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# Degradation of di-(2-ethylhexyl) phthalate by *Bacillus aquimaris* isolated from Ajakanga municipal solid waste leachate

**Olukemi Aromolaran[1]*, Obasola Ezekiel Fagade[2], Hope Miracle Olarenwaju[3] and Adeniyi Adewale Ogunjobi[2]**

[1]Department of Biological Sciences, Bowen University, P.M.B. 284, Iwo, Osun State, Nigeria.
[2]Department of Microbiology, University of Ibadan, Oyo State, Nigeria
[3]Department of Chemistry, Wesley University Ondo, Ondo State, Nigeria
Email: kemiaromolaran@gmail.com

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## ABSTRACT

**Aim:** Di-(2-ethylhexyl) phthalate (DEHP) has been identified as an endocrine-disrupting chemical, commonly found in the environment. The aim of this study was to isolate bacteria from municipal solid waste (MSW) leachates in Nigeria and its ability to degrade DEHP.
**Methodology and results:** The DEHP degrading bacterium was isolated and identified. The degradation process was monitored aerobically at varying temperature and pH and the metabolites were determined using High Performance-Liquid Chromatography and Gas Chromatography-Mass Spectrometry, respectively. Based on the morphology and the 16S rDNA sequence, the bacterial isolate was identified as *Bacillus aquimaris*. *B aquimaris* was able to degrade 99% of 200 mg/L DEHP within 12 days. The optimum pH and temperature for its biodegradation were 8 and 25 °C, respectively and the intermediate metabolites were identified as butyl octyl phthalate and phthalic acid.
**Conclusion, significance and impact of study:** This study showed that *B. aquimaris* could be a useful tool for the biodegradation of DEHP in the environment.

*Keywords:* Di-(2-ethylhexyl) phthalate, *Bacillus aquimaris*, biodegradation, leachate

## INTRODUCTION

Phthalate esters (PEs) are referred to as a group of refractory organic compound commonly use as additives and plasticizer to enhance the flexibility of various plastic products, such as food packs, intravenous bags and tubing, polyvinyl chloride (PCV) and non-polymeric products, such as inks, paints, lubricating oil and cosmetics (Cartwright *et al.*, 2000; Wu *et al.*, 2010; Wang *et al.*, 2013; Wang *et al.*, 2015). The total weight of finished plastic products can comprise up to 20-67% PEs (Staples *et al.*, 1997; Di-Gennaro *et al.*, 2005; Chen *et al.*, 2007). Some PEs have been classified as endocrine disruptors and possible carcinogens in humans (ATSDR, 2000; Wu *et al.*, 2010).

Di-2-(ethylhexyl) phthalate is the most widely used PEs in the production of plastic, but it has been listed as a priority pollutant by the United State Environmental Protection Agency and the European Union (ATSDR, 2000; Fang *et al.*, 2010; Fang *et al.*, 2015). During the manufacturing of plastics, DEHP is added as additive and plasticizer, but it is only physically bound to the polymer chain of the plastics. Therefore, it could easily leach into the environment during usage and disposal and this is responsible for it abundance in the environment (Frenich *et al.*, 2009; Wu *et al.*, 2010). Many of these plastics are disposed into municipal solid waste (MSW) landfill after their usage, which could be responsible for the presence of DEHP in MSW leachates. Leachates from MSW could contaminate other water bodies through percolation and infiltration. Many researchers have reported the presence of DEHP in wastewater treatment plants, sewage sludge, sediments, natural water, soil, aquatic organisms, drinking water, air and food (Juneson *et al.*, 2001; Gu *et al.*, 2004; Chang *et al.*, 2007; Xu *et al.*, 2008; Wu *et al.*, 2010). DEHP is more recalcitrant than short chain PEs because of its long side chain, low solubility in water (0.285 mg/L), high octanol-water portioning coefficient (logKow = 7.5) and is one of the most persistent and stable pollutants among other common PEs (Fang *et al.*, 2015). DEHP could be very hazardous because it can drive cancer risk at very low concentration and could interfere with the reproductive system and development of humans and animals (Roslev *et al.*, 1998; DHHS, 2002). Physical and chemical means of getting rid of DEHP in the environment can be very slow, but biodegradation has been proven to be more effective (Staples *et al.*, 1997; Liang *et al.*, 2008).

