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Review Article

PHTHALATES - A PRIORITY POLLUTANT

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ABSTRACT
Phthalates, also known as phthalate esters, an alkyl aryl esters of 1, 2 benzenedicarboxylic acid. They have a broad range of applications, and are widely used as plasticizer (as vinyl softener). Phthalates act as endocrine disrupters and due to the increased awareness of its adverse effects on environment and health of living organisms, biodegradation of phthalates are now researched at a faster pace. This review highlights the applications of phthalates, their adverse effects on health, regulatory status and biodegradation of phthalates by pure and mixed bacterial cultures and fungi.

KEYWORDS: Phthalates, Xenobiotic compounds, Biodegradation, Endocrine disrupter, Plasticizer

INTRODUCTION

Phthalates, also known as phthalate esters, are the dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid. They are colorless, odorless liquids produced by reacting phthalic anhydride with an appropriate alcohol (usually 6 to 13 carbons). They show low water solubility, high oil solubility, high octanol-to-water partition coefficient (Chen *et al*., 2011) and low volatility. With increasing alkyl chain length, hydophobicity gets increased since the log K_{ow} increases. For example the log K_{ow} value for diethyl phthalate, di-n-butyl phthalate, benzylbutyl phthalate and di (2-ethylhexyl) phthalate are 2.38, 4.45, 4.59 and 7.94 (Staples *et al*., 1997). The polar carboxyl group contributes little to the physical properties of the phthalates, except when R and R' are very small (such as ethyl or methyl groups).

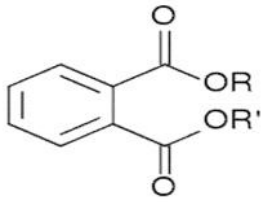

FIGURE 1. Chemical Structure of Phthalate

Phthalates are the primary plasticizer (as vinyl softener) in use today because of performance, cost, durability, and overall product sustainability benefits. When added to plastics, phthalates allow the long polyvinyl molecules to slide against one another and thus increase the plastic flexibility. They are chiefly used to turn polyvinyl chloride (PVC) from a hard plastic into a flexible plastic. Primarily, phthalates are an important ingredient in flexible vinyl products, such as wiring and cabling, wall covering and flooring. They are also used in vinyl blood bags and IV tubing used to help save lives. Other phthalates are used as solvents or fixatives, for example, to make fragrances last long. Di-n- butyl phthalate (DBP) is used as a coalescing aid in latex adhesive, a plasticizer for cellulose plastic and as a solvent for dyes (Liao *et al.,* 2010). Dimethyl phthalate (DMP) is typically used in cellulose ester-based plastics such as cellulose acetate and butyrate (Staples *et al*., 1997); and is also a component of coating food packing, cosmetics, lubricants, decorative clothes etc., (Baikova *et al*., 1999). Di (2-ethylhexyl) phthalate (DEHP) is a high production volume chemical used in the manufacture of a wide variety of consumer food packaging, some children's products, and some polyvinyl chloride (PVC) medical devices.

Phthalates were first produced during the 1920s, and have been produced in large quantities since the 1950s, when PVC was introduced. More than 60 kinds of phthalates are produced nowadays (Lu *et al.,* 2009).The annual worldwide production of phthalates exceeds 5 million tons (Mackintosh *et al*., 2006). Worldwide annually more than 18 billion pounds of phthalate esters are used primarily as plasticizers in flexible PVC products (Blount *et al*. 2000a) and also as inert ingredients in many sprays including insect repellent, pesticides and in many consumer products such as wood finishes and cosmetics (Blount *et al*. 2000b).

