Introduction

Phthalate(1,2-benzene dicarboxylate) and its esters such as **DEHP**(1,2-diethyl hexyl phthalate) are used as plasticizers in plastic industry. Isomers of phthalate such as Isophthalate(m-phthalate) and terephthalate(p-phthalate)are used for the production of PET bottles and packaging industry.

Phthalates are the primary plasticizers 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 flexibility of plastics ,which are then used for a number of uses .

Although they are being used extensively these days all phthalates are harmful in excess and act as pollutants .For example, **DEHP** are tightly bound to plastics and do not dissolve in water easily at room temperature but can accumulate in soil and sediment and in the tissues of various aquatic biota. Due to biomagnifications the maximum exposure is to humans as humans are at the top of food chain.

Phthalates readily releases during the production ,distribution ,waste disposal due to leach out from landfills into water ,soil and groundwater and consequently phthalate & its esters are ubiquitously present in environment and frequently described as man-made environmental priority pollutants. DEHP being long-chain di-esters are less susceptible for biodegradation by microbial enzymes.

Experimental evidence has proved the damaging effect of DEHP on the liver of rats and mice. US EPA have also classified the DEHP as a probable carcinogen. Reports of possible teratogenicity and mutagenicity are also there with US EPA. In pregnant rats and mice exposed to higher amount of DEHP has shown birth defects and fetal deaths.

The biodegradation studies have mostly concentrated on industry-specific degradation approaches. But in terms of efficiency , most of the reported biodegradation are through consortia. Individual bacterial strains capable to degrade this pollutant are very limited. The biodegradability efficiency in microbes differ mainly due to the steric effects of the side ester chains and the chain length.

REVIEW OF LITERATURE

Chang et al.(2007) have reported the degradation of DEHP by *Bacillus* sp.S4 isolated from sludge with a high degradation efficiency.

Chao and Cheng(2007) have put forward the report of biodegradation of DEHP by *Rhodococcus rhodochrous* G2, G7 with an efficient rate of degradation. Nalli et al(2006) also reported degradation of the same pollutant by *R. Rhodochrous* ATCC 21766 though with a lower efficiency than the former.

Nakamiya et al.(2005) reported the successful degradation of DEHP by *Mycobacterium* sp. NK 301 isolated from garden soil.

Quan et al.(2005) have also reported the bacterial degradation of the pollutant by *Bacillus subtilis* no 66.

Thus, the report of successful biodegradation of DEHP is lacking under Indian conditions.

AIMS AND OBJECTIVES

1. To study the nature of degradation of DEHP.
2. To isolate, purify and characterize the soil bacteria capable to degrade DEHP.

MATERIALS AND METHODS

1. Soil sample :

Soil samples were collected from the adjoining areas of Triveni, Distt- Hooghly, West Bengal and Chandrakona, Paschim Medinipur, West Bengal.

2. Ingredients taken for media preparation:

A. Minimal salt medium (MSM) containing –

- Carbon source – DEHP (0.5 % v/v and 0.83% v/v)
    - Salts – NaCl -25mM
      $NaNO_3$ -25mM
      $MgSO_4$ -20mM
    - Major elements -