***Corresponding author**


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Phthalate ester degrading bacteria have been isolated from various sources, such as activated sludge, wastewater treatment processes, river sediments, soil, and landfill sites (Yuan *et al.*, 2002; Quan *et al.*, 2005; Chang *et al.*, 2007; Xu *et al.*, 2007; Li *et al.*, 2012). This study was conducted in search for microorganism that could degrade this toxic pollutant from the environment. The leachates from Ajakanga and Odo-Sida MSW disposal sites in Nigeria were quantified for PEs and screened for DEHP degrading bacteria. To the best of our knowledge, this is the first report of the isolation and identification of DEHP degrading bacteria from MSW leachate in Nigeria. The optimal pH and temperature for the degradation process were determined and a biochemical pathway was proposed.

## MATERIALS AND METHODS

### Chemicals and reagents

Analytical grade chemicals and reagents were used for this work. Di-(2-ethylhexyl) phthalate (DEHP) (CAS: 117-81-7) with 99.5% purity and dichloromethane (HPLC grade) were purchased from Sigma Aldrich Chemicals (Germany). The acetonitrile used was also HPLC grade.

### Quantification of phthalate esters in municipal solid waste leachates

Leachate samples from Ajakanga and Odo-Sida MSW were screened for the presence of phthalate esters. The leachate samples were collected in pre-washed and sterilized 250 mL amber bottles and kept in the refrigerated temperature prior analysis. Thereafter, the samples were filtered into 250 mL separating funnels and analyzed by the modified method of CPSC-CH-C1001-09.1. One hundred milliliters of leachate samples were extracted with 30 mL tetrahydrofuran and 20 mL of re-distilled hexane. Extracts were concentrated to 1 mL by a stream of nitrogen gas and analyzed using GC-FID (HP 68890) powered with Chem Station Rev. A 09.01(1206) software equipped with 4% OV-101 (Chromosorb W) HP capillary column (30 m × 0.32 mm × 0.25 µm). The carrier gas was nitrogen (1.0 mL/min), inlet temperature 250 °C, oven temperature 240 °C, and the detector temperature was set at 320 °C.

### Isolation of DEHP degrading bacteria from municipal solid waste leachate

The culture medium for the isolation of DEHP degrading bacteria was carried out as described by Chen *et al.* (2007). One hundred milliliters of mineral salt medium (MSM), supplemented with varying concentrations of DEHP (50-400 mg/L) was introduced into a 250 mL Erlenmeyer flask and 1 mL of leachate sample from Ajakanga MSW was added. The same was repeated for Odo-Sida MSW leachate sample. The MSM comprised: 1 g/L $K_2HPO_4$, 1 g/L NaCl, 0.5 g/L $NH_4Cl$, 0.4 g/L $MgSO_4$. Hydrochloric acid or sodium hydroxide was used to adjust the pH of the culture medium to 7.0 ± 0.2. The flasks were incubated in the shaker (New Brunswick Scientific, USA) operating at 150 rpm at room temperature (25.0 ± 0.5 °C) in the dark for 12 days. Bacterial isolates that were able to tolerate 200 mg/L DEHP were isolated from the culture medium by pour plate techniques and plated on Nutrient Agar (NA) plates (Difco Lab., USA). The plates were incubated for 24 h at 37 °C, and colonies showing different morphology were streaked on fresh NA plates to obtain the pure culture. The bacterium that showed the highest growth was identified and selected for further studies.

### Identification of bacteria

The bacterial isolates were identified based on the morphology, biochemical tests and the analysis of their 16S rDNA gene. Pure colonies of the isolates were cultured in Nutrient Broth (NB) bottles (Difco Lab., USA) and incubated for 24 h at 25.0 ± 0.5 °C. The bacterial cells were centrifuged, washed and re-suspended in Tris/EDTA (TE) buffer. The bacterial DNA was extracted using Qiagen kit (QIAamp DNA Mini Kit 250, South Africa), as specified by the manufacturer. Applied Biosystem Thermocycler (ABI 9700 Foster City, California, USA) was used for the amplification of the fragments using bacterial universal primers F27 and R1492. Universal primers used were: forward - 5 'GAG TTT GAT CCT GGC TCA G 3' and reverse - 5 'AAG GAG GTG ATC CAG CC 3'. The PCR programme was as follows: Denaturing step at 94 °C for 5 min followed by 36 cycles of denaturation at 94 °C for 30 sec, annealing for 30 sec at 56 °C and extension at 72 °C for 45 sec, followed by a final extension at 72 °C for 7 min and then held at 10 °C. Amplified DNA was examined by electrophoresis in 1.5% agarose gel and sequenced using an ABI 3130 genetic analyzer. The DNA extraction, amplification, and sequencing were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Sequences identification was carried out using the basic local alignment search tool (BLAST) facility of the National Center for Biotechnology Information (NCBI).