Phthalates as Pollutants

As the log K_{ow} of phthalates increases, their hydrophobicity increases. For instance, Butyl Benzyl Phthalate (BBP) log K_{ow} value is 4.45, in comparison of other compounds of same group, so leaching of BBP from the plastic product into the environment increases when used as plasticizers. Talking about the high molecular weight phthalates, hydrophobicity increases as the alkyl chain length increases. For example, DEHP log K_{ow} 7.94, so they are tightly bound to plastics and do not dissolve in water easily but can accumulate in soil and sediment and

in the tissues of various aquatic biota (Yuan *et al*., 2002; Huang *et al*., 2008; Wang *et al*., 2008). Due to biomagnification the maximum exposure is to humans since humans are at the top of food chains.

Phthalates readily releases during the production, distribution, waste disposal and can easily leach out from landfills into water, soil and groundwater, and consequently phthalates are ubiquitously present in environment and have been described as man-made (xenobiotic) environmental priority pollutants (Latini, 2005).

Phthalates as Endocrine Disruptors

Endocrine disruptors (ED) are chemicals that may interfere with the body's endocrine system and produce adverse reproductive, developmental, neurological, and immune effects in both humans and wildlife. A wide range of substances, both natural and man-made, are thought to cause endocrine disruption, including pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, DDT and other pesticides, and plasticizers such as bisphenol A phthalates. Naturally occurring substances such as Phytoestrogens (for example, genistein and daidzein) found in plants have hormone-like activity, act as ED. When absorbed in the body, an endocrine disruptor can decrease or increase normal hormone levels, mimic the body's natural hormones, or alter the natural production of hormones (Gilbert, 2006). Endocrine disruptors may be found in many everyday products– including plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides.

Phthalate esters are considered to be a potential carcinogen, teratogen, and mutagen (Fushiwaki *et al*., 2003; Fatoki *et al*., 2010). Also phthalates acts as endocrine disruptors, which could alter reproductive functions and exert distinct effects on male reproductive organs due to antiandrogenic effects (Latini *et al*., 2006; Lambrot *et al*., 2009; Vo *et al*., 2009; Moral *et al*., 2007). In 2006, the National Toxological Program (NTP) found that DEHP may pose a risk to human development, especially critically ill male infants (NTP-CERHR, 2006). Phthalates showed estrogenic activities (Picard *et al*., 2001; Harris *et al*., 1997; Soto *et al*., 1995; Jobling *et al*., 1995). BBP alters the level of testosterone & other reproductive hormones; toxic to testes, prostrate and seminal vesicle (NTP-CERHR 2003) Phthalates have shown antiandrogenic and teratogenic effects in rats (Bower *et al*., 1970 Ema and Miyawaki, 2002). In humans, BBP increases the severity of endometriosis (Reddy *et al*., 2006). DBP exhibits antagonistic thyroid receptor activity (Li *et al*., 2010).

Phthalates are not only an endocrine disruptor for animals but also an environmental stressor for plants. Phthalates can cause various effects on plants such as carotenoid synthesis disturbance or chlorophyll formation, irregular formation of grana, white leaves, necrosis,etc., (Hemming *et al*., 1981). Chen *et al*., 2011 observed the up and down regulation of genes involved in water celery growth when treated with BBP, affecting plant growth, cell cycle and protein synthesis interference and dwarfism.

Through the combustion of refuse, phthalates may be released in the air, acting as an environmental contaminant (Graedel *et al*., 1986; CICAD 1999). BBP has been detected in stack emissions from hazardous waste combustion facilities and from coal burning power plants in the USA (Oppelt, 1987)

Regulatory Status of Ed's

In recent years, lots of data have been produced on the properties, exposure and toxicity of phthalates, due to regulatory oversight of the manufacture, transport, application and disposal. Such data are essential for the development of safe and acceptable production practices, effluent discharge limits and human exposure limits.

