

# The Problem of Phthalate Occurrence in Aquatic Environment: A Review



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Review

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This review has four major objectives: I) to present the problem of phthalate pollution, II) to highlight common techniques for quantification of phthalate compounds in water, III) to summarize current trends in determination of phthalates toxicity and point out the major adverse effects, and IV) to discuss and critically compare modern approaches in purification of phthalate-polluted water samples and thus reveal the further perspectives. Phthalates are organic compounds that are used extensively as additives in plastics and personal care products. They have high leaching potential and, therefore, they have been detected in various environments, including aquatic environments. Concentrations of phthalates in water are generally low, so their determination usually requires preconcentration. However, phthalates are compounds with very high hazardous potential. Related toxicity studies have been focused mainly on long-term exposures, and the results have shown that phthalates mainly affect the endocrine and reproductive systems. Therefore, phthalates have become a global concern. Their removal from the environment not only ensures environmental protection, but the protection of human health as well. Among various presented approaches for phthalates removal, anaerobic biodegradation has shown the highest potential for further developments because it is a promising technology for using wastewater as a source of green energy.

*Key words:*

phthalates, water medium, quantification, toxicity, removal

## Introduction

The industry of plastics production is increasing constantly. Thus, a production of 265 million tons was reported in 2010, four years later it reached 310 million tons, while in 2019, it exceeded 368 million tons.<sup>1–3</sup> This is not surprising knowing that plastic products have become an inevitable part of human daily routine. Various additives are added to the polymer base during plastics production to facilitate the molding process or to enhance some specific product characteristics.<sup>4</sup> These additives can be generally divided into two major categories: those that modify physical characteristics of the polymer (e.g., plasticizers, fillers, colorants, lubricants, foaming agents...), and those that have a preventive effect on ageing and degradation of the material (e.g., flame retardants, antistatic agents, biocides, UV stabilizers, antioxidants...)<sup>5</sup> Plasticizers are additives

that enhance polymer melt flow and thermoplasticity by loosening the dipolar forces and increasing the distance between the polymer chains. That consequently leads to improved softness, flexibility, durability, and distensibility of a polymer material.<sup>6,7</sup> According to European Chemicals Agency, there are 109 substances identified as plasticizers.<sup>8</sup>

Phthalates are plasticizers primarily used in production of polyvinyl chloride (PVC) plastics.<sup>9</sup> However, they are the most commonly used plasticizer as their share in the global use of plasticizers is around 75%.<sup>10,11</sup> Phthalates is the common name for a group of organic compounds known as phthalic acid esters or simply phthalate esters (Fig. 1). Apart of being used in the production of plastics, phthalates are an integral part of many other products as well. Such products are various care products, cosmetics, paper coatings, paints, etc.<sup>12</sup> Phthalates are prepared by esterification: one mole of phthalic acid anhydride reacts with two moles of an alcohol.<sup>7</sup> That provides a large amount of alcohol combinations and, accordingly, a large number of

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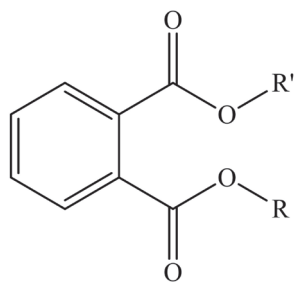


Fig. 1 – Chemical structure of phthalates

different phthalates. However, the range of alcohols used as plasticizers in PVC is usually limited to C6–C13 alcohols. Namely, performance of a plasticizer considerably differs with a change in an alcohol carbon number. As the alcohol chain grows, plasticizer volatility and plastisol viscosity reduces, and its UV aging resistance and low-temperature flexibility are enhanced.<sup>13</sup> Thus, phthalates prepared with <C6 alcohols have a too high volatility, while those prepared with >C13 alcohols have a limited compatibility with PVC.<sup>11</sup> Phthalates of low molecular weight (with C1–C4 alcohols), such as dimethyl phthalate (DMP) and diethyl phthalate (DEP), are mainly used in personal care, cosmetic and cleaning products.<sup>14,15</sup>

The major source of phthalates in the environment is plastic waste from which phthalates are slowly released due to weathering.<sup>16</sup> Because of their low interactions and no chemical bonding with polymer chains in polymer matrices, phthalates are likely to migrate from plastics into a medium (solid, liquid, or gas) with which they are in contact. The migration rate is influenced greatly by: I) properties of the polymer matrix; II) the amount and properties of the phthalate itself; III) the contact area with the surrounding medium; and IV) the properties of the surrounding medium.<sup>17</sup> Because of their low solubility in water, the released phthalates tend to concentrate in soil and sediments.<sup>18</sup> Plants often adsorb the phthalates from the soil, and thus introduce them into the food chain.<sup>19</sup> To date, phthalates have been detected in various samples<sup>20–28</sup> including food and beverages. Therefore, it is not surprising that phthalate metabolites have already been detected in human samples as well.<sup>29,30</sup>

Phthalates have become substances of global concern because of their potential health and environmental risks. The major problem is their endocrine-disrupting behavior.<sup>12</sup> There is a suspicion that exposure to phthalates causes some disorders in humans, such as testicular cancer and reduced sperm quality.<sup>31</sup> Unfortunately, exposure to phthalates is becoming constant. Even patients in hospital care, especially neonates, can be exposed since phthal-

ates can migrate from plastic medical equipment such as blood bags, catheters, nanogastric and intravenous tubes, etc.<sup>32,33</sup> Therefore, many countries are trying to reduce or eliminate the use of phthalates. The European Union (EU) has several phthalate directives and regulations currently in force. Among them, the REACH regulation<sup>34</sup> (Registration, Evaluation, Authorization and Restriction of Chemicals), which dates from December 2006, stands out the most. Annex XVII of this regulation initially limited the use of bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP), and di-*n*-octyl phthalate (DnOP) in toys and childcare articles to concentrations below 0.1 % of the weight of plasticized material. As additional information on the potential adverse effects of phthalates was collected over time, the EU Commission amended REACH regulation. The initial restrictions have been somewhat relaxed for DINP, DIDP, and DnOP, since their application is now limited to toys and childcare articles that children can put in their mouths. The remaining three phthalates (DEHP, DBP, and BBP) are considered toxic for reproduction, along with diisobutyl phthalate (DIBP), diisopentyl phthalate (DIPP), bis(2-methoxyethyl) phthalate (DMEP), dipentyl phthalate (DPP), *n*-penty-isopentyl phthalate (nPIPP), and dihexyl phthalate (DHP), which have been included in the consolidated REACH version. Therefore, deadlines for their application and placing on EU market have been set.<sup>35</sup> Furthermore, DEHP is included in European Pollutant Release and Transfer Register, where its annual release-threshold is 10, 1, and 1 kg for air, water, and land, respectively.<sup>36</sup> Since 2013, it has been listed as a priority substance in the field of water policy.<sup>37</sup> For now, it is the only phthalate on that list, but it is likely that some others will be added very soon. So far, 17 phthalates have been listed as Substances of Very High Concern due to their adverse influence on reproduction, while some of them have endocrine disrupting properties as well.<sup>38</sup>

The increased public awareness of the potentially hazardous activity of phthalates has caused global concern about their environmental fate, and spurred a search for ways to remove them effectively from the environment. Therefore, the aim of this work is to summarize and critically discuss recently published studies on phthalates, covering their chemical analysis, toxicity, and removal techniques. This review is focused on aquatic environment merely because dealing with all three environments (air, water, and soil) would result in a too extensive report.

## Analysis of phthalates

### Quality assurance

Phthalates are ubiquitous substances: they can be present in plastic laboratory equipment, glassware, water, organic solvents, or even in laboratory air.<sup>39,40</sup> Therefore, one of the problems related to the determination of phthalates, especially when analyzing trace concentrations, is the high possibility of sample contamination. To avoid false-positive or overestimated results, some quality assurance steps are required.

One of the steps to prevent contamination is elimination of any plastic equipment from the analytical procedure because plastics are the most likely source of phthalates. Instead, the used equipment should be made of materials such as glass, Teflon, aluminum, or stainless steel. All equipment should be washed with a suitable organic solvent before use (e.g., *n*-hexane, methanol, or isooctane), and stored in a box with a lid (glass, polytetrafluoroethylene, or calcinated aluminum foil) to avoid adsorption of phthalates from the air. The application of high-purity organic solvents is required (HPLC or GC grade) since some scientists have reported the presence of phthalates in organic solvents.<sup>41</sup> In case that some equipment has to be made of plastic (e.g., nitrile gloves, vial caps, filters), it has to be a phthalate-free version. The analysis should not use cosmetic and personal-care products that contain phthalates. If possible, the laboratory should have an air purification filter installed, to reduce the possibility of cross-contamination of equipment and solvents.