### Biodegradation experiment

The pure bacterial colonies were cultured in NB and incubated for 24 h at 25.0 ± 0.5 °C. The cells were collected by centrifugation, washed in normal saline and adjusted to an optical density of 0.2 ($OD_{600}$) using UV spectrophotometer (Jenway 6305, USA). One milliliter of the bacterial suspension was introduced into 100 mL Erlenmeyer flask containing 200 mg/L DEHP and 20 mL MSM. The optimum pH for the degradation experiment was determined by adjusting the pH of the medium to 5, 6, 7, 8, 9 and 10, using HCl or NaOH. The optimum temperature was determined by incubating at different temperatures (15 °C, 25 °C, 35 °C, and 45 °C) and the effect of initial DEHP concentrations was set at varying concentrations of 50 mg/L, 100 mg/L, 200 mg/L and 400



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mg/L. The flasks were incubated in a mechanical shaker (New Brunswick Scientific, USA), operating at 150 rpm and 25.0 ± 0.5 °C for 12 days in the dark. The extraction process was carried out, as described by Wu *et al.* (2010). Twenty milliliters of dichloromethane was added directly into the individual flask at intervals of day 3, 6, 9 and 12. The mixture was shaken vigorously and then left standing to allow the solvent and aqueous phase to separate. The solvent phase was extracted into receiving 100 mL Erlenmeyer flasks and the process was repeated twice. The extract was dried and reconstituted with 1 mL of the solvent before analysis. The microbial growth in the culture flask was determined by measuring the optical density at 600 nm in the UV spectrophotometer.

**Analytical methods**

The DEHP in the culture medium during the biodegradation process was determined by using HPLC (Agilent 1100 Series), equipped with a high-pressure pump (LC-20AD), a degasser (DGU-20A5), a photodiode array detector (DAD, Agilent) and an auto-sampler. An Agilent Eclipse C18 column (4.6 mm i.d x100 mm, 3.5 µm particle size) was used with a mobile phase comprising of acetonitrile/water (98/2, v/v) at a flow rate of 1.0 mL/min. The injector volume was 5 µL and the wavelength was set at 224 nm. The chromatographic data were acquired and the process by a computer equipped with an HP Chem Station and peak area was used as the analytical signal for quantification. The metabolic products of biodegradation were analyzed with 19091S-433HP-5MS gas chromatography coupled to a mass spectrometer (GC-MS) (Agilent Technologies) operating in electron impact and selective ion monitoring modes (SIM) and a DB-5MS capillary column (30 m x 250 µm i.d., 0.25 µm film thickness). Injector and detector temperature were set at 280 °C and 230 °C, respectively. The column temperature program initiated at 80 °C for 1 min, increased to 180 °C at a rate of 10 °C/min, held at 1 min and finally ramped at 2 °C/min to 300 °C and held for 10 min. The injection volume was 1.0 µL of the extract and was injected onto GC-MS in splitless mode with an inlet temperature of 300 °C. Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The mass spectrometer was operated at an electron ionization energy of 70 eV and the total run time was 82 min. The suspected metabolites were identified by comparing the mass spectrum with the published mass spectra from National Institute of Standard and Technology (NIST) Database.

**RESULTS**

**Concentrations of phthalate esters in MSW leachate samples**

The phthalate esters detected in the leachate samples were mono-methyl phthalate (MMP), di-methyl phthalate (DMP), di-allyl phthalate (DAP), di-ethyl phthalate (DEP), di-isobutyl phthalate (DIBP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP) and di-(2-ethylhexyl) phthalate (DEHP) (Figure 1). The concentrations of DEHP (166 µg/L in Ajakanga, and 103 µg/L in Odo-Sida) in the leachate samples were higher than the other six phthalates. DAP and DIBP were the least phthalate esters found in the leachate samples. The concentrations of DAP and DIBP were 7 µg/L, 1 µg/L and 5 µg/L, 1 µg/L, for Ajakanga and Odo-Sida, respectively.

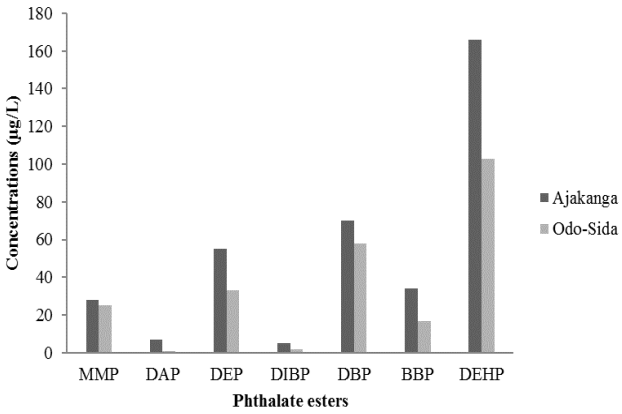


**Figure 1:** Concentrations (µg/L) of phthalate esters in Ajakanga and Odo-Sida MSW leachate samples. MMP, mono methyl phthalate; DAP, di-allyl phthalate; DEP, di-ethyl phthalate; DIBP, diisobutyl phthalate; DBP, di-n-butyl phthalate; BBP, butyl benzyl phthalate; DEHP, di-(2-ethylhexyl) phthalate.