European overview

(http://www.marchem.com/materials/plastisols/phthalate-free.html)

European government banned the use of 6 phthalate esters in toys and children's products that might be potentially placed in the mouth, at levels greater than 0.1% of the total object weight on January 16, 2007. The phthalates subject to this regulation are:

- Di-2-ethylhexyl phthalate (DEHP)
- Dibutyl phthalate (DBP)
- Butyl benzyl phthalate (BBP)
- Di-isononyl phthalate (DINP)
- Di-isodecyl phthalate (DIDP)
- Di-n-octyl phthalate (DNOP)

The EU has also applied limitations to the use of these phthalates in general food contact applications (packaging and closures) and medical device applications. In addition, several phthalates have been listed as "Substances of Very High Concern" (SVHC) requiring reporting of their content in articles exported into the EU under the REACH regulations:

- Di-butyl phthalate (DBP)
- Di-2-ethylhexyl phthalate (DEHP)
- Butyl benzyl phthalate (BBP)

Indian Overview

On April 21st, 2011 the Bureau of Indian Standards (BIS) has circulated a draft amendment No. 3 to IS 9873 (Part 3):1999/ ISO 8124-3:1997 Safety requirements for toys Part 3 Migration of certain elements.

Following are the requirements for phthalates applied only to vinyl toys and childcare article.

Less than or equal to 0.1% of Bis (2-ethylhexyl) phthalate, dibutyl phthalate or benzyl butyl phthalate in vinyl toys or childcare article. Also less than or equal to 0.1%of Di-isononyl phthalate, di- isodecyl phthalate or di-n-octyl phthalate in any part of the vinyl toy or childcare article that can be placed in mouth of a child under 4 years of age (http://www.bis.org.in/sf/pcd/Draft9873_3A3.pdf).

United States overview: (www.cpsc.gov/cpsia.pdf)

In the United States, on August 14, 2008 the Consumer Product Safety Improvement Act (CPSIA) incorporated regulation of phthalate esters as components of children's toys and child care articles for children under the age of 12 that could be "placed in the mouth".

For CPSIA purposes, the following phthalates were permanently banned at levels greater than 0.1%:

- Di-2-ethylhexyl phthalate (DEHP)
- Dibutyl phthalate (DBP)
- Butyl benzyl phthalate (BBP)

The CPSIA also imposed an interim ban on the use of the following phthalates at levels greater than 0.1%

pending the results of further CPSC (Consumer Product Safety Commission) review:

- Di-isononyl phthalate (DINP)
- Di-isodecyl phthalate (DIDP)
- Di-n-octyl phthalate (DNOP)

Biodegradation of Phthalate

Phthalates can be removed from the environment by several methods including hydrolysis (Jonsson *et al .,* 2006), photo degradation (Lau *et al.,* 2005; Yuan *et al*., 2008; Kaneco *et al*., 2006), TiO_2 photocatalysis (Sin *et al*., 2012), pulse radiolysis and electron beam radiolysis (Wu *et al*., 2011), microbial degradation (Li *et al.,* 2006; Chao *et al*., 2006; Gu *et al*., 2009), adsorption (Venkata Mohan *et al.,* 2007) and subcritical water extraction (Chang *et al*., 2011). Microorganisms have a great versatility, simpler, environment friendly and of course less expensive when compared to other non-biological methods for pollutant treatment. Bioremediation involves the degradation of pollutant by the enzymes present in living organisms. Microorganisms may be aerobic (Wang *et al.,* 1995; Jianlong *et al.,* 1995), anaerobic (Wang *et al.,*2000; Shelton *et al.,*1984) or facultative (Zang and Peardon, 1990). Many bacterial strains with the ability to degrade phthalate have been isolated from activated sludge, mangrove sediment, wastewater, river sludge etc. (Roslev *et al.,* 2007; Xu *et al.,* 2007; Liang *et al.,* 2008; Lu *et al*., 2009; Wu *et al.,* 2011), including strains from about 25 genera such as *Sphingomonas (*Chang *et al*. 2002*), Pseudomonas (*Xu *et al*. 2006*), Rhodococcus, Enterococcus (*Chang *et al*. 2007*) Gordonia* (Chatterjee and Dutta (2003)), *Corneybacterium* (Chang *et al., 2004; Chao et al., 2006*), and *Agrobacterium* (Wu *et al*., 2011). Studies have shown esterase as the key enzyme involved in microbial degradation of phthalates esters. Studies have reported isolation and characterization of the phthalate esterases from several bacterial strains comprising *Rhodococcus erythropolis* (Kurane, 1997), *Micrococcus* sp. YGJ1 (Akita *et al.,* 2001; Maruyama *et al*., 2005), *Gordonia* sp.P8219 (Nishioka *et al*., 2006), *Pseudomonas* sp. 054 (Tserovska *et al.,* 2006) and *Ochrbactrum anthoropi* (Zu *et al* ., 2006)*;* fungal strains including *Fusarium* sp. DMT-5-3 (Luo *et al*.,2012). Slow degradation of longer alkyl chains phthalates have been observed in presence of shorter alkyl chains phthalates (Wu *et al.,* 2011). Numerous microorganisms have been studied for the degradation of phthalate which are presented in Table 1.

