SPME in Environmental Analysis: Biotransformation Pathways

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Abstract

Solid-phase microextraction (SPME) is an organic solvent-free sample preparation tool suitable for direct adsorption of analytes from the headspace or the aqueous phase of a matrix followed by desorption into a gas chromatograph (GC) or high-performance liquid chromatograph (HPLC) for subsequent analysis. The SPME technique is designed to accommodate the use of fibers coated with different polymers suitable for the extraction of chemicals with varied hydrophobic and polar properties. Also, the technique can minimize interference from other artefacts associated with complex samples, such as those encountered in biological matrices or reaction mixtures. The preceding characteristics of SPME make the technique suitable for real-time measurements of intermediate reaction products and, thus, able to provide insight into the fate of target chemicals and their degradation pathways. In the present article, the current state of knowledge on the use of SPME–GC and SPME–HPLC in the determination of frequently encountered environmental chemicals and their (bio)transformation pathways are critically reviewed. Future opportunities of SPME in real time in situ process monitoring such as the use of agricultural feed stocks to bio-based industrial products termed henceforth "process analytical chemistry" are also discussed.

Introduction

Most industrial chemicals, including those derived from petroleum and other fossil fuels, polychlorinated solvents, explosives, pesticides, and herbicides, are normally toxic, and are subject to several environmental natural attenuation processes (biodegradation, photolysis, and hydrolysis), leading to the formation of unknown products. To determine the environmental fate and impact of potential environmental contaminants, sensitive analytical methods that do not require the use of organic solvent and are suitable for application in aqueous-based matrices are thus required. Environmental samples are normally complex, and analytical techniques should be able to minimize interference from other co-extracted materials. Solid-phase microextraction (SPME), which was developed by Pawliszyn et al., is a rapid, simple, sensitive, and solvent-free extraction technique (1–6). SPME uses a polymer-coated fiber for the adsorption of organic compounds from an aqueous phase of a matrix or its headspace followed by direct desorption into a gas chromatograph (GC) or high-performance liquid chromatograph (HPLC) for subsequent detection and quantitation. The technique is known for its sensitivity, which enables detection in the µg/L range.

In contrast to the traditional and lengthy sample extraction techniques, such as solid-phase extraction (SPE) and liquid–liquid extraction (LLE), SPME uses a miniature cylindrical coated fused-silica fiber that allows rapid mass transfers during the adsorption and desorption processes. With a smaller volume of aqueous sample required compared with SPE and LLE, SPME acts as an attractive method for the analysis of limited volumes of samples. Moreover, the use of SPME can eliminate interferences present in environmental samples, such as biological matrices, allowing the detection of target analytes that otherwise can't be detected by direct injection of the extracts. Additionally, the SPME method has the advantage of being organic solvent-free and more rapid than SPE or LLE. For example, total SPME analysis of explosives in sediment, including adsorption and analysis, did not exceed 80 min as opposed to approximately 6 h (including time needed for cartridge conditioning, adsorption, elution, and analysis) for the SPE method (7). Additionally, field sampling for SPE may require numerous precautionary safety measures, especially at sites contaminated with hazardous chemicals, such as those derived from pharmaceutical, petroleum, and military industry. Under these circumstances, measures, including use of specialized equipment and restrictions of the amount of sample, must be carefully considered. A technique like SPME that utilizes smaller volumes of samples than SPE or LLE, thus, becomes very attractive. For instance, SPME extraction has been applied in combination with GC–mass spectrometry (MS) and GC–electron capture detection (ECD) for the determination of hazardous chemicals, such as 2,4,6-trinitrotoluene (TNT) and its amino degradation products, in aqueous solutions including seawater (8,9,10) and marine sediment (10). In another study Darrach et al. (11) developed an SPME method for the trace analysis of TNT and its products from World War

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II unexploded undersea ordnance in a shipwreck site near Halifax Harbor. Furthermore, Furton et al. (9) optimized SPME–GC and SPME–HPLC methods for the analysis of explosives from aqueous and post-explosion debris.