### Sample preparation

Sample preparation is perhaps the most important step in analytical procedure. Its importance manifests especially for samples with complex matrices or with low concentrations of analytes. Concentrations of phthalates in water samples are rather low. However, phthalates are hydrophobic substances, meaning that they can be extracted from water samples. At the same time, extraction is a simple and relatively inexpensive technique that is applicable to a variety of water matrices. Therefore, phthalate-containing samples are commonly pretreated by extraction to improve accuracy and quantification levels of the analysis. However, application of extraction generally involves an extensive use of organic solvents, which is not environmentally friendly. Therefore, current research trends are focused on techniques that significantly reduce the use of organic solvents. The most relevant publications<sup>26,42–65</sup> dealing with sample preparation and analysis of phthalates in water samples are summarized in Table 1.

### Extraction by liquids

Table 2 lists the standard methods of the Environmental Protection Agency (EPA) for determination of phthalates in various water matrices.<sup>66–73</sup> As shown in the table, some of these methods<sup>66,68,69,72,73</sup> include liquid-liquid extraction (LLE) with dichloromethane (DCM) as organic solvent.

Usually, more than one extraction step is required to achieve acceptable recovery. Thus, Zhao *et al.*<sup>54</sup> had to perform three LLE-DCM steps for satisfactory extraction of 22 phthalates from river-water samples. Better extraction efficiency is generally obtained for organic solvents immiscible with water, such as DCM or hexane. If water-miscible solvents are used for the extraction (e.g., ethanol, acetone, propanol), addition of inorganic salts could enhance the separation by allowing two phases to separate clearly. Cai *et al.*<sup>53</sup> studied the effect of addition of seven inorganic salts on the separation of aqueous phase from various water-miscible solvents. The results indicated ammonium sulfate as suitable salting reagent for water-propanol and water-acetonitrile systems.

Traditional LLE is a simple, relatively inexpensive, but a time-consuming technique. Unfortunately, it requires large quantities of sample and organic solvent, which is not in accordance with the green chemistry idea; therefore, other LLE techniques have been developed as an alternative. Some of these alternatives offer advantages over the traditional LLE, while still being simple and inexpensive. Liquid-phase microextraction (LPME) was developed as miniaturized version of LLE, which reduced the consumption of organic solvent. LPME can be roughly classified into three categories: single-drop microextraction (SDME), hollow-fiber LPME (HF-LPME), and dispersive liquid-liquid microextraction (DLLME).<sup>74</sup> SDME<sup>75</sup> is probably the simplest and most easily implemented LPME technique. The main problem with SDME is transport of analyte molecules from aqueous phase to the microdrop, which is generally limited by slow diffusion rates of the analyte molecules. Low cost of fiber was pointed out as one of the main advantages of HF-LPME technique because the fibers can be replaced for each extraction.<sup>42</sup> Furthermore, the method is very simple, which indicates the potential for use in routine analysis. DLLME introduces another solvent in LPME system: the so-called dispersant. The role of dispersant is to disperse a small volume of an extraction solvent into the tested water sample.<sup>76</sup> The achieved large surface between two phases (in formed cloudy solution) enables easy and quick mass transfer. For further speed up of the mass transfer, the mixture can be set in an ultrasonic bath or on the vortex; afterwards, the phases can be separated easily by centrifugation.

Table 1 – Recent studies dealing with sample preparation and chromatographic analysis of phthalates in water samples

Extraction					Sample analysis					
Analyte	Matrix	Technique	Extraction phase	Eluent	Method	Column	Mobile phase	Range ( $\mu\text{g L}^{-1}$ )	LOD ( $\mu\text{g L}^{-1}$ )	Reference
DPrP, DIBP, DBP, DIPP, DEHP, DnPP, BBP, DBEP, DCHP	mineral water, tap water, pond water, wastewater	HF-LPME	1-octanol on PP hollow fiber	–	GC-MS/MS (triple quadrupole, EI)	BR–5MS fused silica capillary column (Bruker)	Ar	1–100	–	42
DnOP									0.35	
DEP	tap water, drinking water, mineral water	MSPE	mixture of $\text{Fe}_3\text{O}_4$ -MIL-100 and $\text{Fe}_3\text{O}_4$ - $\text{SiO}_2$ -PT magnetic nanoparticles	ACN	GC-MS	DB5–MS capillary column (Agilent)	He	5–5000	0.72	43
BBP									0.75	
DMP, DEHP									0.91	
DBP										
DEP, DBP, DEHP, DIOP	groundwater	HF-SBSE	PVDF hollow fiber containing C18 silica microspheres	–	GC-MS	–	–	0.01–1000	0.003	44
DBP									0.01	
DEHA									0.02	
DEP, DPP, BBP, DEHP, DnOP	bottled water	RDSE	Teflon disc with Oasis HLB sorbent	MeOH	GC-MS	RTX–5MS fused silica capillary column (Restek)	He	0.25–1000	0.03	45
DMP									0.04	
DnPP								0.01–300		
BBP								0.03–500	0.005	
DEHP	tap water, seawater	SPME	$\text{GO-H}_2\text{NC}_3\text{VI m}^+\text{Br}^-$ fiber	–	GC-MS	–	–	0.05–300	0.010	46
DBP										
DnOP								0.01–500	0.030	
DMP								0.05–500		
DPP, DEHP									0.001	
DIBP									0.008	
DBP									0.009	
DnOP									0.025	
BBP	bottled water	HF-SPME	PSF hollow fiber	–	GC-FID (320 °C)	DB–5 column (Agilent)	$\text{N}_2$	2–1000	0.027	47
DHeP									0.028	
DPrP									0.035	
DEP									0.045	
DMEP									0.130	

Extraction					Sample analysis					
Analyte	Matrix	Technique	Extraction phase	Eluent	Method	Column	Mobile phase	Range ( $\mu\text{g L}^{-1}$ )	LOD ( $\mu\text{g L}^{-1}$ )	Reference
DBP, DEHP, DnOP								0.1–200	0.010	
BBP	river water, pond water	MSPE	magnetic graphene composites	ethyl acetate solution of $\text{Na}_2\text{SO}_4$	GC-MS (quadrupole, EI)	HP-5MS capillary column (Agilent)	He		0.011	48
DIBP								0.032		
DEP								0.034		
DMP								0.056		
DEP								0.08		
DAP	environmental water	DMSPE	MIL-101(Cr) magnetic MOF	<i>n</i> -hexane/acetone (1:1)	GC-MS (ion trap)	VF-5MS fused silica capillary column (Agilent)	He		0.09	49
DBP, DIBP								0.10		
DMP								0.15		
DBP	environmental water	on-line SPME	TRB-5 coated capillary tube	–	HPLC-DAD (230 nm)	Genesis C18 column (Hichrom)	$\text{H}_2\text{O} : \text{ACN}$ (gradient)	3–250	1.0	50
DEHP								7.5–250	2.5	
DIPrP,									0.013	
DAP									0.015	
DPrP									0.019	
DBZP								0.01–100	0.022	
DPP									0.030	
DnOP	bottled water, river water, pond water	SFO-DLLME with addition of NaCl	1-dodecanol with ACN as dispersant	–	HPLC-DAD (225 nm)	Gemini C18 column (Phenomenex)	$\text{H}_2\text{O} : \text{ACN}$ (gradient)		0.031	51
DEP								0.033		
DnPP, DCHP									0.140	
DEHP, DBEP, DBP								0.5–100	0.150	
BBP, DIBP									0.160	
DNP									0.450	
DMP									0.7	
DEP	bottled water	on-line SPE	C18 SPE membrane	ACN	HPLC-UV (230 nm)	C18 column (Agela)	$\text{H}_2\text{O} : \text{ACN}$ (gradient)	2.5–100	1.2	52
DBP								10–100	2.4	
DBP									1–3	
DnOP									1.3	
DEP	environmental water	LLE with addition of $(\text{NH}_4)_2\text{SO}_4$	propanol	–	HPLC-UV (226 nm)	C18 column Acclaim Polar Advantage 2 (Thermo Scientific)	$\text{H}_2\text{O} : \text{ACN}$ (gradient)		1.5	53
DPP								2.3		
DCHP								2.6		
DNP								3.0		