TABLE 1: Studies on Biodegradation of Phthalate

Microorganisms	Descriptions	Comments/ Limitations	References
Gordonia sp. Strain MTCC 4818	Hydrolysed both ester bonds of BBP and utilized the released benzyl alcohol and butanol for growth. End product-Phthalic acid Efficiency of degradation of BBP -1 g/L within four days Aerobic conditions; Neutral pH and 28°C Completely degraded BBP	Degradation of monoesters Mono Butyl Phthalate (MBuP) and Mono Benzyl phthalate (MBzP) was slow; these compounds accumulated in the spent culture in a 1:2 ratio	Chatterjee and Dutta (2003)
Corneybacterium sp. DK4	Efficiency of degradation of BBP 5mg/L within 2 days Aerobic conditions; Neutral pH and 30°C Completely degraded BBP	High concentration of phthalate (30 & 100mg/L) was not degraded	Chang *et al*. 2004
Sphingomonas sp. O18	Efficiency of degradation of BBP- 5mg/L within 3 days Aerobic conditions; Neutral pH and 30°C	*Sphingomonas* sp. O18 could also degrade BBP but more slowly than DK4	Chang *et al*. 2002
Pseudomonas fluorescens B-1	Utilized the butyl group moiety of BBP more readily than the benzyl moiety. Completely degraded BBP. Major metabolites- MBuP, MBzP, phthalic acid (PA), and benzoic acid Efficiency of degradation of BBP - 2.5 to 20 mg/L within 6 days Aerobic conditions; Neutral pH and 30°C	Degradation process could be fitted to a first-order kinetic model	Xu *et al*. 2006
Enterococcus sp. OM1 *Bacillus benzoevorans* (S4)	BBP amount reduced by 98.6% to Efficiency of degradation of BBP - 100% within 5 days	Degradation by these strains was comparable to that by *Sphingomonas* sp. O18 and *Corynebacterium* sp. DK4	Chang *et al*. 2007
Bacillus subtillis strain 3C3	Utilized Diethyl Phthalate (DEP) as sole carbon source at pH 7.0, temperature up to 45°C, DEP concentration 1000mg/L Biodegradation occurred consecutively without lag period and followed a first-order model.	Categorized as a bacterium with biodegradation ability in a moderately to high concentration level of DEP	Navacharoen and Vangnai. 2011

