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# ADSORPTION OF PHTHALATES BY ACTIVATED SLUDGE AND ITS BIOPOLYMERS

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**ABSTRACT**

This study shows diethyl phthalate (DEP) and dibutyl phthalate (DBP) were substantially adsorbed by activated sludge and its extracellular polymeric substance (EPS). The adsorption characteristics followed Freundlich and Langmuir isotherms. According to the Langmuir isotherm, each gram of activated sludge at maximum adsorbed 0.73 mg of DEP and 17.6 mg of DBP, and each gram of centrifugation-extracted EPS adsorbed 14.3 mg of DEP and 10.6 mg of DBP. The adsorption increased with the hydrophobicity of phthalates. This suggests most phthalates, which are of higher hydrophobicity than DEP and DBP, are likely to be removed from wastewater through adsorption by the activated sludge in the biological treatment process.

Keywords: Activated sludge, adsorption, biopolymers, endocrine disruptors, phthalates.

## INTRODUCTION

Phthalates are used not only as additives for paints, adhesives, cardboards and lubricants, but also as plasticizers for polyvinylacetates, polyurethanes and polyvinylchloride. Because they are not chemically attached to the host media, phthalates may be released into the environment over time. Phthalate residues have been detected in landfill leachate [1], surface water, sewage sludge [2], and sediment [3]. Since they are not only toxic to aquatic species, but also known to be endocrine disruptors [4], the presence of phthalates in the water environment has increasingly caused serious concerns.

It has been reported that phthalates in sewage may be removed by the conventional activated sludge process for wastewater treatment [1, 5]. However, little is known about whether phthalates are biologically degraded in the process or simply adsorbed by the biomass [6] or by the extracellular polymeric substances (EPS) of the biomass. This study was thus conducted to investigate the adsorption characteristics of phthalates by activated sludge and by its EPS.

## MATERIALS AND METHODS

### Model Phthalates

Diethyl phthalate (DEP) and dibutyl phthalate (DBP) were selected for this study as two model phthalates. Among phthalates, these two are commonly used by industry, and are relatively soluble in water (1100 mg $l^{-1}$ for DEP and 11 mg $l^{-1}$ for DBP) making them potentially more harmful to the water environment than other phthalates [7].

### Activated Sludge and Extraction of EPS

The activated sludge was sampled from a local municipal wastewater treatment plant. The sludge contained 4.265 g $l^{-1}$ of suspended solids (SS) and 3.505 g $l^{-1}$ of volatile suspended solids (VSS), as measured according to the *Standard Methods* [8]. EPS may be extracted from the activated sludge by centrifugation, heating, and addition of chemicals, such as EDTA, cation exchange resin, formaldehyde and NaOH. The amount of EPS extracted from activated sludge and the characteristics of EPS strongly depend upon the extraction process [9]. The amounts of EPS extracted from an activated sludge sample varied from 25.7 mg $g^{-1}$ by centrifugation alone to 164.9 mg $g^{-1}$ by formaldehyde-NaOH extraction [10].

Since heating and addition of chemicals may affect the chemical nature of EPS and thus their adsorption characteristics, EPS in this study was extracted from the activated sludge by centrifugation alone. After centrifugation at $2x10^4$ G for 20 min, the EPS-rich supernatant was filtered through a 0.2 $\mu$m membrane to remove microbial cells, and then filtered again through a dialysis membrane (3500 Da cut-off; Pierce, USA) to remove low molecular-weight metabolites. The filtrate was finally lyophilized at –80 °C for 12 h.

The carbohydrate content in EPS was measured by the anthrone method using glucose as the standard. Protein and humic content were measured by the modified Lowry method using bovine serum albumin and humic acid as the respective standards. Uronic acid was measured by the *m*-hydroxydiphenyl sulfuric acid method using glucuronic acid

as the standard. DNA content was measured by the diphenylamine colorimetric method using *E. coli* DNA as the standard. Details of all analytical methods were reported previously [10].