Extraction					Sample analysis					
Analyte	Matrix	Technique	Extraction phase	Eluent	Method	Column	Mobile phase	Range ( $\mu\text{g L}^{-1}$ )	LOD ( $\mu\text{g L}^{-1}$ )	Reference
DMP, DEP, DIPrP, DAP, DPrP, DIBP, DBP, DMEP, DIHeP, DEEP, DPP, DHeP, BBP, DBEP, DCHP, DEHP, DPhP, DnOP, DBZP, DNP, DIDP	river water	LLE	DCM	–	GC-MS (quadrupole, EI)	DB–5MS capillary column (Agilent)	He	10–2000	0.005–0.074	54
DIBP, DEHP	mineral water	HLLE with addition of NaCl	ACN	–	GC-FID (300 °C)	SPB–5 capillary column (Merck)	He	1.0–5000	0.02	55
DBP								0.10		
DEP								2.0–5000	0.70	
DMP								10.0–5000		
DBP	environmental water	MSPE	$\text{Fe}_3\text{O}_4/\text{ZIF-67}$ magnetic composite	MeOH/ <i>n</i> -hexane (1:1)	GC-MS	SH–Rtx–5 fused silica capillary column (Restek)	He	1–200	0.005	56
DEP									0.006	
DEHP									0.015	
DPrP, DAP									0.020	
DHeP									0.030	
BBP	0.035									
DEHP	mineral water	DSPE with DLLME	folic acid for DSPE and 1,1,1-TCE with NaCl-water solution as dispersant for DLLME	acetone for DSPE and none for DLLME	GC-FID (300 °C)	Zebron capillary column*, (Phenomenex)	He	0.43–250	0.13	57
DBP								0.96–5000	0.29	
DIBP								1.50–1000	0.46	
BBP, DBP, DCHP, DEHP	tap water	DLLME	[C8MIM] [PF6] and [C6MIM] [PF6]-IL with acetone as dispersant	–	HPLC-UV (224 nm)	SB–C18 column (Zorbax)	$\text{H}_2\text{O} : \text{MeOH}$ (gradient)	50–600	–	58
BBP	bottled water	SPME	DVB/CAR/PDMS 50/30 $\mu\text{m}$ fiber	–	GC-MS (quadrupole, EI)	HP–5MS column (Agilent)	He	0.2–50	0.04	59
DnOP									0.09	
DBP									0.10	
DIDP									0.11	
DINP									0.13	
DEHP									0.18	
DBP	environmental water	SPME	MIP fiber	–	HPLC-DAD (225 nm)	C18 column (J&K Scientific)	$\text{H}_2\text{O} : \text{ACN}$ (gradient)	0.1–125	0.03	60

Extraction					Sample analysis					
Analyte	Matrix	Technique	Extraction phase	Eluent	Method	Column	Mobile phase	Range ( $\mu\text{g L}^{-1}$ )	LOD ( $\mu\text{g L}^{-1}$ )	Reference
DBP, DnOP, DEHP, DEP	greywater**	SPE	Oasis HLB SPE cartridges	5 % MeOH in water	GC-MS	HP-5MS capillary column (Agilent)	He	0.5–500	–	24
DMP								0.005–0.50	0.015	
DBP									0.034	
DEHP	river water	SPE	Oasis HLB	hexane/DCM (1:1) and acetone/DCM (1:1)	HPLC-MS/MS (ESI)	Acquity BEH C18 (Waters)	0.1 % HCOOH : MeOH (gradient)		0.295	61
BBP								0.005–0.25	0.417	
DEP									0.782	
DEHP	river water	SPE	MIP and NIP SPE fibers	chloroform	GC-FID (300 °C)	DB-1 column (Agilent)	N <sub>2</sub>	35–3000	11	62
DEP								1.06–21.20	0.001	
BBP								1.23–24.72		
DBP								1.08–21.64	0.007	
DPrP	river water	sublation	<i>n</i> -hexane (flotation with bubbling nitrogen gas)	–	HPLC-UV (224 nm)	Eclipse XDB-C18 (Agilent)	H <sub>2</sub> O : ACN (gradient)	1.04–20.86	0.034	63
DEHP								1.05–20.92	0.136	
DAP								1.03–20.54	0.213	
DCHP								1.01–20.16		
DMP								1.12–22.40	0.225	
DEP								0.01–20	0.002	
DMP									0.003	
DBP	lake water	SPE	nylon6 nanofibers mat	acetone	HPLC-UV (230 nm)	C18 column (Dikma)	H <sub>2</sub> O : ACN (gradient)	0.02–20	0.006	64
DEHP								0.10–20	0.010	
DnOP									0.033	
DMP								<0.0515	0.0026	
DEP								<0.0450	0.0004	
DBP						two connected fused silica capillary columns:		<0.0359	0.0003	
BBP	distilled water	SDME	toluene	–	GC-FID	HP-5 and HP17 (Varian)	N <sub>2</sub>	<0.0320	0.0006	65
DEHP								<0.0256	0.0019	
DnOP								<0.0256	0.0026	
DNP								<0.0239	0.0060	

\*30 m × 0.25 mm i.d.; 0.25  $\mu\text{m}$  film of: 5 % phenyl, 95 % dimethylpolysiloxane

\*\*greywater is wastewater from showers, wash basin, washing clothes, and dishwashing

Selection of an appropriate extraction solvent is a very important step in DLLME, as the solvent should not only be able to efficiently extract the analytes and to have low solubility in water, but also to form a cloudy solution in the presence of dispersant after their mixture is injected into the aqueous phase. It is also preferable for the solvent to have higher density than water, but unfortunately, such solvents are usually toxic; therefore, some new methods using low-density solvents have been proposed. Thus, Yang *et al.*<sup>51</sup> applied 1-dodecanol as extraction solvent and acetonitrile (ACN) as dispersant to extract 15 phthalates from water samples. The method was based on solidification of floating organic droplet (DLLME-SFO). After shaking, the mixture was cooled in refrigerator (4 °C), which resulted in solidified organic droplets floating on the top of the solution; the droplets were transferred with spatula into a vial for direct analysis on HPLC. The proposed method showed satisfactory results while being rapid, sensitive, cost-effective, and environmentally friendly at the same time.

Homogeneous LLE (HLLE) applies homogeneous solution of water and water-miscible solvent (e.g., ACN, acetone, isopropanol), which provides an infinite contact surface among the solvents and thus enhances the mass transfer.<sup>55</sup> In addition, there is no need for intensive stirring. The phases are separated later by adding an appropriate salt into the solution.

Sublation is another extraction technique used for phthalate extraction. It is a technique where gas bubbles are streaming through a column filled with liquid. The liquid consists of two phases: aqueous phase (the lower phase) and water-immiscible organic phase (the upper phase). The sublation re-

quires no additional stirring due to the passing of the bubbles. As the bubbles pass through the aqueous phase, the solute adsorbs on liquid-gas interface. Carried by the bubbles, the solute is transferred into the organic phase in which it dissolves.

#### Extraction on solids

Solid-phase extraction (SPE) is also part of EPA standard methods for phthalates determination in water matrices (Table 2).<sup>66,67,70–73</sup> It is the most popular and the most often used sample preparation technique for phthalate analysis due to its simplicity and rapidity; in addition, there is a possibility of process automation. However, most of the reported SPE methods are used off-line, which increases the risks of sample loss and contamination. SPE device usually consists of a short column filled with a solid sorbent on which the analyte is extracted. Sorbent is thereafter washed with appropriate organic solvent (usually methanol, DCM, hexane, or acetone) and analyzed.<sup>77</sup> Generally, SPE has higher efficiency compared to LLE. It overcomes most problems associated with LLE, such as incomplete phase separation, use of expensive special glassware, and disposal of large quantities of toxic organic solvents. Salazar-Beltran *et al.*<sup>52</sup> developed an on-line SPE/HPLC-UV method for selective extraction of phthalates from bottled-water samples. After extraction, the analyte was eluted and automatically transferred into analytical column using a two-position column switching valve. The authors pointed out that, compared to the off-line SPE, the developed on-line method reduced the influence of the analyst, as well as the use of organic solvents; this made the method less susceptible to experimental error and more environmentally friendly.

Table 2 – Standard EPA methods for determination of phthalates in various water matrices

Phthalate	Matrix	Sample preparation		Quantification	EPA method no.
		Technique	Solvent		
DEHP BBP DBP DEP DMP DnOP	Drinking water	LLE	dichloromethane	GC-PID	506 <sup>66</sup>
		SPE			
		SPE			
	Municipal and industrial wastewater	LLE	dichloromethane	GC-ECD	606 <sup>68</sup>
				GC-MS	625 <sup>69</sup>
		SPE	hexane	GC-ECD	8060 <sup>70</sup>
				GC-MS	8250 <sup>71</sup>
	Groundwater	LLE	dichloromethane	GC-MS	8270d <sup>72</sup>
				SPE	
		LLE	dichloromethane	GC-ECD	8061a <sup>73</sup>
SPE				ACN	

\*Method is not applicable for DnOP.