SPME has been applied in the analysis of several other classes of contaminants, including new substances. Typical examples on the versatile use of SPME in environmental analysis include organometallic compounds, such as those containing Hg, Pb, As, Sn, Sb, Cr, and Te (12–18); petroleum hydrocarbons (19–24); chlorinated solvents, including polychlorinated biphenyls (25–27); industrially used phenolic compounds (28–30); volatile fatty acids (31,32); bactericides (33,34); herbicides (35,36); pesticides (37,38); insecticides (39); fungicides (40); surfactants (41); explosives (7,9–11); and products from thermal degradation of polymers (42–45).

More recently, Monteil-Rivera et al. (7,10) has demonstrated that SPME–GC–ECD and SPME–HPLC can be used both as a purification technique for sediment extracts and as an extraction technique for water samples prior to analysis. The authors successfully applied SPME–GC–ECD and SPME–HPLC coupling for the trace analysis of nine explosives, including nitroaromatics and the nonaromatic cyclic nitramine explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), in real seawater and marine sediment samples. In the case of SPME–GC–ECD coupling, the highest extraction efficiencies of the tested analytes were obtained in the presence of a high concentration of salt [30% (w/v)] in the aqueous medium by use of a carbowax–divinylbenzene coating. Method detection limits ranged from 0.05 to 0.81 µg/L in water and from 1 to 9 µg/kg in dry sediment. Spike recoveries in seawater were satisfactory (89–147%) when samples were fortified at 2 µg/L of each analyte. In general, lower recoveries were obtained in the case of sediment when fortified at 10 µg/kg for each explosive, which was attributed to the potential occurrence of sorption and degradation of the chemicals into the sediment matrix. However, the authors concluded that with a smaller volume of aqueous and sediment samples required compared with SPE, SPME is an attractive method for the analysis of limited volumes of samples such as sediment.

Discussion

Biotransformation pathways by SPME

Although SPME has been widely used for the trace analysis of organic and organometallic compounds in several aqueous-based matrices, little is known on the applicability of the technique for monitoring chemical and biochemical transformation pathways of organic compounds (46). Until recently, lengthy sample preparation and separation techniques (SPE and LLE) followed by chromatographic clean-up procedures were required to isolate and identify intermediates produced during (bio)transformation processes (47). When such intermediates are formed in trace amounts, the previously mentioned traditional SPE and LLE techniques are not fast or sensitive enough for their detection, thus, leading to the loss of valuable information on the transformation pathways.

The applicability of the SPME technique to perform rapid and sensitive analysis of chemicals makes the technique suitable for the detection of trace amounts of relatively short lived intermediate products (metabolites) in chemical and biochemical reactions. Also, the ability of SPME to sample both the headspace and the aqueous media, combined with simplicity for coupling with several detection tools such as GC and HPLC (without having to stop the chemical reaction) makes the technique suitable for real-time in vitro and in vivo measurements. Last but not least, the option to use SPME fibers coated with different polymers having different polarities, such as carbowax, polydimethylsiloxane, and polyacrylate, allows the technique to assess a wide range of analytes related to the (bio)transformation process in question.

Recently, several workers realized the usefulness of the SPME technique and used it to elucidate the (bio)transformation pathways of various chemicals. For instance, Barreiros et al. (48) reported a novel pathway for the mineralization of the thiocarbamate herbicide by a mixed bacterial culture using SPME. On the other hand, Peller (49) used SPME to investigate the breakdown mechanism of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) by hydroxyl radical, whereas De Visscher et al. (50) employed SPME–GC–MS to study the degradation of ethyl benzene using sonication (520 kHz). Several products, including mono substituted monocyclic and dicyclic aromatic hydrocarbons, were detected and were suggested to originate from the initially formed dehydrogenated styrene product. SPME has also contributed to the understanding of processes related to environmental remediation (51–53) and to the provision of data useful to the understanding of the environmental fate and toxicity mechanisms (54) of chemicals. For instance, Pierini and Mastrogiacomo (55) applied SPME to analyze methyl-*t*-butyl ether and its degradation products, *t*-butyl alcohol and *t*-butyl formate, in water samples.

