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Sorption of hydrophobic organic contaminants on functionalized biochar: protagonist role of π-π electron-donor-acceptor interactions and hydrogen bonds

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GRAPHICAL ABSTRACT
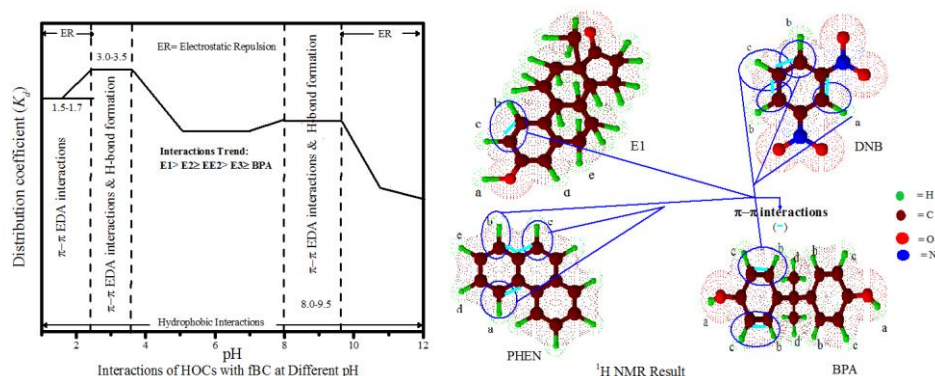
Highlights

- Potential mechanisms for HOC sorption were studied

- H-bond formation and EDA interactions were the main sorptive mechanism

- Specific direction of π-π EDA interactions was identified by ^{1}H NMR analysis

- fBC domains were responsible for different sorptive mechanisms at different pH

- fBC showed excellent removal efficiency of hydrophobic organic contaminants

Abastract

The sorption of five potent endocrine disruptors as representative hydrophobic organic contaminants (HOCs) namely estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2) and bisphenol A (BPA) on functionalized biochar (fBC) was systematically examined, with a particular focus on the importance of π-electron-donor (phenanthrene: PHEN) and π-electron-acceptors (1,3-dinitrobenzene: DNB, p-amino benzoic acid: PABA) on sorption. Experimental results suggested that hydrogen-bond formation and π-π-electron-donor-acceptor (EDA) interactions were the dominant sorption mechanisms. The sorption of HOCs decreased as E1 > E2 > EE2 > E3 > BPA based on the Freundlich and Polanyi-Mane-models. The comparison of

adsorption coefficient (K_d) normalized against hexadecane-water partition coefficient (K_{HW}) between HOCs and PHEN indicated strong π-π-EDA interactions. π-π interactions among DNB, PHEN and HOCs were verified by the observed upfield frequency (Hz) shifts using proton nuclear magnetic resonance (^{1}H NMR) which identified the specific direction of π-π interactions. UV-vis spectra showed charge-transfer bands for π-donors (PHEN and HOCs) with the model π-acceptor (DNB) also demonstrating the role of π-π EDA interactions. The role of π-electron-donor and π-electron-acceptor domains in fBC was identified at different solution pH.