To improve the SPE efficiency, researchers usually reduce the size of cartridge particles and thus increase the total active surface. Unfortunately, this increases the column back-pressure as well, which may lead to the blocking of the cartridge flow. One of the techniques that overcome this problem is dispersive solid-phase extraction (DSPE) in which the sorbent is mixed into the sample solution (with no use of cartridge). After removing the sorbent from the sample, the adsorbed analytes are eluted with suitable solvent. Farajzadeh *et al.*<sup>57</sup> tested folic acid as sorbent for removal of phthalates from water samples. The authors pointed out low enrichment factor as the main drawback of DSPE method. Therefore, they applied DLLME posttreatment. The use of folic acid offered a possibility for sorbent recovery, which reduced the environmental impact of DSPE.

Another technique that avoids the SPE blockage problem is magnetic solid-phase extraction (MSPE) in which magnetic material is used as a sorbent; this allows separation by applying a magnet. Separation is simpler and faster compared to DSPE approach, since no additional filtration or centrifugation is required.

Solid-phase microextraction (SPME) enables an integration of sampling and sample preparation. It is a technique commonly used prior to phthalate analysis for shortening the sample preparation time. The technique is primarily applied for liquid samples, but it can be used for gasses as well. It uses a special device containing fibers capable of adsorbing the analytes of interest. The device is immersed in the sample to adsorb the analyte. After adsorption, the analytes can be eluted with an appropriate organic solvent or desorbed directly in the instrument (pyrolizer) for GC analysis. Chafer-Pericas *et al.*<sup>50</sup> successfully automated SPME for on-line extraction of two phthalates from environmental water samples. Huang *et al.*<sup>47</sup> proposed the use of a polysulfone hollow fiber for extraction of 10 phthalates from water samples. The analysis of fiber was performed directly by flash vaporization GC. By excluding the use of solvent, the authors minimized the possibility of contamination. The performance of SPME mainly depends on the sorbent used; therefore, the development of new fiber materials with increased extraction efficiency and high selectivity is of great relevance. Recent studies related to sorbents for phthalates have focused on molecularly imprinted polymers (MIP),<sup>46</sup> polymeric ionic liquids<sup>79</sup> and various nanocomposite-based sorbents.<sup>80</sup> In addition to the benefits, SPME methods have some disadvantages as well. Namely, SPME devices must be replaced frequently, which makes the method more expensive, and the possibility of reus-

ing the fibers increases the chance of cross-contamination.

Stir-bar sorptive extraction (SBSE) uses a glass-coated magnetic bar, which is additionally covered with a layer of appropriate sorbent. As the bar is rotating in a sample, the analytes are adsorbed on the sorbent. After the bar is dried, the analyte is desorbed. Some scientists<sup>44</sup> replaced glass-coated magnetic bar with a hollow fiber bar. By using hollow fiber, they minimized the chromatographic interferences, since the fiber prevented contact between sorbent and macromolecules from the sample. In general, SBSE is based on the same principles as SPME, but the amount of sorbent material is much greater. Accordingly, it can produce lower LOD, which makes it superior when dealing with trace concentrations. However, desorption in the case of SBSE is slower.<sup>76</sup> Manzo *et al.*<sup>45</sup> modified SBSE by embedding the stirring bar into a sorbent-coated Teflon disc; they named the approach rotating-disk sorptive extraction (RDSE). Compared to the common SBSE approach, RDSE showed less time consumption, and considerably smaller sample volume was required.

### Phthalates determination and quantification

Awareness of potential adverse effects of phthalates on the environment, and especially on humans, has resulted in very demanding directives in most developed countries. Accordingly, it is important to develop methods that are able to quantify phthalates at low concentration levels, and with high precision and accuracy. The methods should be highly sensitive and selective. Various analytical approaches are available in literature (Table 1).

#### Chromatography

Water samples may contain very complex matrices and more than one phthalate compound. Therefore, pretreatment techniques described in the Sample preparation chapter are commonly followed by chromatographic separation, which allows higher selectivity of the analysis. The EPA has set GC as a standard technique for phthalate analysis in various water matrices (Table 2). Nevertheless, LC methods are very common in literature as well.

To achieve acceptable sensitivity, LOD, and LOQ (limit of quantification), the chromatography is coupled with various detectors. According to data summarized in Table 1, HPLC is usually coupled with diode array detector (DAD), UV detector, and mass spectrometry (MS), while GC comes in combination with flame ionization detector (FID), electron capture detector (ECD) or MS detector. Among these, MS detectors offer the highest sensitivity and

selectivity. However, FID and DAD are less expensive detectors with performances sufficient for numerous analytical tasks, what makes them widely applied. In the case of DAD detectors, all phthalates are determined in relatively short wavelength range of 224–230 nm.

Due to their high volatility and thermostability,<sup>18</sup> phthalates can be analyzed by GC without prior derivatization, which highly simplifies the analytical procedure and minimizes the costs. However, some authors<sup>24</sup> have reported on the use of derivatization methods when phthalates had been analyzed together with some other compounds (e.g., some other endocrine disrupting compounds). Derivatization was performed to allow analysis of compounds with inadequate volatility or stability, and to improve detectability or chromatographic behavior of the analytes. Namely, it can provide more symmetrical and less broad chromatographic peaks with a decrease in the retention times.<sup>77</sup>

GC separation of phthalates is generally conducted on capillary columns with a stationary-phase film containing 5 % diphenyl and 95 % dimethylpolysiloxane; the carrier gas is helium or argon. GC is simple, rapid, and more sensitive compared to HPLC.<sup>76</sup> Nevertheless, it might have problems with analysis of phthalate isomers.<sup>81,82</sup> LC can be used instead in such cases.<sup>77,82</sup> HPLC separation of phthalates is done on C18 stationary phase, while mobile phase is commonly a mixture of ACN and water. To achieve fast analysis with no peak-overlapping, HPLC analyses are mostly done in gradient mode.

#### *Other methods for determination of phthalates in water*

Chromatography is currently the dominant technique for determination of phthalates in water. Yet, some other techniques have been tested as well in order to obtain cheaper and less time-consuming approaches or approaches that can be performed in-situ. This mainly includes the use of electrochemical methods.

Polarography was used as one of the techniques for phthalate determination<sup>84,85</sup> during the 20<sup>th</sup> century. This technique had difficulties analyzing phthalate mixtures, and was not environmentally friendly.<sup>86</sup>

Voltametric methods, which overcome some of the polarographic disadvantages, are still used for phthalate analysis. These methods have good LOD values and are quicker, more sensitive, and more environmentally acceptable compared to those polarographic.<sup>86</sup>

Electrochemical immunosensors based on high specific antigen-antibody interactions have drawn

great attention as well. The immunochemical methods are characterized by specificity and rapid response.<sup>87</sup> Their application for phthalate analysis is simple and rather inexpensive. In addition, these methods are portable, which allows their *in-situ* application. He and Li<sup>88</sup> developed electrochemical immunosensor based on AuNCs/PEI-wCoSe<sub>2</sub> nanocomposite for quantitative determination of dipropyl phthalate (DPrP). The results were comparable with those achieved by some chromatographic techniques. Zhang *et al.*<sup>89</sup> proposed another immunosensor for DPrP detection: they used chemiluminescent enzyme dipropyl 4-aminophthalate-ovalbumin-horse-radish peroxidase complex, where chemiluminescence intensity was directly proportional to the amount of DPrP present in the sample. The method showed excellent specificity for DPrP in the presence of five different structurally similar phthalates. The authors reported LOD and recovery comparable to chromatographic analysis.

Molecular imprinting technology is another newly developed technology that has been applied in the field of phthalate analysis. It is based on preparation of MIPs, which have high specificity to analyte molecule.<sup>90</sup> Generally, MIP sensors have a significant drawback of complicated and time-consuming preparation, low binding capacity, and poor site accessibility.<sup>91</sup>

Compared to the mentioned methods, spectrophotometry is less selective. Therefore, it is combined frequently with various preconcentration and separation techniques. However, it is still a less expensive and faster approach than chromatography, and can be used for phthalate monitoring in environmental samples.<sup>92</sup> Jayshree and Vasudevan<sup>93</sup> used bacterial-enzyme-based spectrophotometry for DEHP determination in bottled water. They compared the results with those obtained by GC-MS with DLLME sample-prep, and found only slightly higher LOD and LOQ values. The applied enzyme greatly depended on pH value and the temperature, which complicated the analysis; however, beside this, no significant shortcomings were reported. In general, spectrophotometry overcomes many deficiencies related to chromatographic determination of phthalates. Therefore, further developments are expected in this field.

The methods discussed in this chapter are not frequently used for phthalate analysis, although they have sufficient advantages to be used in many practical applications. The methods need no special sample preparation, and the equipment is less complex compared to those chromatographic, which makes the analysis faster and less expensive. Finally, some of them have great potential for further improvements.