The previously mentioned characteristic features associated with SPME (e.g. rapidity, sensitivity, and suitability for direct application in the headspace or the aqueous solution without the use of solvent) also made the technique suitable for a wider application in areas related to food and agrochemical industry. For example, the SPME technique has been used for on site assessment of the freshness of food by determining degradation products and other released volatile chemicals. In this respect, Brunton et al. (56) used SPME to measure aldehydes, such as hexanal and pentanal, in turkey, and Koprivnjak et al. (57) used the technique to study the influence of olive fruit storage in bags on oil quality by determining the composition of volatile compounds.

There are many other applications of SPME, but only a few specific examples were selected to demonstrate the suitability of the technique for in situ monitoring of chemical and biological assays. Data gathered from real-time measurements of reaction intermediates (metabolites) during reactions can then be used to determine (bio)transformation pathways. The selected examples include biodesulfurization of dibenzothiophene, biotransformation of petroleum hydrocarbons, and biotransformation of two widely used explosives (RDX and TNT). We would like to emphasize that the objective of the present

article is to discuss the applicability of SPME–GC and SPME–HPLC couplings for real-time measurements of chemical changes in chemical and biological assays. The time profile of detected intermediates can then be used to provide insight into the (bio)transformation pathways.

Example 1. Biodesulfurization pathway of dibenzothiophene.

Bitumen is a fossil fuel rich in sulfur, which produces sulfur oxides known as acid rain agents upon burning. To improve the fuel value of bitumen, sulfur must first be removed to keep the carbon skeleton intact. Several group of researchers (58–60) used bacteria to biodesulfurize bitumen. However, to be able to optimize biodesulfurization, the identity of intermediates and end products must be known. McPherson et al. (61) employed *Rhodococcus* sp. strain ECRD-1 to desulfurize dibenzothiophene (DBT), a model thiophenic compound commonly found in fossil fuel, and employed SPME–GC–MS to identify key metabolites formed during desulfurization. Biodesulfurization was conducted by incubating DBT (10 mg/L) with strain ECRD-1 in a mineral salt medium (MSM) supplemented with glucose as a carbon source in special flasks as described by McPherson et al. (61). Aliquots of the cell suspension (2 mL) were acidified (pH 2) and filtered through a Millex-HV 0.45-µm filter for subsequent SPME adsorption using fused silica fiber coated with polyacrylate polymer using 4-ethyl DBT as the recovery standard, followed by desorption inside the injector port of a GC–MS (Varian Saturn II Ion Trap). First, an SPME–GC–MS method was developed to determine the thermodynamic equilibrium of sorption of the starting material (DBT) and its final product (2-hydroxy-biphenyl) in aqueous solution. Sorption of both chemicals reached equilibrium after 10 min (Figure 1A). A sorption time of 20 min and desorption time of 10 min were selected for subsequent analysis of the metabolites formed during DBT biotransformation with strain ECRD-1.