Arthrobacter sp. WY	Utilize both the monoesters (MBuP and MBzP) as well as phthalic acid for growth. End product-Side chain alcohols (benzyl alcohol and butanol) Efficiency of degradation of BBP - Degraded 50% BBP (initial concentration 1 g/L, at pH7 and 28°C) within 16 days and 95% within 39 days; no BBP was detected after 44 days Initial concentration 1 g/L, at pH7 and 28°C	Cell surface hydrophobicity of strain WY was very low, which reduced the contact with the hydrophobic substrate and thereby slowed down the degradation in the aqueous medium.	Chatterjee and Dutta 2008a
Arthrobacter sp. WY with addition of Tween 80 at 0.05mM	Total degradation of BBP in 20 days		Chatterjee and Dutta 2008b
Agrobacterium sp.	Complete degradation of DBP within 48hrs at pH 8.0, 30^{o}C, substrate concentration lower than 200mg/L DBP degradation was exponential Half- life of degradation was about 10.4 h at less than 200mg/L concentration of DBP	Potential candidate for removing phthalates	Wu *et al.,* 2011
Enterobacter sp. T5	Optimum degradation pH 7.0 and temperature 35ºC Half- life of degradation was about 20.9 h at less than 1000mg/L concentration of DBP Major products- Phthalic Acid and Mono Butyl Phthalate (MBP)	Also grew on DMP, DEP; suggesting its ability to resist pthalic acid diester toxicities.	Fang *et al*., 2010
Flavobacterium sp. strain No. A-1	Complete degradation of phthalic acid (1660mg/L) in less than 2 days	But could not degrade dimethyl, diethyl phthalate ester and phthalic anhydride	Tanaka *et al*., 2006
Corynebacterium sp. DK4	99.2% BBP was degraded after 7 days of incubation but DEP, DPP and DBP degraded completely	Degradation was slower; could be due to their low bioavailability in sediments.	Chang *et al*. 2004
Fusarium oxysporum f. sp. pisi strain	Almost 60% of the initial BBP (500 mg/L) within 7.5h	Fungal cutinase (10mg protein/L) was the enzyme responsible for degradation	Kim *et al*. 2002
Pleurotus ostreatus, Irpex lacteus, Polyporus brumalis, Schizophyllum commune, Fomitella fraxinea, Merulius tremellosus, Trametes versicolor, & T. versicolor MrP1, MrP13 (transformant of the Mn-repressed peroxidase gene of *T. versicolor*) & MnP2-6 (transformant of the Mn-dependent peroxidise gene of *T. versicolor*)	80 to 100mg/L of BBP was degraded within 6 to 12 days	Among these, P. brumalis, T. versicolor, and the transformants of T. versicolor, MrP1 and MnP2-6, degraded 100 mg/L of BBP much faster than the other strains tested.	Seok *et al*. 2008

Generally phthalates have been detected as a mixture in environment, so phthalates coexistence can also affect their concurrent biodegradation. Since for selecting a bioaugmented microbial culture for bioremediation application, the information about each phthalate interaction with each other can lead to alteration of rate and extent of microbial degradation (Chang *et al*., 2004; Navacharoen and Vangnai, 2011; O'Grady *et al.,* 1985(6)). Also addition of certain co-metabolic substrate like yeast extract (Navacharoen and Vangnai, 2011), Tween 80 etc., enhance the cell ability to cope with pollutant toxicity and increase the degradation efficiency. Co-metabolic substrate can either stimulate the cell growth or act as an inducer for certain enzymatic reactions (Arp *et al*., 2001; Grant and Betts, 2004)

Studies have shown that a microbial consortium shows more compound degrading capacity. This may be due to synergistic relationship that allows microbial population to produce enzymes that are not produced by either population alone (Atlas and Bartha, 1998). Also phthalate degradation requires diverse metabolic machinery comprising sets of distinct degradative genes. Since

phthalate hydrolysis results into phthalate esters, side-chain alcohols and phthalic acid, and full utilization of phthalate esters, side-chain alcohols and phthalic acid by single microbial species is rather slow and incomplete