## Toxicity of phthalates

Water solubility of phthalates decreases with increasing molecular weight. Therefore, only phthalates of lower molecular weight can be present in water at concentrations that are considered sufficient to cause acute effects. Furthermore, even for these “lower” phthalates, high concentrations in polluted water are rarely achieved. This explains why an analysis of the literature published over the last 10 years showed a very limited number of studies dealing with acute toxicity of phthalates. More specifically, we managed to find five papers only: two using *Vibrio fischeri* as the target organism,<sup>94,95</sup> one with *Danio rerio*,<sup>96</sup> and two with *Daphnia magna*.<sup>97,98</sup> The remaining studies were focused on long-term exposures and observation of resulting malformations or abnormalities in behavior of the target organism.

There are numerous toxicological reports on the effects of phthalates on animals and humans. Some of them include teratogenic, mutagenic, and carcinogenic effects. In addition, male reproductive system can be affected as well, which includes infertility, low sperm count and motility, hypospadias, and others.<sup>99</sup> Chen *et al.*<sup>100</sup> demonstrated that phthalates could disrupt spermatogenesis and elicit reproductive toxicity in male zebrafish (*Danio rerio*). Tests were carried out by exposing adult male zebrafish to DBP, DIBP and their mixtures for 30 days. The authors were observing adverse effects on plasma hormone secretion, testis histology, and transcriptomics. As expected, the highest mixture concentration provoked the most severe testicular damage. Hannas *et al.*<sup>101</sup> found that exposure of fetal male rats to phthalates causes malformations of reproductive tract by reducing testosterone production and affecting steroidogenesis. They exposed the fetuses to 6 phthalates: DIBP, DnPP, dihexyl phthalate (DHeP), diheptyl phthalate (DHpP), DINP, and DIDP. The authors applied real-time polymerase chain reaction array containing key target genes. All the phthalates tested, with exception of DIDP, reduced testicular testosterone production in fetus. The authors also tested an influence of a mixture containing 9 antiandrogenic phthalates: DIBP, DnPP, DHeP, DHpP, DINP, BBP, DBP, DEHP, and DIHP (diisooheptyl phthalate). Data for single-compound toxicities for the last three phthalates were used from previous studies;<sup>102,103</sup> toxicity of BBP was assumed to be equal to the mean toxicity of remaining phthalates in the mixture. Additive model (also known as concentration addition model) was used to investigate the mode of the joint toxicity action in the mixture. The authors found similar mode of action for all phthalates from the

mixture; nevertheless, they were unable to understand completely the related toxicity mechanism.<sup>101</sup>

Generally, studies dealing with joint toxicity of phthalates are welcomed, since phthalates in water media are rarely present as single-component solutions; mostly, they come in combination with other toxic compounds. Wei *et al.*<sup>94</sup> investigated joint toxicity of DBP in binary combinations with five antibiotics: oxytetracycline hydrochloride, chlortetracycline hydrochloride, sulfamethazine, sulfamerazine, and sulfadiazine, toward luminescent bacteria *Vibrio fischeri*. All tested mixtures showed synergistic deviation from the additive behavior (meaning from the results predicted by additive model). Toxicity study of binary mixtures of six phthalates was conducted by Hamid *et al.*<sup>96</sup> They applied three toxicity models to reveal the modes of toxicity action: additive model (which was presented in form of the combination-index model), independent action model, and molecular docking model. Molecular docking model highlighted the affinity of DEHP to bind on estrogen receptors. In fact, among six tested compounds, DEHP revealed the highest *in vivo* and *in vitro* toxicity, followed by DEP, DBP, and DMP. Some binary mixtures proved to have higher toxicity potential than their constituents individually: a synergistic deviation from additivity was observed for *in vitro* analysis of three tested mixtures (DMP–DEP, DMP–DBP, and DEP–BBP).

Numerous studies have reported negative effects of prenatal exposure to phthalates. Xu *et al.*<sup>104</sup> investigated the effect of DEHP on the gene expression profiles in rat placenta. They reported adverse pregnancy outcomes that occur due to suppression of placental growth and development. Another study on pregnant rats<sup>105</sup> revealed the underlying mechanisms of placental size reduction after maternal exposure to DEHP. The study showed that DEHP altered the endocrine function of the placenta. In addition, DEHP and its active metabolite mono(2-ethylhexyl) phthalate (MEHP) enhanced maternal progesterone secretion. Unfortunately, the presence of DEHP and MEHP in human amniotic fluid was already confirmed.<sup>106,107</sup> A recent study<sup>108</sup> has shown that exposure to phthalates (DEHP and DBP at 50 and 250  $\mu\text{g L}^{-1}$ ) can cause spinal birth defects in zebrafish embryos, induced by transcriptional alterations of the spinal developmental genes. The results showed that exposure of the embryos to DEHP and DBP in concentrations 50 and 250  $\mu\text{g L}^{-1}$  inhibited spontaneous movement after fertilization, caused spine curvature, and decrease in body length. In addition, alteration in locomotor activity was observed, probably due to abnormal development of the spine and skeletal system. Adverse skeletal effects caused by embryonic exposure to phthalates were reported for rats also.<sup>109</sup>



Pu *et al.*<sup>110</sup> tested acute toxicity of six phthalates on zebrafish embryos and found different abnormalities, not only spinal curvature, but abnormal movement, decreased heart rate, and pericardial edema as well. Among six tested compounds, DBP and BBP showed highest toxicity, causing mortality even at low doses.

Toxicity studies of phthalate effects on humans are performed *in vitro*. Like the results obtained for animals, the observed adverse effects are related with reproductive system. Thus, Pant *et al.*<sup>111</sup> and Sun *et al.*<sup>112</sup> noticed compromised sperm functions, while Fang *et al.*<sup>113</sup> reported embryonic toxicity. Yet, some other effects were found as well. Kruger *et al.*<sup>114</sup> linked exposure to phthalates and eye irritation. Sicińska and associates did an extensive study<sup>115–117</sup> of changes in blood-constituent cells. They confirmed adverse effect of DBP, BBP, and their metabolites mono-*n*-butyl phthalate (MBP) and monobenzyl phthalate (MBzP) on human erythrocyte cells. All four compounds have strong oxidative potential, and therefore disturbed the erythrocytes redox balance. As a result, eryptosis can occur, which consequently leads to faster removal of erythrocytes from the circulation. Gutiérrez-García *et al.*<sup>118</sup> studied the effects of DEP, DBP, BBP and DEHP in umbilical cord blood. DBP, BBP and DEHP acted adversely by significantly reducing the expansion of hematopoietic cell; DEP showed no influence on cell expansion.

Generally, conclusions based on toxicity results obtained for one target organism may not be applicable for other organisms. They are only indicators of a potential threat, so species-specific test must be conducted for the final risk assessment.<sup>119</sup>

## Removal of phthalates from water samples

Phthalates have become ubiquitous environmental pollutants and can be found in almost all aquatic environments, where they tend to leach and volatilize from various solid products. Wastewater treatment plants (WWTPs) are another source of phthalates in the environment, since they contain a wide range of different wastewaters that are potentially heavily polluted with phthalates (e.g., leachate from plastic waste landfills, toilet water, house drainage water containing personal care and cleaning products, manufactory effluents like plastic and cosmetics industry, etc.).<sup>120</sup> Unfortunately, WWTPs mostly apply biological treatment focusing on carbon and nitrogen removal, while micropollutants are not specially targeted.<sup>121</sup> Consequently, they are unable to significantly reduce the amount of present phthalates but release them into the environment.<sup>122</sup>

Therefore, finding a method for successful removal of phthalates from water samples has become a challenge for scientific community and for the industry.

There are numerous studies dealing with the problem of phthalates removal from water samples. The methods can be classified into three main groups: physical-chemical treatments, biological treatments, and advanced oxidation processes. Each of the mentioned methods will be briefly discussed in following chapters.