Adsorption of Activated Sludge

In order to avoid adsorption of phthalates by plastic lab-ware, only glassware were used in this study. In order to ensure that phthalates were removed by adsorption instead of biodegradation, 1000 mg $l^{-1}$ of sodium azide was added to the activated sludge to suppress its microbial activity prior to the experiments. All experiments were conducted at ambient temperature in 200 ml glass bottles containing 50 ml of activated sludge. The bottles were dosed with DEP and DBP individually at six concentration levels, i.e. 0, 0.5, 1, 3, 5 or 10 mg $l^{-1}$. The bottle without phthalate addition served as control. After 24 hrs of mixing in a shaking water bath, each mixed liquor was filtered through a 0.2 $\mu$m membrane to remove the sludge which had been presumably saturated with adsorbed phthalates. Content of residual phthalate in each filtrate was compared with the content of phthalate in the initial solution. The difference was the quantity of phthalates adsorbed by the activated sludge.

Adsorption of EPS

Experiments on EPS adsorption were similar to those conducted for activated sludge, except without the addition of sodium azide. A 50 ml aqueous solution containing 20 mg $l^{-1}$ of EPS was added to each bottle. The initial phthalate concentrations varied from nil to 50, 100, 200, 300 and 500 $\mu$g $l^{-1}$. After mixing for 24 hrs, the mixed liquor was filtered through a Millipore YM3 membrane with a molecular weight cutoff level of 3000 Da. The reduction of phthalate in the filtrate represented the amount adsorbed by the EPS.

Extraction and Analysis of Phthalates

In order to reduce background contamination of phthalates, hexane was distilled twice and ultra-pure water was twice treated with activated carbon before use. After extracting each filtrate (40 ml) twice with 5 ml of hexane, contents of DEP and DBP in the combined hexane solution were measured by a gas chromatograph (Agilent 6890) fitted with a 5973 mass spectrometer detector with an EI of 70 ev as the ionization source and a quadrupole mass filter. The chromatographic column was a 30 m x 0.25 mm Supelco SPB-5 capillary with a 0.25-$\mu$m film thickness. The column temperature program was kept at 80 °C for 1 min, heating at 20 °C $min^{-1}$ up to 280 °C, and holding at 280 °C for 3 min. The inlet and detection temperatures were 220 °C and 300 °C, respectively. The flow rate of the helium carrier gas was 1.2 ml $min^{-1}$. The injection volume was 1 $\mu$l, splitless. The selected ion mass modes were 149, 167, 177, 188, 212, and 223. Phenanthrene-$d_{10}$ was used as the internal standard with a detection limit of 0.05 $\mu$g $l^{-1}$. The blank levels in purified water were 0.39±0.19 $\mu$g $l^{-1}$ for DEP and 0.56±0.28 $\mu$g $l^{-1}$ for DBP. Figure 1 illustrates a typical chromatogram of phthalate analysis, in which peaks 1, 2 and 3 respectively represent DEP, phenanthrene-$d_{10}$ and DBP.

## RESULTS AND DISCUSSION

Characteristics of Activated Sludge and EPS

Each gram of activated sludge (as volatile suspended solids - VSS) produced 23 mg EPS in the supernatant after centrifugation at $2x10^4$ G for 20 min. After filtering the

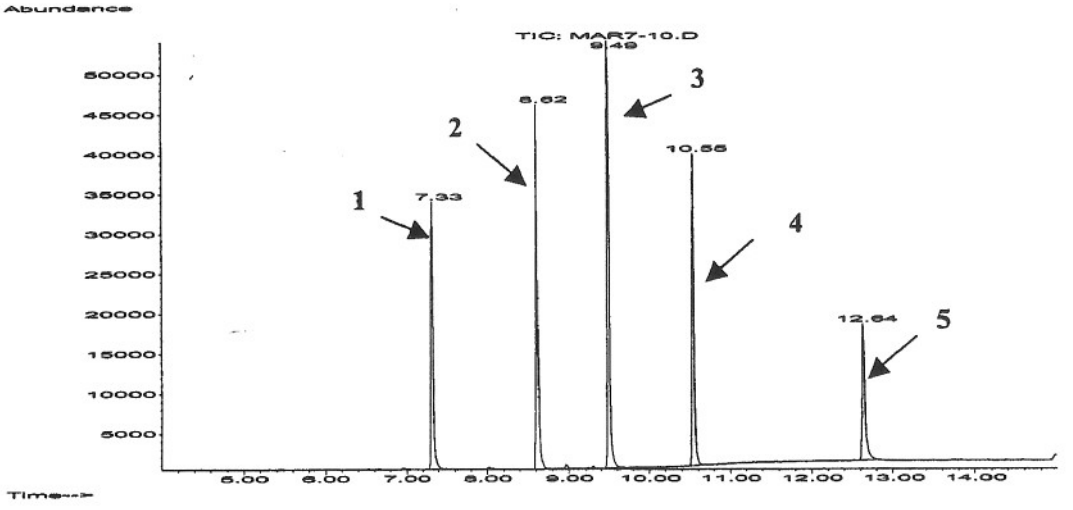


Figure 1. A typical chromatogram of phthalate analysis.

