 14-h dark at 19 ± 3 °C, 1.0 kg of soil, 80 d.	Both Tween 80 and salicylic acid increased the degradation of PAHs. Salicylic acid could alleviate the growth inhibition caused by Cd toxicity.	[95]
Medicago sativa	Pollution-free/sandy soil	0.25–3 × CMC of Tween 80, plastic pots: 10 cm diameter, 8 cm height, 200 g of soil, 16–30 °C, 56 d.	Tween 80 did not affect plant germination and chlorophyl levels. Tween 80 showed a trend to increase plant biomass.	[29]
Brassica juncea	Engine oil; $PbCl_2$/each 500 $mgkg^{-1}$/spiked sandy soil	0.5, 1 and 2 × CMC of surfactants, 1 kg of soil, day at 32 °C, night at 21 °C, 50 d.	Plant biomass yields were in order of Triton X-100 < Tween 80 < control. At the same concentrations, Tween 80 was more effective than Triton X-100 in facilitating rhizodegradation of engine oil.	[96]
Brassica juncea	Engine oil; $PbCl_2$/each 500 $mgkg^{-1}$/spiked sandy soil	0.5, 1 and 2 × CMC of surfactants, 2 kg of soil, day, 32 °C; night, 21 °C, 50 d.	Tween 80 slightly increased the basal soil respiration. Tween 80 is able to remove Pb (II) and thereby increase the rhizodegradation of engine oil under aerobic conditions.	[97]
Medicago sativa	Hydrocarbons/3600 $mgkg^{-1}$/ spiked sandy soil (commercial soil)	0.036 $mmolkg^{-1}$ of Tween 80, 15 $mmolkg^{-1}$ of citric acid, 200 g of soil, 16-h light at 22 °C and 8-h dark at 18 °C, 90 d.	The combination of citric acid and Tween 80 significantly improved alkane-degrading microorganisms (2.4 times) and lipase activity (5.3 times) in the rhizosphere of plants.	[98]

In summary, research in this field is at a critical stage. Most studies to date have been conducted at the laboratory scale using pot experiments. Recall that phytoremediation is a cost-effective and secure technology that can be applied for the in-situ cleanup of contaminated sites. If the Tween 80-enhanced phytoremediation can be performed *in situ*, less labor and transport would be required and the process would be ecologically acceptable. Thus, with the in-depth study in this field, Tween 80-enhanced phytoremediation can be a promising technology for the clean-up of soil contaminated with HOCs.

Conclusions

Due to its low cost, low toxicity, low polarity and high solubilization capacity characteristics, Tween 80 has frequently been used to enhance the removal of HOCs from soil. The micelle-solubilized HOCs in aqueous phase have better mobility, thus exhibited superior availability to subsequent biological treatments. In most studies, Tween 80 showed no obvious toxicity to the microorganisms or plants. Moreover, due to Tween 80 having potentially bioavailable carbon, in some cases, the application of Tween 80 could lead to higher biomass yield. In particularly, Tween 80 can enhance the removal efficiency by promoting the degradation ability of the microorganisms in question. Tween 80 is able to enhance the affinity of the pollutant-degrading bacterial cells with HOCs molecules and promote the oxidation ability of fungi's extracellular enzymes. Greenhouse studies have shown that Tween 80 could significantly promote plants uptake of HOCs from soil. Besides, with some improvements, Tween 80-enhanced phytoremediation can be applied for the cleanup of heavy metals-HOCs cocontaminated soils. Very promising results obtained in using these Tween 80-enhanced bioremediation techniques means that a solution to soil HOCs contamination is in progress. However, most of these studies have been conducted on the laboratory scale: thus, it would be injudicious to extrapolate these results to real applications without first performing scale-up studies. The current problems of Tween 80-enhanced bioremediation that hinder their utilization at the field level mainly includes: (1) Tween 80 solubilization may be not efficient for desorption of HOCs from aged soil especially with high SOM content; (2) Tween 80 might inhibit the biodegradation of HOCs due to its biotoxicity or the preferential microbial uptake of Tween 80 as a substrate; (3) most of these technologies have not been tested by field studies, in which the soil properties, weather and other environmental issues need to be taken into consideration, and (4) the system issues, such as economic or operational problems are not considered by most of the studies. In future studies, more attention should be devoted to scaling up these technologies to investigate their economic feasibility and problems arising from the scale-up.

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