### Physical-chemical treatments

Physical-chemical treatments of water include flotation, coagulation/flotation, adsorption, and membrane-based processes (filtration). Among them, adsorption has attracted special attention due to its low operating and maintenance costs in combination with relatively high removal efficiency. Furthermore, it produces no hazardous by-products. Activated carbon (AC) is probably the most commonly employed sorbent.<sup>123</sup> Recently, some other materials, such as clays, metal-organic frameworks, bioadsorbents, biochars, and agro-industrial waste and byproducts, have been studied as new sorbents.<sup>124–126</sup> The intention was to additionally lower the expenses of the treatment and to make it more environmentally acceptable. Membrane-based processes are another approach that has great popularity in wastewater treatment. The role of the membranes is to control permeation of different substances. Great separation efficiency, low energy consumption and, commonly, reduction in the number of processing steps are the main advantages of this approach.<sup>127</sup> Membrane designs can vary chemically and morphologically; each design provides some specific physical-chemical characteristics of the membrane. The variety of designs allows application of membrane technology for a wide range of organic molecules<sup>128</sup> including phthalates.<sup>129</sup> Despite the advantages, the membranes are rather expensive and have limited life cycle. Therefore, their use for highly concentrated solutions is commonly avoided. Wang *et al.*<sup>130</sup> combined two approaches: they used AC adsorption as a pre-step to nanofiltration. The intention was to improve the efficiencies of DMP, DEHP, and DnOP removal from the river water while preserving the membranes at the same time. They found that the obtained removal-rate values of this combined approach (above 99 %) significantly exceeded the values of each process individually. In addition, application of adsorption as a pre-step to membrane treatment increased life cycle of the membranes, since it removed the majority of dissolved organic matter and inorganic particles.

Once removed, the phthalates remain on the sorbent, in the sludge (if coagulation or flotation is

used), and partially on the membranes as well. The sludge as well as the used-up sorbents and membranes must be stored, and because they contain high concentrations of phthalates, they become a new potential source of pollution. Therefore, some other methods that overcome this imperfection have also been considered.

### Biological treatments

Biological treatment, or simply biodegradation, is the most common approach used for removal of phthalates from water media. The approach is based on the metabolic degradation of phthalates by microorganisms under aerobic, anoxic, or anaerobic conditions. Compared to other (non-biological) approaches, biodegradation is cost-effective and environmentally friendly method.

The primary degradation step usually includes hydrolysis of phthalate bonds, which results in side-chain alcohols and free phthalate residue. A conversion of longer alkyl chains to shorter chains has been reported as well. The side-chain alcohols are easily degraded by most microorganisms, but the phthalate residue often accumulates due to the lack of essential enzymes for its degradation. Therefore, total mineralization commonly requires synergistic action of diverse microorganisms, where one of them must be able to metabolize the phthalate residue.<sup>131</sup>

Numerous studies can be found on the biodegradation of phthalates. Therefore, only a few of the most representative ones will be emphasized in following sections.

#### Aerobic degradation

Strict aerobic bacteria: *Gordonia* sp.,<sup>132,133</sup> *Burkholderia* sp.,<sup>134,135</sup> *Pseudomonas* sp.,<sup>136–138</sup> and *Sphingomonas* sp.,<sup>139,140</sup> are most common pure cultures used for aerobic degradation of phthalates. Nevertheless, some facultative anaerobic bacteria (such as *Serratia* sp.<sup>141</sup> and *Bacillus* sp.<sup>142</sup>), or even algae<sup>143</sup> and fungi,<sup>144</sup> can be used as well.

Researchers report that pure cultures generally degrade short-chain phthalates effectively, while they are less effective in the case of longer-chain phthalates. Thus, Chen *et al.*<sup>145</sup> studied biodegradation of DBP with *Camelimonas* sp. M11 and found a decrease in efficiency with increasing number of carbon atoms in the chain. This suggested that esterases produced by the strain *Camelimonas* sp. M11 have highly specific activity. However, as an exception, the authors found no degradation in the case of DMP (with only one carbon atom in the chain). Zhang *et al.*<sup>142</sup> studied metabolic pathways of biodegradation of seven phthalates by *Bacillus mojavensis* B1811. The phthalates tested were:

DBP, BBP, DnOP, DPP, DEHP, DEP, and DMP. The results showed that all tested phthalates, including even DMP, could be degraded by the strain B1811. However, the degradation rates differed significantly: once again, phthalates with long alkyl chains degraded more easily than the short-chain phthalates. Obviously, the esterases produced by *Bacillus mojavensis* B1811 were also highly specific. All phthalates were primarily degraded to phthalate monoesters and then to phthalic acid. However, chemical analysis showed that *Bacillus mojavensis* B1811 caused no accumulation of phthalic acid in the medium, indicating that this strain was able to use phthalic acid effectively as an energy source. Such behavior was not detected in the case of *Camelimonas* sp. M11.<sup>145</sup>

Wu *et al.*<sup>132</sup> isolated four strains of *Gordonia* sp. (JDC2, JDC13, JDC26, and JDC33) from river sediments, and used them for biodegradation of phthalates. They performed experiments in medium-salt medium (MSM) at neutral pH and 30 °C. The research was divided in two parts. In the first one, biodegradation of DBP was examined. Strain JDC2 showed the best degradation of 96 % for 18 h contact-period. Other strains achieved good degradation of DBP as well, but for a longer contact-period. The range of phthalate chains that can be used as supplements to substrates was analyzed as the second step of the research. The analysis showed good growth of all four strains in substrates supplemented with short-chain phthalates (DMP, DEP), while only JDC2 and JDC13 showed good growth in the case of longer-chain phthalates (DnOP, DIOP). Sakar *et al.*<sup>133</sup> also tested *Gordonia* sp. for biodegradation of phthalates. More specifically, they tested ability of strain Dop5 to degrade DnOP isolated from municipal waste contaminated soil. They determined optimal degradation conditions: MSM with pH value 7.0 and 28 °C. Under these conditions, the tested strain completely degraded 0.75 g L<sup>-1</sup> of DnOP within a contact-period of 40 h. The authors pointed out that strain Dop5 had hydrophobic interaction with DnOP because it was found attached to the DnOP droplets surface. Obviously, this characteristic of *Gordonia* sp. plays an important role in its biodegradation ability because the strain was found to be similarly effective in degrading short- and long-chain phthalates.

Li *et al.*<sup>134</sup> studied biodegradation of seven phthalates: DEHP, DBP, BBP, DnOP, DMP, DPP and DEP, with strain B1213 of bacterium *Burkholderia pyrrocinia* in MSM. The concentration of the phthalates tested was 500 mg L<sup>-1</sup>. The optimal conditions included pH 7.0 and temperature 30 °C with addition of 1.0 % yeast. The addition of yeast enhanced the growth rate of the bacterium cells, and thus the biodegradation of phthalates as well. Even



though long-chain phthalates generally show resistance to biodegradation, the authors reported the opposite behavior of the tested strain. Namely, for 6 days contact-period, the strain biodegraded 98.05 % of DEHP and 88.74 % of DnOP (long-chain phthalates), but it showed weak degradation effect on other five tested phthalates (short-chain phthalates). This indicated that *Burkholderia pyrrocinia* B1213 could be a potential solution for removal of long-chain phthalates from polluted waters.

Feng *et al.*<sup>138</sup> exposed different DBP concentrations, ranging from 50 to 2000 mg L<sup>-1</sup>, to *Pseudomonas* sp. strain YJB6 for five days. YJB6 strain was isolated from phthalate-contaminated soil collected from plastic-film greenhouses. High levels of degradation (> 80.0 %) were achieved at all the concentrations tested, indicating the applicability of the tested strain to environments heavily contaminated with DBP. Further, the authors tested the strain's ability to grow on five other phthalates: DMP, DEP, BBP, DEHP, and DnOP, but also on phthalate degradation intermediates: MBP, phthalic acid, benzoic acid, and protocatechuic acid. The concentration of residual phthalate and the increase in biomass showed that the tested strain was using short-chain phthalates (DMP, DEP, DBP, BBP) as the sole carbon source more easily than the longer-chain phthalates (DEHP, DnOP). Furthermore, the strain showed growth ability on intermediates as well. Finally, Feng and co-workers tested the applicability of two environmentally friendly materials: polyvinyl alcohol and sodium alginate, to be carriers for the YJB6 cells. The materials were tested alone and in combination. Cells immobilized on the materials showed greater DBP degradation in comparison to the free cells: determined DBP residual after three days of incubation was 32.4, 21.8, 16.6, and 11.7 % for free strain cells, and cells immobilized on sodium alginate, polyvinyl alcohol, and sodium alginate-polyvinyl alcohol combination, respectively. Additionally, it was shown that carriers with immobilized cells could be reused several times with no significant reduction in DBP degradation potential (depending on the matrix used and the initial DBP concentration). This favors application of immobilized-cells approach over the free-cells approach.