**DEHP degrading bacterial strains**

Two bacteria isolates were able to tolerate 200 mg/L DEHP in their growth medium as a carbon source and these were strains AJA40 and OD32 isolated from Ajakanga and Odo-Sida MSW leachates, respectively. AJA40 is a Gram-positive rod and formed round colonies on Nutrient agar plate. It is positive for catalase and starch hydrolysis test but exhibits negative reaction for methyl red and Voges-Proskauer test. It could neither utilize lactose nor glucose. OD32 is a Gram-negative rod, positive for catalase, methyl red reaction and citrate utilization, but showed negative reactions for starch hydrolysis and Voges-Proskauer test. By comparing the bacteria 16S rDNA with the bacteria nucleotide sequences in GenBank using BLAST search, strain AJA40 showed 93.0% similarity to *B. aquimaris* and OD32 showed 96.0% similarity to *Pseudomonas aeruginosa*. The accession number for *B. aquimaris* and *P. aeruginosa* were KP 866219 and KP 282446, respectively. The result of the growth profile of *B. aquimaris*, *P. aeruginosa* and the combination of the two strains (i.e. 1 mL each of *B. aquimaris* and *P. aeruginosa* cells) is shown in Figure 2. *B. aquimaris* showed a profuse growth in the MSM medium supplemented with DEHP (200 mg/L) and the optical density (OD) reading at day 12 was 0.171 whereas, *P. aeruginosa* did not grow very well in the medium. Optical density reading of 0.066 was observed for *P. aeruginosa* at day 8 and declined to



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0.046 at day 12. The mixed culture (combination), comprising 1 mL each of washed cells of *B. aquimaris* and *P. aeruginosa* did not show significant growth, indicating that, there could be an antagonistic reaction between the two isolates and could not be combined for the biodegradation studies. Therefore, only *B. aquimaris* was used for the biodegradation experiments. The result of the growth profile of *B. aquimaris* in 200 mg/L DEHP showed its ability to effectively degrade DEHP, thus utilizing it for cell growth and maintenance (Figure 3). More than 80% of DEHP was consumed in less than eight days, while the un-inoculated flask did not show any bacteria growth.

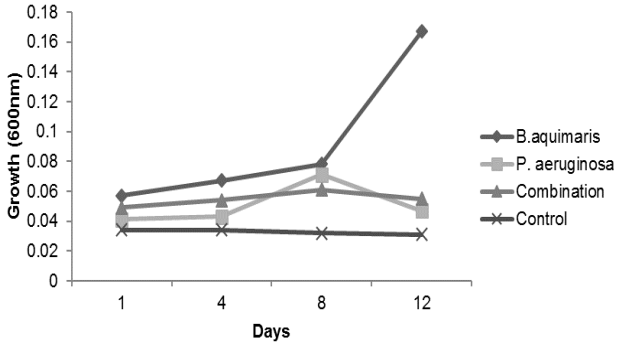


**Figure 2:** Growth profile of *B. aquimaris* and *P. aeruginosa* in MSM supplemented with 200 mg/L DEHP at pH 7 and 25 °C.