TABLE 2. List of mixed bacterial cultures degrading BBP

Mixed bacterial cultures	Individual cultures	Utilization of		Description	References
		A	**B**		
Arthrobacter sp. Strain WY and *Acinetobacter* sp. strain FW	*Arthrobacter* sp. Strain WY	Yes	No	Completely assimilated 1 g/L of BBP in aqueous solution within 44 days at 28°C with no appreciable accumulation of intermediate metabolites. Degradation was slow.	Chatterjee and Dutta 2008a
	Acinetobacter sp. strain FW	No	Yes		
Gordonia sp. strain MTCC 4818 and *Arthrobacter* sp. WY	*Gordonia* sp. strain MTCC 4818	No	Yes	Completely mineralized BBP without identifiable intermediates within 108 h. This co-culture was able to completely degrade a mixture of phthalates of environmental concern	Chatterjee and Dutta 2008b
	Arthrobacter sp. WY	Yes	No		
Corynebacterium sp. and Sphigomonas sp.	*Corynebacterium* sp.	No	Yes	The degradation rate of eight phthalates were higher for *Sphigomonas* sp. than *Corynebacterium* sp. In the simultaneous presence of both strains, the degradation rate was enhanced.	Chang *et al*. 2004
	Sphigomonas sp.	Yes	No		

A- MBuP, MBzP, phthalic acid,or protocatechuic acid
B- benzyl alcohol or 1-butanol

Based on alkyl chain length, studies have suggested that phthalates with shorter alkyl chains are rapidly degraded whereas phthalates with longer alkyl chains are poorly degraded (Chang, 2004; Xia, 2004; Wang, 2000; Eljertsson, 1997). Also phthalate degradation is affected by changes in environmental conditions like change in pH value, temperature, and phthalate concentration and by addition of nonylphenol and polycyclic aromatic hydrocarbon (Chang *et al*., 2004).

With the use of syntropic consortia it can be suggested that phthalates with longer alkyl chains might be completely degraded by biochemical cooperation of different strains. Wu *et al*., (2010) recently described a dual culture performing better than the single species alone. *Gordonia* sp. strain JDC-2 and *Arthrobacter* sp. strain JDC-32 isolated from activated sludge showed the biochemical cooperation in complete degradation of di-n-octyl phthalate (DOP). DOP was rapidly degraded into phthalic acid by *Gordonia* sp. strain JDC-2, which accumulated in the culture medium. *Arthrobacter* sp. strain JDC-32 degraded phthalic acid but not DOP. Vega and Bastide [2003] reported that *Arthrobacter* sp. transformed Di Methyl Phthalate to Mono Methyl Phthalate (MMP), and then *Sphingomonas paucimobilis* hydrolyzed MMP to Phthalic Acid. Another study demonstrated that *Klebsiella oxytoca* Sc rapidly transformed dimethyl isophthalate to monomethyl isophthalate, which was further converted to isophthalic acid by *Methylobacterium mesophilicum* Sr (Li and Gu, 2007).

Phthalate esters have also been de novo synthesized by freshwater algae and cyanobacteria (Babu & Wu, 2010), and marine alga (Chen, 2004). DBP and Mono Ethyl Hexyl Phthalate (MEHP) were synthesized by the cells themselves, and the synthesized phthalates was stored in the cells and not released to the extracellular medium under normal growth conditions. However release of phthalates from algal cells might occur when they grow under stress conditions giving rise to phthalate leaching, thus affecting the aquatic ecosystem. The study showed the kind and quantity of phthalates is dependent of species and display inter-generic, inter-specific, and intra-specific variations.

CONCLUSION

With the increase in population and their demands and thus the advancement of technology, releases of xenobiotic compounds into the environment are increasing at a much faster pace. However the natural biodegradation capability of microorganisms evolves at a much slower rate. Phthalates are one of the xenobiotic compounds, proven as an endocrine disrupter**.** In future more research emphasizing on different improvement strategies like addition of surfactants, immobilization on Activated Carbon, Genetic Engineering etc., will explore the new metabolic pathways for the safe disposal and complete mineralization of phthalates without any dead end product formation.

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