Various enzymes are required for complete mineralization of phthalates, and they can be obtained by applying a combination of various microorganisms. Therefore, research related to the application of mixed cultures of microorganisms in biodegradation of phthalates is a particular scientific interest. In fact, the joint activity of microorganism is inherent in the natural environment in which different microorganisms live and act in synergy. Yang *et al.*<sup>146</sup> applied a mixed culture of microor-

ganisms for biodegradation of DBP in a batch reactor. Activated sludge from a WWTP and soil from rice paddy fields was mixed to obtain the mixed culture. The sludge/soil combination was shaped into pellets for simple resuspension in wastewater loaded with DBP. The pH was not adjusted but only monitored throughout the process and the temperature was 30 °C. The contact-period varied from 30 to 70 h, depending on DBP concentration. The experiments were performed on real wastewater and model solutions. Generally, a higher biomass increase was obtained for higher initial DBP concentrations. The removal values in all cases were from 91 to 100 % with slightly better results obtained for model solutions; the exception was 600 mg L<sup>-1</sup> solution, where better result was obtained for real wastewater samples. This indicates that some compounds from real wastewaters might inhibit the growth of biomass and DBP removal. Furthermore, cultures obviously need more time to adapt to the real wastewater environment.

#### Anaerobic degradation

In environments rich with oxygen, aerobic processes are usually the most important biodegradation processes. However, when it comes to deep waters, water sediments or water-rich soils, the oxygen content is reduced to zero within just a few millimeters of water body. There is a lot of organic material in landfills, so the oxygen available in a thin surface layer is consumed very quickly due to the initial aerobic biodegradation, leaving the area under the layer in complete or almost complete anaerobic conditions. Obviously, biodegradation of phthalates in such environment is also limited by the level of oxygen. Therefore, it is not surprising that there is a growing interest in research and development of anaerobic biodegradation processes.<sup>131,147</sup> Moreover, anaerobic biodegradation requires no aeration, which minimizes energy consumption and reduces reactor dimensions compared to aerobic processes. In addition, anaerobic biodegradation results in the production of significant levels of methane, which makes organic-rich wastewaters a promising source of bioenergy.<sup>148</sup> Therefore, anaerobic treatment is preferable in biodegradation of heavily polluted waters such as wastewater. Yousefzadeh *et al.*<sup>148</sup> analyzed application of two anaerobic biofilm bioreactors for DEP removal from model wastewater; these were anaerobic fixed film baffled reactor and up-flow anaerobic fixed film fixed bed reactor. Both selected bioreactors achieved nearly 90 % of DEP removal, which is still insufficient. Therefore, anaerobic biodegradation should be considered only as a pretreatment to some other removal technologies. Thus, Tomei *et al.*<sup>149</sup> treated waste sludge by such anaero-

bic/aerobic combination to remove various pollutants, including phthalates. Anaerobic phase of the treatment removed 69 % of DEP and 85 % of DEHP. The next (aerobic) phase of the treatment removed 38 and 77 % of the remaining DEP and DEHP, respectively.

### Advanced oxidation processes

As mentioned previously, it is difficult to degrade biologically complex organic molecules such as long-chain phthalates. Advanced oxidation processes (AOPs) are commonly used as an alternative approach due to their ability to transform complex structure pollutants into simpler and more biodegradable substances.<sup>150</sup> AOPs are based on generation of free radicals, which are highly reactive; the radicals react with molecules of pollutants to mineralize them, *i.e.*, to convert them into CO<sub>2</sub>, H<sub>2</sub>O, and mineral acids.<sup>151</sup> In many cases, the pollutants decompose only into simpler intermediates (complete mineralization is not achieved). The rate of phthalate removal by AOPs generally depends on chemical and physical characteristics of treated phthalate, but also on the characteristics of the oxidant applied. AOPs based on ozone, UV, or H<sub>2</sub>O<sub>2</sub> are the most common and most frequently studied AOPs because they have proved to be effective in oxidation and mineralization of a wide range of water pollutants. In fact, the efficiency of phthalate removal by UV or H<sub>2</sub>O<sub>2</sub> process is not very high. However, the combined action of these two processes results in photolysis of H<sub>2</sub>O<sub>2</sub>, which generates highly reactive hydroxyl radicals ( $\bullet$ OH). Thus, high phthalate removal rates have been successfully achieved with combined UV/H<sub>2</sub>O<sub>2</sub> treatment for some time.<sup>152,153</sup> The main parameters that affect the degradation are irradiation intensity, initial phthalate concentration, added oxidant concentration, and pH value of the solution. However, real polluted-water samples usually have heavy matrices that contain a number of other factors that can affect phthalate degradation and give results different from those obtained for phthalates in pure water. Therefore, some recent research has studied phthalate degradation with different matrices included. Thus, De Almeida *et al.*<sup>154</sup> compared UV-C/H<sub>2</sub>O<sub>2</sub> and UV-C/H<sub>2</sub>O<sub>2</sub>/AC approaches in removing DEP from three different matrices: tap water, tap water with phenol included, and a model solution of polluted water. They noticed a decrease in DEP degradation rate with increasing matrix complexity. Obviously, the presence of radical scavengers or competitive reactions with other matrix substances led to a significant decrease in kinetic constants of phthalate degradation. Wang *et al.*<sup>155</sup> tested UV/H<sub>2</sub>O<sub>2</sub> treatment in removal of DBP. Together with initial DBP concentration, dosage of oxidant, and pH value, the authors tested the influence of some

additional factors: alkalinity, presence of various inorganic anions and cations, and content of organic matter. They reported that increased alkalinity, presence of inorganic anions and organic matter had negative impact on DBP degradation due to the scavenging effect and related reactions with hydroxyl radicals. The effect of added metal ions was tested with the addition of Fe<sup>3+</sup>, Zn<sup>2+</sup> and Cu<sup>2+</sup> ions, which commonly exist in natural waters. It was found that Fe<sup>3+</sup> and Zn<sup>2+</sup> slightly decreased DBP removal, while addition of Cu<sup>2+</sup> ions promoted the reaction and resulted in higher DBP removal efficiency.

Application of ionizing irradiation may offer some other advantages, since both oxidizing and reducible species can be formed. Şolpan and Mehrnia<sup>156</sup> combined ionizing gamma rays with various concentrations of H<sub>2</sub>O<sub>2</sub> to treat DMP solution. They compared the results and found that increasing the concentration of added H<sub>2</sub>O<sub>2</sub> promoted the degradation of DMP and its intermediates.

Despite their high efficiency, the mentioned AOPs could potentially face some limitations with the scale-up of the process, like rapid decomposition of H<sub>2</sub>O<sub>2</sub> in the environment. Therefore, Wang *et al.*<sup>157</sup> proposed using UV/persulfate system as a better alternative. This approach generates sulfate radicals whose redox potential is somewhat lower than that of the hydroxyl radical, but with longer lifetime, they have a better potential to react with the organic matter in the matrix. The authors studied the effects of various factors on UV/persulfate degradation of DBP. The results showed that application of UV and persulfate alone resulted in no significant degradation of DBP. However, as was the case with UV/H<sub>2</sub>O<sub>2</sub> treatment, the combination of UV and persulfate resulted in high removal rate of DBP. Initial DBP concentration, added amount of oxidant, pH value of solution, and the presence of natural organic matter and inorganic ions were pointed out as parameters that affected the removal process. Finally, the authors performed additional tests with two different radical scavengers, and confirmed the existence of both sulfate and hydroxyl radicals in the system. Analysis of the results indicated that hydroxyl radical had greater influence on DBP degradation than the sulfate radical. The same researchers obtained compliant results for DEP degradation.<sup>158</sup>

Ozone is widely used for disinfection and decomposition of organic matter in drinking water. Mohan *et al.*<sup>159</sup> studied degradation of DEP in model leachate. Authors compared results obtained for O<sub>3</sub> and O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> treatments. O<sub>3</sub> treatment degraded 21 % of DEP within 120 min, while the addition of various concentrations of H<sub>2</sub>O<sub>2</sub> increased the degradation rate significantly (up to 99.9 % for the same contact period).

In photo-Fenton process,  $\bullet\text{OH}$  radicals are produced in reaction between  $\text{H}_2\text{O}_2$  and ferrous ions ( $\text{Fe}^{2+}$ ) in acidic medium under UV irradiation.<sup>160</sup> Acidity is required to avoid precipitation of  $\text{Fe}(\text{OH})_3$  and for scavenging of  $\bullet\text{OH}$  radicals. Careful adjustment of  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  doses is extremely important for effectiveness of the process. Dbira *et al.*<sup>161</sup> studied degradation of DAP in aqueous solutions by Fenton oxidation. They reported that lower pH values favored DAP degradation. Greater generation of hydroxyl radicals at acidic conditions enhanced DAP oxidation. However, the increase in pH value supported formation of ferric hydroxide complexes as well as the auto-decomposition of peroxide to oxygen and water; this reduced generation of the radicals and thus decreased degradation efficiency of the process. Further, different degradation values were obtained for the same amount of added  $\text{Fe}^{2+}$  but various iron sources; thus, 100, 91.49, 85.98, and 77.17 % was obtained for ferrous sulfate, pyrite,  $\text{FeF}_2$ , and ferric oxide, respectively. This indicated the great influence of the origin of iron on the degradation.