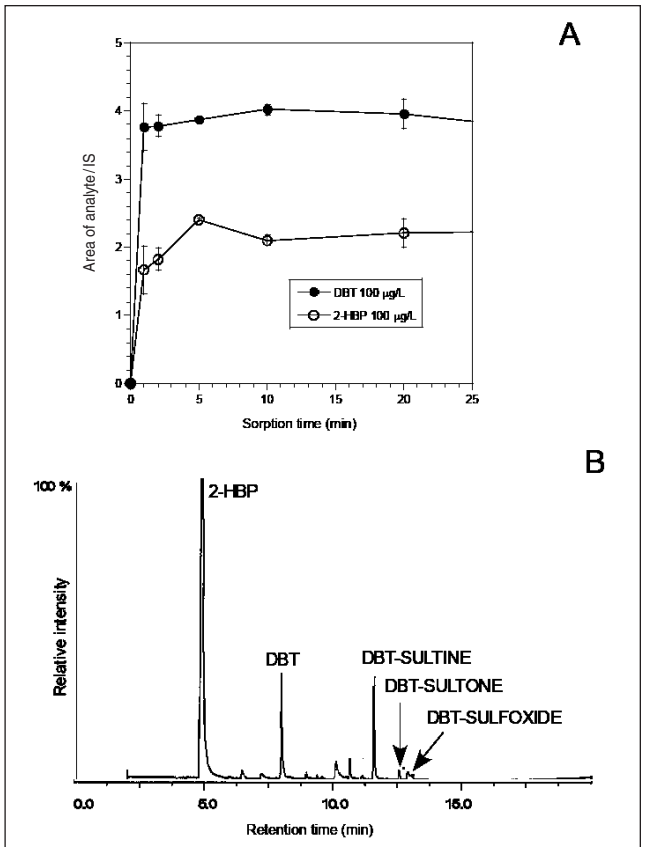

Figure 1. Representative adsorption isotherms of dibenzothiophene (DBT) and its metabolite, 2-hydroxybiphenyl (2-HBP), using polyacrylate coated SPME fiber with stirring at room temperature (A). SPME–GC–MS chromatogram obtained during biotransformation of DBT by *Rhodococcus* sp. strain ECRD-1 (B). Modified from the literature (61).

Figure 1B represents a typical SPME–GC–MS chromatogram of the metabolites formed during biodesulfurization of DBT that were identified as DBT-sulfoxide, DBT-sulfone, dibenz[c,e][1,2]oxathiin 6-oxide (DBT-sultine), dibenz[c,e][1,2]oxathiin 6,6-dioxide (DBT-sultone), and 2-hydroxybiphenyl (2-HBP) (61). The profile of products observed in the SPME–GC–MS chromatogram (Figure 1B) indicates the occurrence of a stepwise metabolism of DBT showing that DBT-sulfoxide was the first metabolite formed. The second metabolite, probably DBT-sulfone, is seemingly converted rapidly to DBT-sultine and DBT-sultone, which eventually would convert to the sulfur free product 2-HBP.

All four intermediates detected by McPherson et al. (61), using the rapid, solvent-free SPME, were also detected earlier by Olson et al. (62) and Denome et al. (63). Both the SPME data obtained by McPherson et al. (61) and those reported in the literature (62,63), using lengthy analytical procedures, basically show the following sequence of events during biotransformation of DBT: DBT, DBT-sulfoxide, DBT-sulfone, DBT-sultine (or its sulfinic acid), DBT-sultone (or its sulfonic acid), and 2-HBP.

Example 2. Biotransformation routes of hydrocarbons

Microbial biotransformation of benzene, a highly carcinogenic hydrocarbon and a major contaminant of groundwater, soil, and sediment, has been extensively investigated as a potential technology to remove the chemical from affected environments, but little information is available on its metabolites and degradation pathway. Caldwell and Suflita (64) highlighted the importance of knowing intermediates produced during in situ benzene biodegradation to help understand the migration potential of generated intermediates, particularly polar ones. Traditionally, intermediate metabolites of hydrocarbon biodegradation have proven to be difficult to detect because of the inherent chemical properties of these compounds, including volatility, polarity, and the high reactivity. As was mentioned earlier, the SPME technique used fibers coated with polymers having several polarities suitable for direct immersion either inside the aqueous phase or in the headspace of a reaction mixture. These unique properties of SPME make the technique suitable for the detection of several volatile and nonvolatile products that also vary in their polar properties. For instance, Yerushalmi et al. (65) reported the use of SPME-GC–MS to identify intermediate metabolites produced during the biological transformation of benzene under limited oxygen conditions to match predominant anoxic environments found in subsurface soil and sediments. Using uniformly labeled [^{13}C]-benzene, the authors were able to confirm the presence

of phenol, catechol, and resorcinol (1,3-dihydroxybenzene) as intermediates of degradation.

In another study, Rhofir and Hawari (66) used SPME–GC–MS to determine the biodegradation pathway of biphenyl, another typical component of petroleum hydrocarbons, by incubating the chemical (10 mg/L) with *Pseudomonas pseudoalcaligenes* sp. strain KF707 in a MSM. Aliquots (2 mL) of the culture medium are acidified (pH 2) and filtered through a 0.45-µm membrane. Before applying SPME to determine biphenyl metabolites in the reaction mixture, the authors first established optimal conditions for sorption of biphenyl and its suspected products from aqueous standard solutions. Approximately 30 to 40 min was found optimal for sorption of biphenyl and two of its hydroxylated derivatives, 2-hydroxybiphenyl and 2,2'-dihydroxybiphenyl. Based on a signal-to-noise ratio of 3, the detection limit was lower than 1 ppb (66). A fused-silica fiber coated with polyacrylate polymer was then immersed in the aqueous phase of the filtered reaction mixture (30 min) followed by desorption (10 min) into a GC (Varian Saturn II Ion Trap), and three key metabolites were identified, including 2-hydroxybiphenyl, 2,3-dihydroxybiphenyl, and benzoic acid (66). No relevant products were detected when the SPME fiber was used in the headspace of the reaction mixture. The authors used knowledge of the metabolites discovered by SPME–GC–MS and suggested the occurrence of two initial routes for the degradation of biphenyl: one route involved an initial monooxygenase attack on biphenyl to first produce metabolite 2 and the other one involved a dioxygenase reaction to produce product 3. Eventually the two hydroxylated intermediates 2 and 3 decomposed further to serve as carbon sources for bacteria as determined by liberation of $^{14}CO_2$ (Figure 2).