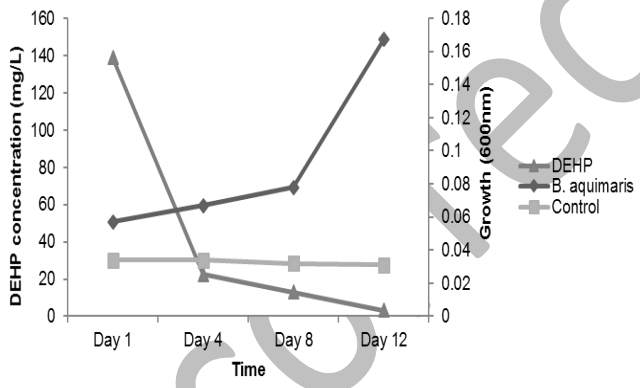


**Figure 3:** Biodegradation of DEHP by *B. aquimaris* at pH 7 and 25 °C.

**Effect of pH and temperature on DEHP degradation by *B. aquimaris***

The effects of pH and temperature on degradation of DEHP by *B. aquimaris* are presented in Figures 4 and 5, respectively. The degradation rate increased rapidly from 45% to 98.25%, when the pH of the culture medium was increased from 5 to 6. The highest degradation value (99.82%) was achieved at pH 8. This study showed that the optimum pH for DEHP degradation by *B. aquimaris* was 8. The growth of the bacterium was influenced by temperature. *B. aquimaris* showed 63.65% DEHP degradation at 15 °C. The degradation rate increased from 63.65% to 99.96%, when the temperature was increased from 15 °C to 25 °C. The highest DEHP degradation rate (99.96%) was observed at 25 °C. The rate of degradation decreases when the temperature was increased from 25 °C to 35 °C and significantly decreased (48.08%), when the temperature was increased to 45 °C.

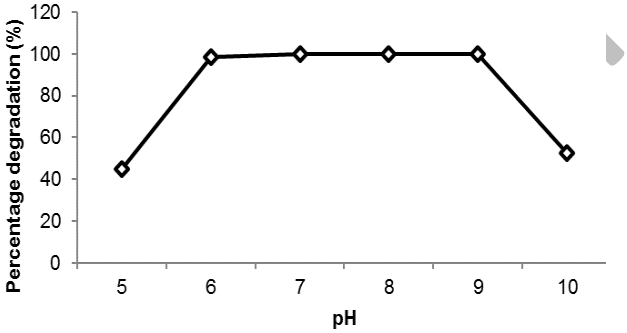


**Figure 4:** Effect of pH on DEHP degradation by *B. aquimaris* at 25 °C.

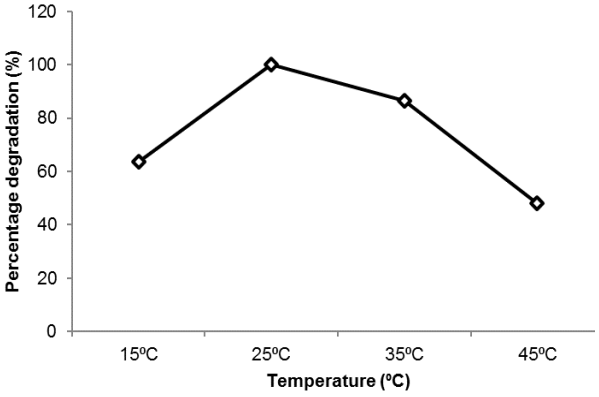


**Figure 5:** Effect of temperature on DEHP degradation by *B. aquimaris* at pH 7.

**DEHP biodegradation kinetics**

The effect of initial DEHP concentration on the biodegradation process is presented in Table 1. The result shows that the degradation rate is not directly related to the concentration of DEHP.

**Table 1:** DEHP degradation kinetics equation in different initial concentrations.

| Initial concentrations (mg/L) | Kinetic equation | Half-life (day) |
|---|---|---|
| 50 | ln C = -0.6003t + 4.3140 | 1.15 |
| 100 | ln C = -0.5624t + 4.5129 | 1.23 |
| 200 | ln C = -0.6095t + 3.9601 | 1.14 |
| 400 | ln C = -0.5422t + 4.801 | 1.28 |



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The degradation of DEHP by *B. aquimaris* was fitted into the Monad first-order kinetic model.

First-order kinetic equation:

$$\ln C = -kt + A$$

Where ln C is the initial concentration of DEHP at a particular time, k is the kinetic constant, t is the time and A is a constant. The half-life of the DEHP biodegradation by *B. aquimaris* is expressed as:

$$T_{1/2} = \ln C/K$$

The rate constant K obtained from the result ranged from 0.5422 to 0.6095 and the biodegradation half-life was observed between 1.14 and 1.28 day.

In order to determine the effect of different initial concentrations of DEHP on the biodegradation efficiency, *B. aquimaris* was inoculated and cultured at a range of DEHP concentration (50-400 mg/L). At the end of 24 h, 100 mg/L DEHP has reduced to 38.01 mg/L. It was also observed that 400 mg/L DEHP has been reduced to 31.86 mg/L. At day 12, the remaining DEHP of all the different concentrations were less than 1 mg/L.