Heterogeneous AOPs with application of solid catalysts are used for phthalate removal as well.  $\text{TiO}_2$  holds a leading position among the catalysts due to its high chemical stability and activity, low production costs, and non-toxicity.<sup>162</sup> A systematic overview of photocatalytic materials used for phthalates removal is provided by Gmurek *et al.*<sup>162</sup> and Pang *et al.*<sup>163</sup>

Manosuri *et al.*<sup>151</sup> studied AOP degradations of DEP in aqueous solutions. They tested nine different approaches: UV,  $\text{H}_2\text{O}_2$ ,  $\text{O}_3$ ,  $\text{O}_3/\text{H}_2\text{O}_2$ ,  $\text{O}_3/\text{TiO}_2$ ,  $\text{O}_3/\text{AC}$ ,  $\text{O}_3/\text{Al}_2\text{O}_3$ ,  $\text{O}_3/\text{Fe}^{2+}/\text{H}_2\text{O}_2$ , and UV/ $\text{TiO}_2$ . The individual treatment by UV photolysis and  $\text{H}_2\text{O}_2$  oxidation resulted in very low degradation values. Degradation by ozonation highly depended on pH value of the solution: the degradation was very high in neutral and alkaline environments, while relatively low values were obtained in acidic environments. The optimal pH was found to be 9, where practically complete DEP degradation was achieved. Nevertheless, TOC values at pH 9 decreased only slightly (21 %), which indicated accumulation of DEP degradation products in the system. It would be interesting to see what occurs with TOC values at pH 11, where equal removal efficiency was achieved. Unfortunately, the authors did not provide this information.

Compared to ozonation, all combined processes tested showed similar pH dependence, with pH 9 as an optimal value. Combinations of ozonation with heterogeneous catalysts showed better results than UV/ $\text{TiO}_2$  process. Among them,  $\text{O}_3/\text{TiO}_2$  proved to be less efficient than the ozonation alone. Finally, the best approach for DEP removal was  $\text{O}_3/$

$\text{Al}_2\text{O}_3$ ; it provided complete degradation even though it was the cheapest and had the lowest energy consumption.

As discussed, application of AOPs can result in very high removal efficiencies. Unfortunately, these processes usually require costly equipment (e.g., ozone generators, UV lamps) and consumption of chemicals (e.g.,  $\text{H}_2\text{O}_2$ , persulfate, ferrous compounds). Therefore, it seems better not to use AOPs as the only treatment but as a posttreatment after some other less efficient but cheaper and more environmentally friendly approach, such as biodegradation. Such a combination would not only reduce consumption of energy and chemicals, but would extend the life of AOP equipment used. At the same time, the combination would retain the same removal efficiency as the single AOP.

## Conclusion

Phthalate pollution is a global problem. Phthalates can be found in practically all aquatic environments, including drinking water, wastewater, surface water, etc. Accordingly, various organisms, including humans, are in daily contact with phthalates. Phthalates are considered to be endocrine disruptors; they have a negative influence on reproductive system and development of organism.

Phthalates in the environment are rarely present as single-compound solutions, but as complex mixtures, which is an additional problem. Namely, the joint action of compounds can potentially increase their toxicity. Therefore, it is necessary to focus more intensively on their joint toxicity actions.

The fact that phthalates are ubiquitous in the environment, which includes their presence in a variety of analytical equipment as well, complicates their analysis because it increases the probability of sample contamination. Therefore, a high level of quality assurance must be implemented. Furthermore, the analysis of phthalates commonly requires extraction as a pretreatment. SPE and SPME are the most common techniques for extraction of phthalates. Beside those, liquid extraction methods that use micro-volumes of organic solvents are gaining more attention; they have lower price compared to the solid-phase extractions, while retaining simplicity and effectiveness. Analysis of phthalates is commonly performed by liquid chromatography with DAD, UV, or MS detection, or by gas chromatography coupled with FID, ECD, or MS detection. Spectrophotometric and electrochemical methods are used as well, due to their low cost and simplicity with no need for special sample preparation.



Various approaches have been tested for efficient removal of phthalates from different water solutions. Among physical-chemical processes, adsorption and membranes have relatively high removal efficiencies, and it has been shown that more effective removal can be achieved by combining these two approaches. Furthermore, the use of adsorption as a pre-step in membrane treatment prolongs the life cycle of the membranes, as it significantly reduces the concentrations of pollutants in solutions coming to the membranes. However, disposal of the sludge, as well as the used-up membranes is an additional problem, since they contain removed phthalates. In addition, application of membranes requires rather high investment, maintenance, and operating costs. AOPs have probably the highest potential for degradation of phthalates. However, investment and operating costs are deficiencies of these processes as well, accompanied with high chemical consumption. In contrast, biological treatment is a cost-effective and environmentally friendly approach. Its efficiency is mostly below those of membranes or AOPs, but it can be used as an excellent supplement to those approaches. Current trends in biological degradation of phthalates are focused on anaerobic processes, since such conditions are common in lower layers of solutions highly polluted with organics. In addition, the application of methanogenic organisms results in formation of methane, which indicates a significant potential of anaerobic bio-treatment in green energy production.

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#### List of abbreviations

AC	– activated carbon	DEHP	– bis(2-ethylhexyl) phthalate
ACN	– acetonitrile	DEHA	– 2-ethylhexyl adipate
AOP	– advanced oxidation process	DEP	– diethyl phthalate
BBP	– benzyl butyl phthalate	DHeP	– dihexyl phthalate
DAD	– diode array detector	DHpP	– diheptyl phthalate
DAP	– diallyl phthalate	DIBP	– diisobutyl phthalate
DBEP	– bis-2- <i>n</i> -butoxyethyl phthalate	DIDP	– diisodecyl phthalate
DBP	– dibutyl phthalate	DIHeP	– diisohexyl phthalate
DBZP	– dibenzyl phthalate	DIHpP	– diisoheptyl phthalate
DCHP	– dicyclohexyl phthalate	DINP	– diisononyl phthalate
DCM	– dichloromethane	DIOP	– diisooctyl phthalate
DEEP	– bis(2-ethoxyethyl) pEUththalate	DIPrP	– diisopropyl phthalate
		DIPP	– diisopentyl phthalate
		DLLME	– dispersive liquid-liquid microextraction
		DLLME-SFO	– dispersive liquid-liquid microextraction-solidification of floating organic droplet
		DMEP	– bis(2-methoxyethyl) phthalate
		DMP	– dimethyl phthalate
		DMSPE	– dispersive magnetic solid-phase extraction
		DnOP	– di- <i>n</i> -octyl phthalate
		DNP	– dinonyl phthalate
		DnPP	– di- <i>n</i> -pentyl phthalate
		DPP	– diphenyl phthalate
		DPrP	– dipropyl phthalate
		DSPE	– dispersive solid-phase extraction
		ECD	– electron capture detector
		EPA	– Environmental Protection Agency
		FID	– flame ionization detector
		GC	– gas chromatography
		HF-LPME	– hollow-fiber liquid-phase microextraction
		HF-SBSE	– hollow-fiber stir-bar sorptive extraction
		HF-SPME	– hollow-fiber solid-phase microextraction
		HLLE	– homogenous liquid-liquid extraction
		HPLC	– high performance liquid chromatography
		HRT	– hydraulic retention time
		i.d.	– inside diameter
		LC	– liquid chromatography
		LLE	– liquid-liquid extraction
		LOD	– limit of detection
		LOQ	– limit of quantification
		LPME	– liquid-phase microextraction
		MBP	– mono- <i>n</i> -butyl phthalate
		MBzP	– monobenzyl phthalate
		MEHP	– mono(2-ethylhexyl) phthalate
		MeOH	– methanol
		MIP	– molecularly imprinted polymer
		MOF	– metal-organic framework
		MS	– mass spectrometry
		MSPE	– magnetic solid-phase extraction

NIP – non-imprinted polymer  
 nPIPP – *n*-pentyl-isopentyl phthalate  
 o.d. – outside diameter  
 PP – polypropylene  
 PSF – polysulfone  
 PVC – polyvinyl chloride  
 PVDF – polyvinylidene fluoride  
 RDSE – rotating-disk sorptive extraction  
 REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals  
 SBSE – stir-bar sorptive extraction  
 SDME – single-drop microextraction  
 SPE – solid-phase extraction  
 SPME – solid-phase microextraction  
 TOC – total organic carbon  
 UV – ultraviolet  
 WWTP – wastewater treatment plant  
 ZIF-67 – zeolitic imidazolate framework-67

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