Keywords: HOCs; EDCs; π-π-EDA interaction; Hydrogen bonds; fBC

3

1. Introduction

Pyrogenic carbonaceous materials (CMs) such as mesoporous carbon, biochar, activated carbon, graphene and carbon nanotubes (CNTs) exhibit strong sorption affinities for a wide range of organic contaminants including polycyclic aromatic hydrocarbons, benzene derivatives, phenolic compounds, and pharmaceuticals such as antibiotics, endocrine disrupting chemicals (EDCs) and pesticides [1-7]. The interactions of such contaminants with CMs in water, sediments and soil may result in strong or weak bindings that can significantly affect the environmental fate of contaminants and their remediation rates [8-10]. The underlying physical and chemical phenomena potentially responsible for these apparent interactions of contaminants with CMs are of great importance. Further, their persistence and potential risks to aquatic life is also important. Organic contaminants comprise broad classes of chemicals, some of which are persistent in soils, sediments and water, with potential for long-term impacts. The conventional idea for ionic organic species is that they may undergo Coulombic attraction/repulsion at charged sites on the adsorbent in addition to the weak forces available to uncharged molecules, including London-van der Waals force, H-bonding, and the hydrophobic effect (solute exclusion from water) [2, 11]. However, noncovalent forces are ubiquitous between chemical interactions as they control diverse phenomena such as boiling points of liquids, solvation energies, and the structures of molecular crystals. π-π interactions constitute one of the most important classes of noncovalent interactions, contributing to biomolecular structure, chemical bonding, and the structure and properties of π-conjugated materials (such as biochar and CNTs) of interest having π-structure benzene or aromatic ring [12]. The elucidation of molecular-level interactions controlling sorption of non-ionic compounds and the influence of solution-phase composition on sorption are of considerable theoretical and practical importance. This study is therefore aimed to compare the sorptive mechanism of five non-ionic endrocrine disruptor compounds (EDCs) such as E1, E2, E3, EE2 and BPA as representative HOCs on funcitionized biochar (fBC) based on their physicochemical properties (**Table 1**). fBC was selected for its higher soprtion capacity of organic pollutants (in both single

4

and competative moods) due to the presence of multifuctional groups on its surface. In addition, the production and operation cost of fBC is relatively low. Further, fBC can be used repetatively after regeneration **[14]**.

In this paper, the interaction mechanisms of HOCs and fBC were evaluated based on theoretical, experimental and analytical findings. The magnitude of non-hydrophobic interactions was assessed by "normalizing" the hydrophobic effect. The fundamental role of non-hydrophobic interactions namely hydrogen-bond formation and π-π interaction based on adsorption-pH profiles of HOCs by fBC was examined. Further, we also identified the probes of structure-adsorption property relationships for different π-electron-donor rings of HOCs (due to –OH groups on arene units) and PHEN (as model HOC) *vs* π-electron-acceptor rings of model compounds (DNB and PABA). The potential role of π-electron-donor-acceptor domains in fBC at different pH was predicted based on equilibrium sorption distribution coefficient and sorption capacity in the π-electron-donor-acceptor systems. We also evaluated several potential causes of sorption interactions, including pH effects on sorbent hydrophobicity, π-H-bonding, effects of solution acidity on solute activity coefficient and the occurrence of π-π EDA interactions with the π-electron-acceptor and donor sites of fBC. The role of π-π interactions was assessed by solution-phase proton nuclear magnetic resonance (^{1}H NMR) and ultraviolet-visible (UV-vis) spectroscopic studies by inspecting the behaviour of π-electron-donor compounds (HOCs and PHEN) in comparison with π-electron-acceptor (DNB) systems.

2. Experimental

2.1. Chemicals

E1 (99%), E2 (> 98%), E3 (> 97%), EE2 (98%), BPA (99%), phenanthrene, 1,3-dinitrobenzene, *p*-aminobenzoic acid, hexadecane, 2-nonanone (NON-2), and organic solvents such as methanol, methanol-d$_4$, formic acid (99.9%), and acetonitrile of HPLC grade were purchased from Sigma-Aldrich, Australia.

5

2.2. Materials

Eucalyptus globulus wood was used to produce biochar by heating the wood particles at 380 °C for 2 h in a reactor under continuous nitrogen gas supply and then activated using phosphoric acid according to our previous studies [13-15]. Brifely, biomass and phosphoric acid mixture was then heated at 600 °C for 2 h under continuous nitrogen supply at 2.5 psi, cooled at room temperature, and washed with distilled water 4 times while adjusting pH to 7, followed by drying overnight at 120 °C, to obtain the activated biochar. The activated biochar product named as functionalized biochar (fBC) based on its surface characteristics [14-15]. Structural analysis of carbon network showed that fBC was composed of mesopore (2-50 nm) and macrospore (> 50 nm) structure. X-ray photoelectron spectroscopic (XPS) results indicated that fBC surface was rich in different functional groups especially graphitic carbon (~57%), phenolic or alcoholic (C-O-, ~13.5%), carbonyl or quinone (C=O, ~4%), carboxylic or ester (COO-, ~3%), π-π^* transition (~1%), quarternary nitrogen (~1%), and polyphosphates and/or phosphates (C-O-PO$_3$, ~1%) (**Table A1**). The point of zero charge for fBC was pH 2.2.