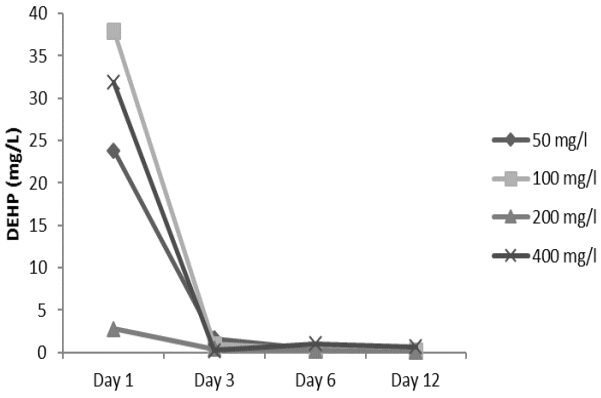


**Figure 6:** Effect of different initial concentrations of DEHP on biodegradation by *B. aquimaris*.

**Metabolic products from degradation of DEHP by *B. aquimaris***

The result of the GC-MS indicated the presence of butyl octyl phthalate (BOP) and phthalic acid (PA) as intermediate products during the biodegradation experiment. PA was detected at retention time 6.63 min. The second peak at 14.58 was identical to BOP. Based on the result, a tentative metabolic pathway for biodegradation of DEHP by *B. aquimaris* was proposed (Figure 7).

**DISCUSSION**

Seven PAEs were identified in the MSW leachates, which ranged from 1 µg/L to 166 µg/L and the most abundant was DEHP. Bauer and Herrmann (1997) reported that DEHP represent 91% of the total diesters leaching from household waste. The DEHP concentrations in this study were comparable with those observed in other municipal wastewater from other countries (Marttinen *et al.*, 2003; Lin *et al.*, 2004). Marttinen *et al.* (2003) reported the concentration of 98-122 µg/L DEHP in sewage treatment plant from Finland. In this study, DEHP is of primary concern because it has been grouped as class B carcinogen and it is difficult to remove from the environment (Abdel-Daiem *et al.*, 2012; Ferreira and Morita, 2012; Liu *et al.*, 2013). At very low concentration, it has an adverse effect on human and the environment and can drive cancer risk at the concentration of $1.4\times10^{-2}$ mg/kg/d (DHHS, 2002; Shailaja *et al.*, 2008).

Di-(2-ethylhexyl) phthalate degrading bacteria were isolated from Ajakanga and Odo-Sida MSW leachates in Nigeria and were identified as *B. aquimaris* and *P. aeruginosa*, respectively. Some researchers have also reported the presence of PEs degrading bacteria in municipal wastewater (Fang *et al.*, 2010; Li *et al.*, 2012). *Bacillus* species have been responsible for the breakdown of some recalcitrant pollutants in the environment. *Bacillus* sp. S4 isolated from sludge and *Bacillus subtilis* No. 66 isolated from soil, had shown the capability to degrade DEHP (Quan *et al.*, 2005; Chang *et al.*, 2007). This is in agreement with the report of this study which showed that *B. aquimaris* was capable of degrading more than 99% 200 mg/L DEHP within 12 days. The result showed that the pH of the DEHP supplemented medium and the incubation temperature had significant impacts on the bacterial growth. The organism grew very well in the pH range of 6 to 8 and temperature of 25 °C, but the degradative enzyme was deactivated at pH < 6 and pH > 8. Based on this result, the optimum pH and temperature for biodegradation of DEHP by *B. aquimaris* were 8 and 25 °C, respectively. Phthalate esters degradation by bacteria is usually affected by low pH (Xu *et al.*, 2007; Fang *et al.*, 2010).

First order kinetic model is often applied in describing the biodegradation kinetics of many xenobiotic compounds (Fang *et al.*, 2010). In this study, DEHP biodegradation by *B. aquimaris* was fitted into the first order kinetic model and the constant ranged between 0.5422 and 0.6095. Shailaja *et al.* (2008) in a similar study on biodegradation of DEHP in a bio-slurry reported kinetic equation constant ranging from 0.003 to 0.0935 of the first-order kinetic model and half-life 5.34 days. Butyl octyl phthalate and phthalic acid were the major metabolic products found during the degradation of DEHP by *B. aquimaris*. The presence of BOP in the culture medium of PEs with longer alkyl chain was consistent with the report of Wu *et al.* (2010). Phthalic acid is an intermediate product in the degradation of PEs, followed by the release of carbon dioxide and methane gas (Liang *et al.*, 2008). Microbial degradation has been very helpful in the removal of PAEs from the environment. The degradation of DEHP by *B. aquimaris* in this study did not follow the common de-esterification pathway as described by Staples *et al.* (1997), when diester is transformed to PA