      KH_2PO_4- 0.1mM

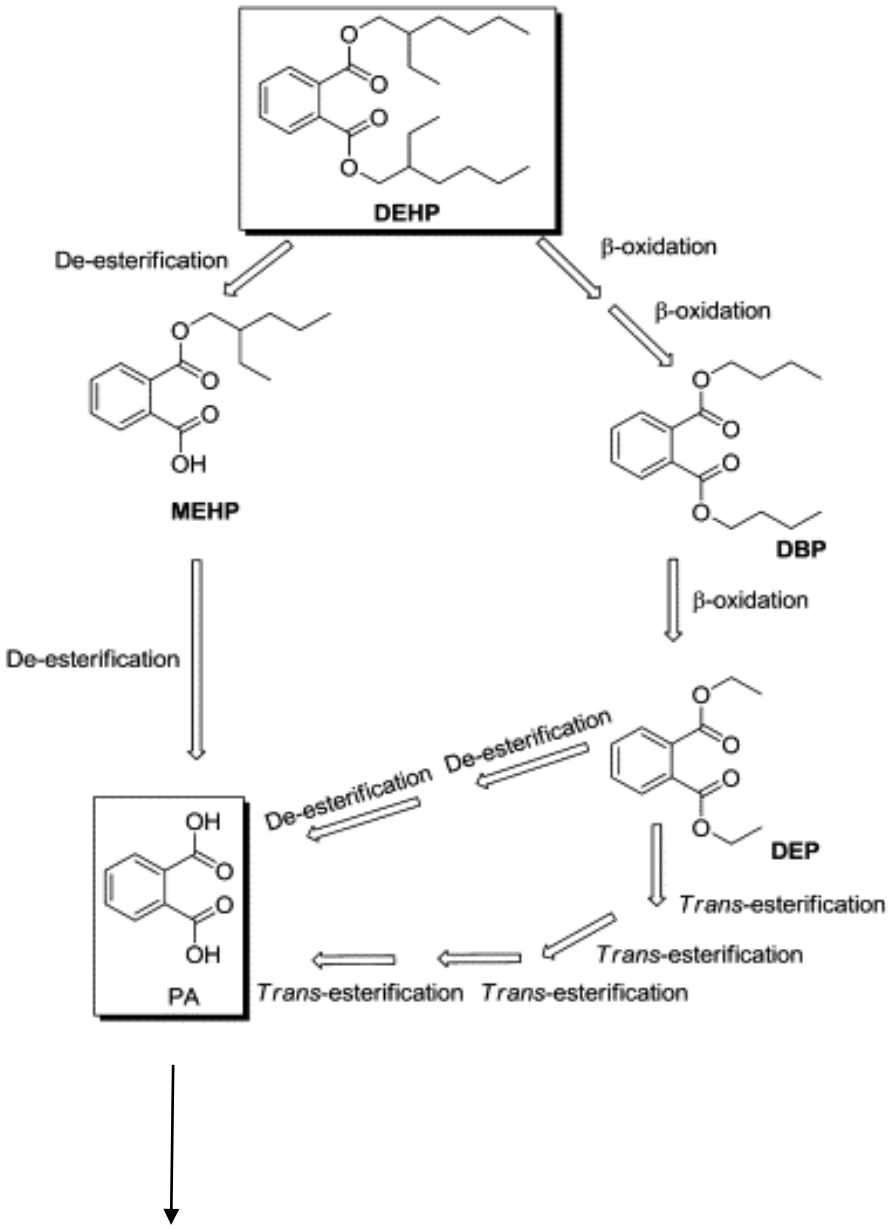
      Na_2HPO_4- 2.5mM

    - Trace element solution added @0.1ml /100ml .
    - Trace element solution composition ($FeSO_4$, $ZnSO_4$, $MnSO_4$ & $CuCl_2$)

- For better understanding of metabolic efficiency the strains were also grown on the respective phthalate isomers such as , o-phthalate , m-phthalate (isophthalate) and p-phthalate (terephthalate) @ 0.1% w/v.

3. **Broth culture** – Soil samples were added to the MS medium supplemented with 0.5 % and subsequently 0.83% (v/v) of DEHP and incubated for 36 hours at 37° C within the rotary –shaker incubator @ 150rpm.
4. **Isolation by plating** – Plates were prepared with the spreading of the same media as in broth supplemented with 2% agar and the sole carbon source (DEHP). Plates were incubated at 37° C.
5. Glasswares used –
    a. Erlenmeyer's flasks (100ml, 250ml)
    b. Petri plates
    c. Measuring cylinder
    d. Test tubes
    e. Spreader
    f. Beaker (500ml)
    g. Pipettes (5ml & 10ml)
6. Instruments used –
    a. Autoclave
    b. Laminar air flow
    c. Compound microscope
    d. Micro-pipette
    e. Rotary Incubator – shaker
    f. Electronic weighing balance
7. Hi-media reagents and discs were used for the biochemical tests.