supernatant through a 0.2 μm membrane, the filtrate contained 120 mg $l^{-1}$ of EPS, including carbohydrate (30.4 mg $l^{-1}$), protein (40.6 mg $l^{-1}$), humic substance (28.9 mg $l^{-1}$), uronic acid (5.6 mg $l^{-1}$), DNA (0.019 mg $l^{-1}$), and 14.3 mg $l^{-1}$ of unidentified substances, most of which were likely to be lipids or phenols [11]. The corresponding contents of carbohydrate, protein, humic substance, uronic acid, DNA and unidentified substances in EPS were 25.3%, 33.8%, 24.1%, 4.7%, 0.2% and 11.9%, respectively. The EPS was relatively hydrophobic judging from its rich contents of protein and humic substance.

Phthalates Removal

Figure 2a illustrates the adsorption efficiencies of DEP and DBP with initial concentrations ranging from 0.5 mg $l^{-1}$ to 10.0 mg $l^{-1}$ by activated sludge which contained 3505 mg $l^{-1}$ of VSS. The adsorption efficiency of DEP decreased from 46.2% to 24.5% with the increase of initial concentration, whereas removal efficiency of DBP was consistently over 99% for all batches. Similarly, Figure 2b illustrates the adsorption efficiencies of DEP and DBP with initial concentrations ranging from 50 μg $l^{-1}$ to 500 μg $l^{-1}$ by the centrifugation-extracted EPS at the concentration of 20 mg $l^{-1}$. The adsorption efficiencies of both DEP and DBP decreased with the increase of initial concentration, from 38.8% to 31.5% for DEP and from 80.0% to 58.8% for DBP. The removal efficiencies of DBP were substantially higher than those of DEP by both activated sludge and EPS. Since the EPS in activated sludge was slightly hydrophobic, the higher adsorption of DBP, which is more hydrophobic than DEP, was as expected.

Adsorption

Adsorption isotherms are equations correlating the specific adsorption capacity of the adsorbent with the equilibrium concentration of the adsorbed species. The two most common adsorption isotherms are Freundlich and Langmuir isotherms, as expressed in the following:

Freundlich: $Q = k C_e^n$ (i)

Langmuir: $Q = Q_m b C_e (1 + b C_e)^{-1}$ (ii)

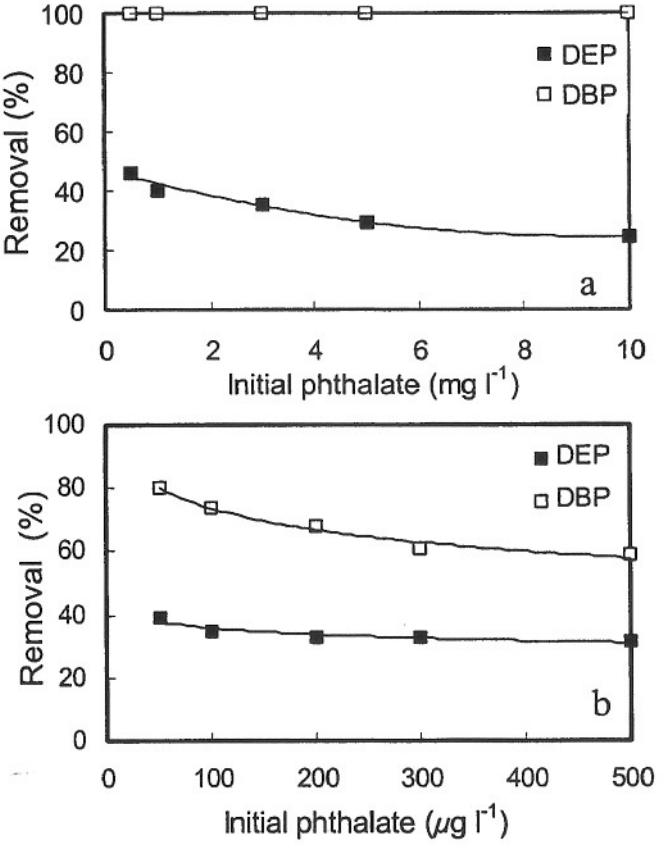


Figure 2. Adsorptions of DEP and DBP by (a) activated sludge and (b) EPS.