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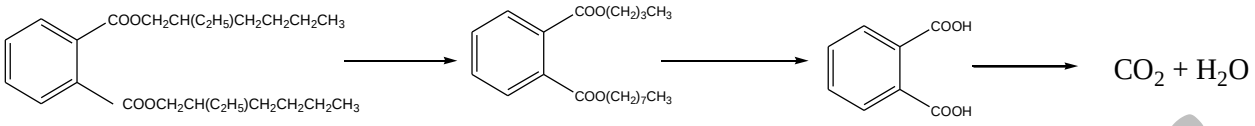


**Figure 7:** Proposed DEHP degradation pathway by *B. aquimaris*.

through monoester. Based on this result, a tentative biodegradation pathway for DEHP by *B. aquimaris* was proposed, which involve beta-oxidation of the parent compound to yield BOP, then PA, which can be further converted to $CO_2$ and $H_2O$.

## CONCLUSION

Di-(2-ethylhexyl) phthalate could be rapidly degraded by *B. aquimaris* isolated from Ajakanga MSW leachate. The optimum pH and temperature for the biodegradation was 8 and 25 °C, respectively. However, its degradation did not appear to be influenced by different initial concentrations. The GC-MS analysis showed that *B. aquimaris* degraded DEHP to less toxic intermediate products – butyl octyl phthalate (BOP) and phthalic acid (PA). The results of this study suggest that DEHP can be degraded by microorganisms in MSW leachates. Therefore, bacteria in MSW leachates can be harnessed for bioremediation of contaminated sites.

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## REFERENCES

**Abdel-Daiem, M. M., Rivera-Utrilla, J., Ocampo, P. R. and Mendez-Diaz, J. D. (2012).** Environmental impact of phthalic acid esters and their removal from waste and sediments by different technologies. *Journal of Environmental Management* **109, 164-178.**

**Agency for Toxic substances and Disease Registry (ATSDR) (2000).** Toxicological profile for di-(2-ethylhexyl) phthalate (DEHP). Agency for Toxic substances and Disease Registry. Atlanta, Georgia. Available at: http://www.atsdr.cdc.gov

**Bauer, M. J. and Herrmann, R. (1997).** Estimation of the environmental contamination by phthalic acid esters leaching from the household wastes. *Science of the Total Environment* **208, 49-57.**

**Cartwright, C. D., Owen, S. A., Thompson, I. P. and Burns, R. G. (2000).** Biodegradation of diethyl phthalate in soil by a novel pathway. *FEMS Microbiology Letters* **186, 27-34.**

**Chang, B. V., Wang, T. H. and Yuan, S. Y. (2007).** Biodegradation of four phthalate esters in sludge. *Chemosphere* **69, 1116-1123.**

**Chen, J. A., Li, X., Li, J., Cao, J., Qiu, Z., Zhao, Q., Xu, C. and Shu, W. (2007).** Degradation of environmental endocrine disruptor di-2-ethylhexyl phthalate by a newly discovered bacterium, *Microbacterium* sp. strain CQ0110Y. *Applied Microbiology and Biotechnology* **74, 676-682.**

**Department of Health and Human Services (DHHS) (2002).** Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR), Division of Toxicology and Rural Medicine, Tox FAQs TM; DEHP US Department of Health and Human Services 2002. Available at: http://www.atsdr.cdc.gov

**Di-Gennaro, P., Collina, E., Franzetti, A., Lasagni, M., Luridiana, A. P. and Bestetti, G. (2005).** Bioremediation of di-ethylhexyl phthalate contaminated soil: A feasibility study in slurry and solid phase reactors. *Environmental Science Technology* **39, 325-330.**

**Fang, C. R., Long, Y. Y. and Shen, D. S. (2015).** Degradation of dibutyl phthalate in refuse from different phases of landfill by its dominant bacteria strain *Enterobacter* sp. T1. *Chemistry and Ecology* **31(3), 265-272.**

**Fang, C. R., Yao, J., Zhen, Y. G., Jiang, C. J., Hu, L. F., Wu, Y. Y. and Shen, D. S. (2010).** Dibutyl phthalate degradation by *Enterobacter* sp. T5 isolated from municipal solid waste in landfill bioreactor. *International Biodeterioration and Biodegradation* **63,732-738.**

**Ferreira, I. D. and Morita, D. M. (2012).** *Ex-situ* bioremediation of Brazilian soil contaminated with plasticisers process wastes. *Brazilian Journal of Chemical Engineering* **29(1), 77-86.**

**Frenich, A. G., Bonilla, M. N. B., Martinez, J. C. L., Vidal, J. L. M. and Romero-Gonzalez, R. (2009).** Determination of di-(2-ethylhexyl) phthalate in environmental samples by liquid chromatography coupled with mass spectrometry. *Journal of Separation Science* **32, 1383-1389.**