DEHP Degradation Pathway



Phthalate degradation pathway

RESULTS

The isolated strains were tested for their biochemical characterization. The results are summarized in the table below:

Name of the Strain	Indole	MR	VP	Citrate	ONPG	Nitrate Reduction	Catalase	Oxidase
DCB41	-	+	-	-	-	-	-	-
DCB42	-	+	-	-	+	+	+	-
DCB43	-	+	-	-	-	-	+	-
DCB44	-	-	-	-	-	-	+	-
DCB45	-	-	-	-	-	-	+	-

Morphological , structural and colony characteristics of individual strains

DCB41 – Gram –ve short rods, with biofilm formation, small shiny colonies with regular margin.

DCB42 - Gram –ve rods, without biofilm formation, medium colony, irregular margin.

DCB43 - Gram –ve rods with biofilm formation, bluish , dull colonies with regular margin.

DCB44 - Gram –ve short rods, without biofilm formation, bluish, dull colony with regular margin.

DCB45 – Gram –ve short rods, without biofilm, shiny colonies with regular margin.

All the cultures showed convex elevation in the colony.

In addition to the growth on DEHP . some strains have also shown the growth on the parent molecules like phthalate, terephthalate etc.

DCB41 grows on phthalate (0.1 % w/v) ; DCB 44 and DCB 45 also showed growth on 0.1% (w/v) terephthalate.

DISCUSSION

The isolated strains have shown the growth on such a pollutant which has not been reported in India. The growth was also favoured at **very high concentration** of pollutants viz. 0.5% and 0.83%(v/v) which corresponds **to 5 x 10^3 ppm** and **8.33 x 10^3 ppm** respectively.

These findings are thought to be promising.

In addition to DEHP , strains which are capable to degrade other pollutants have the metabolic flexibility to adapt such changes in nutrient source. Though we did not have the strains metabolizing isophthalate.

CONCLUSION

The reported strains have shown promising properties in the degradation of DEHP, as a pollutant. The results of degradation were found to be high and encouraging.

Hence, the microbial degradation of DEHP studied in the said strains are novel in terms of efficiency and metabolism.

Future study can put the emphasis on enzymatic characterization and further genetic characters for the said strains in degradation.

References

Chang BV, Wang TH, Yuan SY (2007) Biodegradation of four phthalate esters in sludge. Chemosphere 69:1116–1123

Chao WL, Cheng CY (2007) Effect of introduced phthalate-degrading bacteria on the diversity of indigenous bacterial communities during di-(2-ethylhexyl) phthalate (DEHP) degradation in a soil microcosm. Chemosphere 67:482–488

Chen J.E., Li X., Li J., Cao, J., Qiu Z., Zhao Q., Xu C., Shu W., 2007 Degradation of environmental endocrine disruptor di-2 –ethyl hexyl phthalate by a newly discovered bacterium *Microbacterium* sp. Strain CQ0110Y. Applied Microbiology Biotechnology 74: 676 – 682

Graham P.R., 1973 Phthalate ester plasticizers – why and how they are used. Environ.Health. Perspect. 3 : 3 - 12

Juneson C, Ward OP, Singh A (2001) Biodegradation of bis-(2-ethylhexyl) phthalate in a soil slurry-sequencing batch reactor. Process Biochem 37:305–313

Nakamiya K, Hashimoto S, Ito H, Edmonds JS, Yasuhara A, Morita M (2005) Microbial treatment of bis (2-ethylhexyl) phthalate in poly-vinyl chloride with isolated bacteria. J Biosci Bioeng 99:115–119

Nalli S, Cooper DG, Nicell JA (2006) Metabolites from the biodegradation of di-ester plasticizers by *Rhodococcus rhodochr-ous*. Sci Total Environ 366:286–294

Quan CS, Liu Q, Tian WJ, Kikuchi J, Fan SD (2005) Biodegradation of an endocrine-disrupting chemical, di-2-ethylhexyl phthalate, by *Bacillus subtilis* no. 66. Appl Microbiol Biotechnol 66:702–710