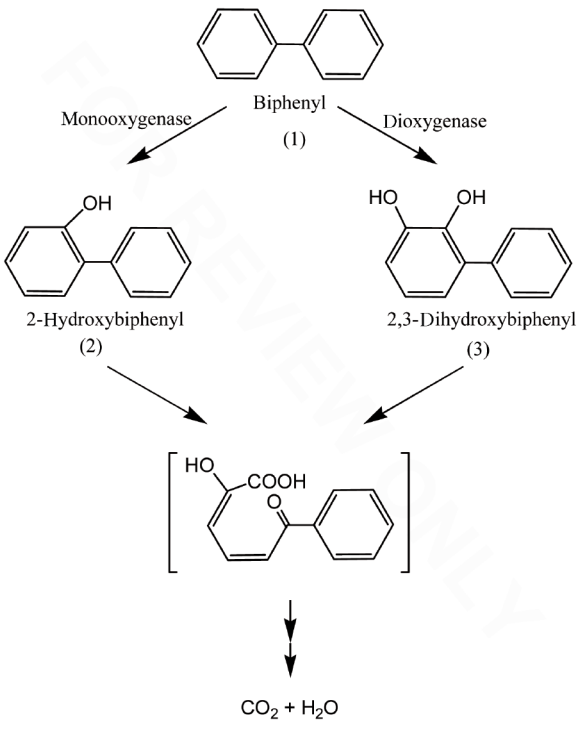

Figure 2. A schematic representation of biotransformation of biphenyl as determined by a time course study using SPME–GC–MS. Modified from the literature (66).

Recently, Whyte et al. (67) employed psychrotrophic bacteria, *Rhodococcus* sp. strain Q15, to biodegrade a variety of alkanes (dodecane and hexadecane) and employed SPME to determine intermediate products (68). Cells from strain Q15 were first grown in MSM supplemented with yeast extract in the presence of dodecane or hexadecane at 24°C. The culture broth (1 m) was sampled and clarified from bacterial cells for subsequent analysis by SPME using fibers coated with polyacrylate for adsorption (20 min) and desorption (10 min) in the injector port of a GC–MS (Varian Saturn Ion trap, mass range of 30 to 400 amu at a scan rate of 0.5 s per). Figure 3 shows a typical SPME–GC–MS total ion chromatogram of hexadecane after 24 h of incubation with *Rhodococcus* sp., which shows two main metabolites of hexadecane. The two intermediates were identified by matching the retention times and mass spectra with authentic standards, such as 1-hexadecanol and 2-hexadecanol (68). Peaks corresponding to metabolic intermediates are not seen in the SPME–GC–MS of parallel sterile controls containing hexadecane with no bacteria added.

The detection of 1-hexadecanol and 2-hexadecanol from the biotransformation of hexadecane by SPME–GC–MS indicates that strain Q15 can initiate attacks on the alkane on at least at two positions. The first one occurs by an enzymatic attack on a terminal carbon to produce the primary alcohol and the second one by an enzymatic attack on a secondary carbon atom leading to the production of a secondary alcohol. The resulting alcohols are proposed to undergo further enzymatic oxidation, leading to the formation of acids prior to mineralization. SPME–GC–MS analytical data on the use of alkanes by *Rhodococcus* sp. strain 15 as carbon source is confirmed by measuring respiration (liberated $^{14}CO_2$) using ^{14}C-labeled alkanes (67).