**Gu, J. D., Li, J. and Wang, Y. (2004).** Degradation of endocrine disrupting dimethyl phthalate ester isomers by aerobic microorganism isolated from mangrove sediment. *In: European Symposium on Environmental Biotechnology, ESEB*. Verstraete, W. (e.d.). A. A Balkema Publishers, London. **pp. 557-561.**



 ISSN (print): 1823-8262, ISSN (online): 2231-7538

**Juneson, C., Ward, O. P. and Singh, A. (2001).** Biodegradation of bis (2-ethylhexyl) phthalate in a soil slurry-sequencing batch reactor. *Process Biochemistry* **37, 305-13.**

**Li, C., Tian, X. L., Chen, Z. S., Yu, D., Deng, J. Y. and Xu, H. (2012).** Biodegradation of an endocrine disrupting chemical di-n-butyl phthalate by *Serratia marcescens* C9 isolated from activated sludge. *African Journal of Microbiology Research* **6(11), 2686-2693.**

**Liang, D. W., Zhang, T., Fang, H. H. P. and He J. Z. (2008).** Phthalates biodegradation in the environment. *Applied Microbiology and Biotechnology* **80, 183-198.**

**Lin, X., Chen, A., Wang, X., Wu, S., Chen, S., Wang, G. and Ren, R. (2004).** Analysis of phthalate esters of environmental hormones in water samples of wastewater treatment plant. *Environmental Science and Technology* **27, 79-81.**

**Liu, Y., Chenz, Z. and Shen, J. (2013).** Occurrence and removal characteristics of phthalate esters from typical water sources in Northern China. *Journal of Analytical Methods in Chemistry* **2013, 1-8.**

**Marttinen, S. K., Kettunen, R. H., Sormunen, K. M. and Rintala, J. A. (2003).** Removal of bis (2-ethylhexyl) phthalate at a sewage treatment plant. *Water Resources* **37, 1385-1393.**

**Quan, S., Liu, Q., Tian, W., Kikuchi, J. and Fan, S. (2005).** Biodegradation of an endocrine disrupting chemical, di-(ethylhexyl) phthalate by *Bacillus subtilis* No 66. *Applied Microbiology and Biotechnology* **66, 702-710.**

**Roslev, P., Madsen, P. L., Thyme, J. B. and Henrikson, K. (1998).** Degradation of phthalate and DEHP by indigenous and inoculated microorganisms in sludge-amended soil. *Applied Environmental Microbiology* **64 (12), 4711-4719.**

**Shailaja, S., Mohan, S. V., Krishna, M. R. and Sarma, P.N. (2008).** Degradation of di-(2-ethylhexyl) phthalate (DEHP) in bioslurry phase reactor and identification of metabolites by HPLC and MS. *International Biodeterioration and Biodegradation* **62, 143-152.**

**Staples, C. A., Peterson, D. R., Parketron, T. F. and Adams, W. I. (1997).** The environmental fate of phthalate esters: A literature review. *Chemosphere* **4, 667-749.**

**Wang, L. P., Li, X. Y. and Li, X. M. (2013).** Dioctyl phthalate esters wastewater treatment by Fenton Reagent/Fly Ash Combine Process. *Advance Material Research* **777, 122-126.**

**Wang, M. Y., Zhou, N. Q. and Hu, J. (2015).** Effect of plasticizer (DOP) on cell structure and mechanical properties of extrusion-formed PVC sheet. *Material Science Forum* **815, 601-606.**

**Wu, X., Liang, R., Dai, Q., Jin, D., Wang, Y. and Chao, W. (2010).** Complete degradation of di-n-octyl phthalate by biochemical cooperation between *Gordonia* sp. strain JDC-2 and *Arthrobacter* sp. strain JDC-32 isolated from activated sludge. *Journal of Hazardous Material* **176, 262-268.**

**Xu, G., Li, F. S. and Wang, Q. H. (2008).** Occurrence and degradation characteristics of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in typical agriculture soils of China. *Science of the Total Environment* **393 (2-3), 333-340.**

**Xu, X., Li, H. and Gu, J. (2007).** Metabolism and biochemical pathway of n-butyl benzyl phthalate by *Pseudomonas fluorescens* B-1 isolated from a mangrove sediment. *Ecotoxicology and Environmental Safety* **68, 379-385.**

**Yuan, S., Liu, C., Liao, C. and Chang, B. (2002).** Occurrence and microbial degradation of phthalate esters in Taiwan river sediment. *Chemosphere* **49, 1295-1299.**



 ISSN (print): 1823-8262, ISSN (online): 2231-7538