where Q (in $\mu g\ mg^{-1}$ or $mg\ g^{-1}$) is the amount of individual phthalate adsorbed by a unit weight of biomass (as measured by VSS) or EPS, $C_e$ (in $\mu g\ l^{-1}$) is phthalate concentration in the mixed liquor at equilibrium, k and n are the Freundlich parameters, whereas $Q_m$ and b are the Langmuir parameters. The two isotherms may be re-arranged as follows:

Freundlich: $$\text{Ln } Q = \text{Ln } k + n \text{ Ln } C_e \quad \text{(iii)}$$

Langmuir: $$Q^{-1} = (Q_m\, b\, C_e)^{-1} + Q_m^{-1} \quad \text{(iv)}$$

Based on Equations (iii) and (iv), the Freundlich parameters may be obtained from the plot of Ln Q vs Ln $C_e$, whereas the Langmuir parameters from the plot of $Q^{-1}$ vs $C_e^{-1}$. The Freundlich isotherm is an empirical equation, and thus the two parameters, k and n, have no physical meaning. On the other hand, the Langmuir isotherm can be derived from statistical thermodynamics with the assumption of mono layer adsorption. Accordingly, $Q_m$ in the Langmuir isotherm represents the maximum specific adsorption capacity of the adsorbent.

The results of this study show that adsorption of DEP and DBP by either activated sludge or EPS may be characterized by both the Freundlich and Langmuir isotherms ($R^2 > 0.99$). Figure 3a illustrates the Freundlich isotherms of DEP and DBP adsorption for activated sludge, whereas Figure 3b illustrates the corresponding plots for EPS. Similarly, Figures 4a and 4b illustrate the Langmuir isotherms of DEP and DBP adsorption for activated sludge and EPS, respectively. The best-fit values of k and n for the Freundlich isotherm and those of $Q_m$ and b for the Langmuir isotherm are summarized in Table 1.

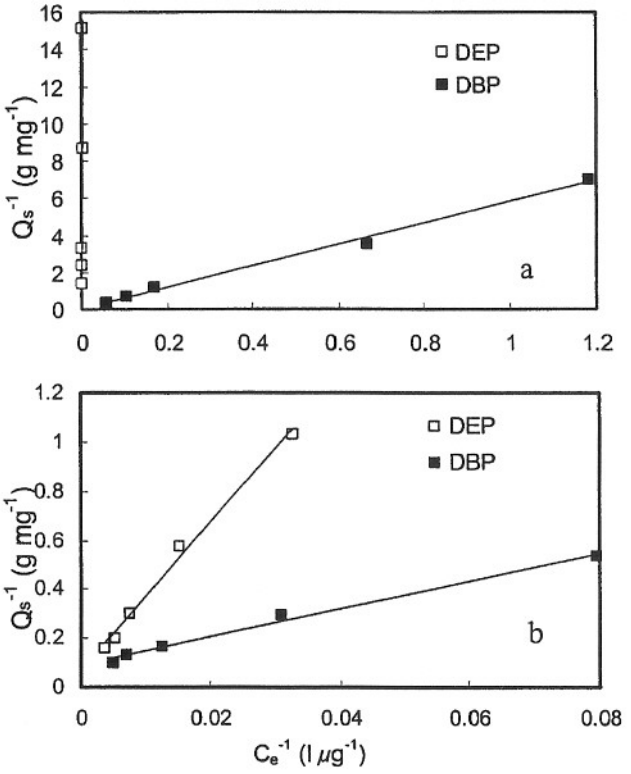


Figure 4. Langmuir isotherms of DEP and DBP adsorption by (a) activated sludge and (b) EPS.