The biotransformation pathways of hexadecane obtained by SPME (68) are in line with the other bacterial and fungal microbial degradation pathways of *n*-alkanes obtained by other

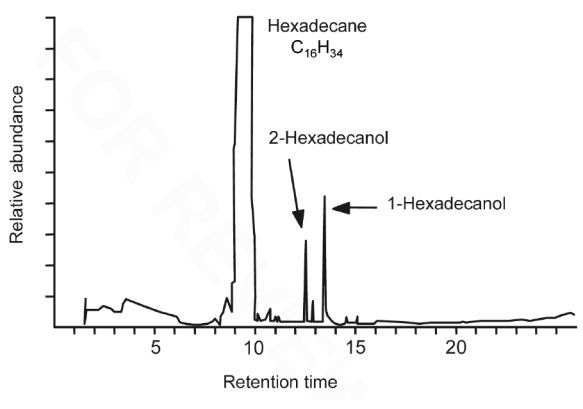

Figure 3. A typical SPME–GC–MS total ion chromatogram of biodegradation of hexadecane with *Rhodococcus* sp. Q15 strain showing the formation of the two metabolites: 1-hexadecanol and 2-hexadecanol. Modified from the literature (68).

groups of researchers using lengthy sample preparation and separation techniques (69).

Example 3. SPME–GC–MS investigation of biotransformation of explosives

Another family of common environmental pollutants is nitrogenous-based munitions compounds, such as RDX and TNT. Presently, contamination by explosives generated as wastes from the munitions and defense industries is a significant worldwide environmental problem. Most munition compounds are mutagenic, toxic, and have the tendency to persist in the environment (70,71). To understand the fate and toxicity mechanisms of explosives, it is necessary to first understand their biotransformation pathways. Hawari et al. (72) reported the successful use of indigenous bacterial consortia in anaerobic sludge to biotransform TNT and then employed SPME–GC–MS to determine biotransformation products. Experimental procedures for conducting biotransformation experiments, SPME sampling, and GC–MS analysis have been described previously (72). A fused-silica capillary fiber (1 cm) coated with polyacrylate or polymethylsiloxane polymer fitted to an auto sampler assembly were first employed to determine the sorption equilibria of TNT and all of its suspected products (Figure 4A). In general, it was found that monoamino-dinitrotoluenes, 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT), sorb faster than those of the starting material TNT and the two diamino products,2,4-diamino-6-nitrotoluene (2,4-DANT) and 2,6-diamino-4-nitrotoluene (2,6-DANT) (Figure 4A). Based on data obtained from the sorption isotherms in Figure 4A, an SPME sorption time of 20 min and desorption time of 10 min in the injector port of a GC are selected as typical time events for SPME–GC–MS analysis of TNT biotransformation.

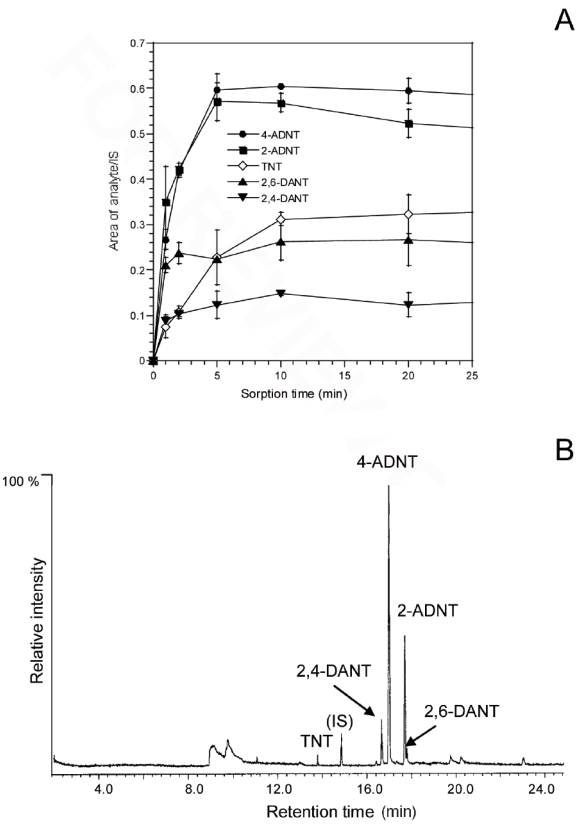

Figure 4. Representative absorption isotherms of TNT and its metabolites 2-ADNT, 4-ADNT, 2,4-DANT, and 2,6-DANT determined by immersion of SPME fibers coated with polyacrylamide polymer under stirring at room temperature (A). SPME–GC–MS total ion chromatogram of metabolites observed during incubation of TNT with anaerobic sludge (B). The internal standard (IS) was 2,3,5-trinitrobenzene, modified from the literature (72).