Table 1. Adsorption parameters of Freundlich and Langmuir isotherms.

| Phthalate / Adsorbent | Freundlich isotherm | | Langmuir isotherm | |
|---|---|---|---|---|
| | k | n | $Q_m$ ($mg\ g^{-1}$) | b |
| DEP / activated sludge | 1.203 | 0.7176 | 0.73 | 0.000358 |
| DBP / activated sludge | 174.5 | 0.9394 | 17.6 | 0.00987 |
| DEP / EPS | 48.87 | 0.8646 | 14.3 | 0.00234 |
| DBP / EPS | 440.9 | 0.5805 | 10.6 | 0.01661 |

The $Q_m$ values in Table 1 show that the maximum adsorption capacities of activated sludge were 17.6 mg-DBP g-$VSS^{-1}$ and 0.73 mg-DEP g-$VSS^{-1}$. The adsorption of phthalates by activated sludge appeared to increase with hydrophobicity. DBP having longer alkyl branches is more hydrophobic than DEP. The hydrophobicity of DBP is also reflected in its low water solubility (11 mg $l^{-1}$) and high $K_{ow}$ value ($10^{4.45}$), as compared to corresponding 1100 mg $l^{-1}$ and $10^{2.38}$ for DEP [7]. Since DEP and DBP are less hydrophobic than most other phthalates, it is highly likely that phthalates, which are known endocrine disruptors, in wastewater may be effectively removed through adsorption by the activated sludge process.

On the other hand, Table 1 also shows that the extracted EPS had similar potential adsorption capacities for DEP (14.3 mg g-$EPS^{-1}$) and DBP (10.6 mg g-$EPS^{-1}$). The slightly higher capacity for DEP suggests that the centrifugation-extracted EPS, which was of low molecular weight, was more hydrophilic than those high molecular-weight biopolymers in activated sludge that could not be extracted by simple centrifugation.

## CONCLUSION

Activated sludge and its EPS are strong adsorbents for DEP and DBP. The adsorption may be characterized by either the Freundlich isotherm or the Langmuir isotherm. According to the latter, each gram of activated sludge could at maximum adsorb 0.73 mg of DEP and 17.6 mg of DBP. The adsorption increased with the hydrophobicity of phthalates. Since DEP and DBP are less hydrophobic than most other phthalates, it is highly likely that phthalates, which are known endocrine disruptors, in wastewater may be effectively removed through adsorption by the activated sludge process.

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## REFERENCES

1. Jonsson, S., Ejlertsson, J., Ledin, A., Mersiowsky, I. and Svensson, B.H., Mono- and di-esters from *o*-phthalic acid in leachates from different European landfills, *Water Res.* **37**, 609-617 (2003).
2. Fauser, P., Vikelsøe, J., Sørensen, P.B. and Carlsen, L., Phthalates, nonylphenols and LAS in an alternately operated wastewater treatment plant—fate modeling based on measured concentrations in wastewater and sludge, *Water Res.* **37**, 1288-1295 (2003).
3. Yuan, S.Y., Liu, C., Liao, C.S. and Chang, B.V., Occurrence and microbial degradation of phthalate esters in Taiwan river sediments, *Chemosphere* **49**, 1295-1299 (2002).
4. Rhodes, J.E., Adams, W.J., Biddinger, G.R., Robillard, K.A. and Gorsuch, J.W., Chronic toxicity of 14 phthalate esters to *Daphnia magna* and rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **14**, 1967–1976 (1995).
5. Marttinen, S.K., Kettunen, R.H., Sormunen, K.M. and Rintala, J.A., Removal of bis(2-ethylhexyl) phthalate at a sewage treatment plant, *Water Res.* **37**, 1385-1393 (2003).
6. Liu, Y., Lam, M.C. and Fang, H.H.P., Adsorption of heavy metals by EPS of activated sludge. *Water Sci. Technol.* **43**, 59-66 (2001).
7. Staples, C.A., Peterson, D.R., Parkerton, T.F. and Adams, W.J., The environmental fate of phthalate esters: a literature review. *Chemosphere* **35**, 667-749 (1997).
8. APHA, AWWA and WEF, *Standard Methods for the Examination of Water and Wastewater*, 17th edn. **2**, 75-77, American Public Health Association, Washington, D.C. (1989).
9. Fang, H.H.P. and Jia, X.S., Extraction of extracellular polymer from anaerobic sludges, *Biotechnol. Techniq.* **10**, 803-808 (1996).
10. Liu, H, and Fang, H.H.P., Extraction of extracellular polymeric substances (EPS) of sludges. *J. Biotechnol.* **95**, 249-256 (2002).
11. Schmidt, J.E. and Ahring, B.K., Granular sludge formation in upflow anaerobic sludge blanket reactors. *Biotechnol. Bioeng.* **49**, 229-246 (1996).