Aliquots (2 mL) from the TNT culture medium were first centrifuged to remove the suspended material, including biomass. The remaining clear broth was subjected to SPME-GC–MS analysis (Varian Saturn II Ion Trap GC–MS) using the adsorption–desorption time profile described previously. Figure 4B represents an SPME–GC–MS chromatogram for the disappearance of TNT and the appearance and disappearance of its metabolites. They include 2-ADNT, 4-ADNT, 2,4-DANT, and 2,6-DANT. The intermediates were identified by comparing their chromatographic retention time and mass data with their corresponding reference standards. Figure 4B shows that the disappearance of TNT is accompanied by the sequential formation of its amine metabolites, 4-ADNT, 2-ADNT, 2,4-DANT, and 2,6-DANT formed by the reduction of the peripheral NO_2 groups (72–74). When the TNT culture medium was analyzed by HPLC (EPA 8330), a similar product distribution was observed, thus, supporting the applicability of the SPME technique for the direct detection of intermediate products during a reaction event.

On the other hand, RDX has been found to be more reactive than TNT during incubation with anaerobic sludge (75). For instance, incubation of RDX with domestic anaerobic sludge leads to effective mineralization as determined by liberated $^{14}CO_2$.

Removal of RDX is accompanied by the concurrent formation of nitrite, suggesting that initial enzymatic denitration of the chemical leads to its instability and subsequent degradation in water (75). Using SPME–GC–MS, the authors were able to detect the key ring cleavage product formaldehyde, HCHO, in the headspace of the reaction mixture. The aldehyde is detected using an on fiber derivatized method developed by Martos and Pawliszyn (76). The authors concluded that the aldehyde is the RDX metabolite leading to mineralization by the bacteria. For instance, when $H^{14}CHO$ is incubated with the sludge under the same conditions, $^{14}CO_2$ is formed (75).

Conclusion

SPME–GC–MS is a robust analytical tool suitable for the analysis of various chemicals and their corresponding intermediate products in complex matrices. The technique is organic solvent free, rapid, and sensitive, thus, allowing the detection of trace amounts of chemicals that are short lived during biotransformation, thus, providing insight into degradation pathway(s).

Future Work

With concerns about the toxic effects of petroleum-based products combined with limited oil reserves, industry and other government departments worldwide are focusing on the use of agricultural feedstocks as a renewable source of energy and biobased chemicals for industrial applications (e.g., manufacturing of plastics, nylons, polymers, and fibers). Thus, expected massive use of agricultural-based chemicals necessitates the understanding of the environmental fate and impact of extracted new substances on various biological receptors. Therefore, rapid techniques suitable for on-site and in situ measurements of new substances and their transformation products will be one of the important future challenges that analytical chemists must provide. Analytical methods will, thus, be needed that can be used to monitor chemicals and their transformation products (metabolites) in various aqueous-based processes, including in vivo biological assays. In vivo measurements of analytes and their transformed products (metabolites) are needed to provide insight into metabolic pathway(s) in various biological receptors (bacteria, marine algae, earthworms, plant tissues, and other mamalian tissues). Chemical and bio-analytical knowledge and data acquired from biological systems can then be used to provide insight into natural environmental processes, such as in situ natural attenuation in groundwater and marine sediments, uptake by plant, and phytoremediation/phytoextraction. Also, chemical and bioanalytical data obtained from such biological assays can be used to understand the toxic effects and toxicity mechanisms of emerging chemicals. Therefore, future analytical tools must be able to perform real-time in situ process monitoring, termed henceforth "process analytical chemistry", to optimize product yields and to minimize or eliminate the formation of unwanted products.

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