2.3. Batch sorption experiments

HOC sorption experiments in distilled water were conducted in 50 mL glass vials with Teflon-lined screw caps at 25 ºC in triplicate on an orbital shaker over 48 hours using fBC. The sorption of HOCs using prestine biochar was not studied due to its lower sorption capacity. The effects of pH and sorption isotherm experiments of HOCs (at pH 3.0-3.5) were performed at the same conditions. The control experiments without sorbents were also conducted. The fBC dosage was selected for 15-99% sorption of each HOC at different concentrations. Single sorption experiments of PHEN, NON-2; DNB and PABA were carried out at three different pH ranges i.e. pH 1.5-1.7, 3.0-3.5, and 8.0-9.5, in triplicate. The effects of competitors such as PHEN, PABA, and DNB for HOC sorption were also carried out under the same conditions. The supernatants were filtered through a 0.45 μm PTFE filter and analyzed by high-performance liquid chromatography (HPLC)

6

and by UV-vis spectroscopy. Detailed methods for all solutes are presented in **supporting information.**

2.4. ^{1}H NMR and UV-Vis studies for π-π EDA interactions in solution

UV-Vis Spectroscopic spectra of test solution of HOCs, π-donor-DNB and π-acceptor-PHEN in water were measured at room temperature using Shimadzu (UV-1700) instrument. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at a specific concentration of HOCs and donor-acceptor solutes at room temperature using an Agilent 500 MHz NMR instrument. Samples were prepared in methanol-d$_4$ and measured after 24-30 hours. The chemical shifts (δ) were recorded by internally referenced to methanol-d$_4$ solvent and observed proton frequency shift was calculated based on NMR instrument frequency and chemical shift.

2.5. Data fitting

Different models employed to fit the adsorption isotherms are as follows:

Freundlich model: $S_e = K_f\, C_e{}^n$ (1)

Polanyi-Mane model (PMM): $S_e = S_{max}^{p} + (Z \times \left(RT \ln\left(\frac{C_s}{C_e}\right)\right)^{d})$ (2)

where S_e is the solid-phase sorbed capacity (μg kg^{-1}) of HOCs, S_{max}^{p} is the maximum adsorption capacity (μg kg^{-1}) from PMM, n is a dimensionless number related to surface heterogeneity, and K_f is the Freundlich affinity coefficient (μg^{1-n} L^n kg^{-1}). C_e represents the aqueous-phase concentration of solute (μg L^{-1}) at 25 ^{0}C, whereas C_s (μg L^{-1}) stands for solubility of each HOC at 25 ^{0}C. Z and d are PMM adsorption fitting constants, R is universal gas constant (8.314 × 10^{-3} kJ mol^{-1} K^{-1}), and T (K) is absolute temperature. All model equations were fitted by origin-pro, with model parameters being obtained with standard coefficient of determination (r^2) and adjusted coefficient of determination (r_{adj}^2).

The single point adsorption distribution coefficient (K_d) of HOCs was calculated using equation (3):

Partition model: $K_d = S_e/C_e$ (3)

where $C_e = 0.002C_s$ based on the fitting results using PMM. We used C_e/C_s ration of 0.002 to get maximum distribution coefficient.

Equilibrium sorbed volume of each HOC can be calculated based on initial and equilibrium concentrations using following equation:

$$S_v = (C_0-C_e)\ V/\ (1000\ m\ \rho_{HOC})\qquad (4)$$

where C_o and C_e ($\mu g\ L^{-1}$) are the initial and equilibrium concentration of HOCs; V (L) is the solution volume in the system; m (g) is the mass of fBC sorbent in the system; and ρ_{HOC} ($cm^3\ g^{-1}$) is the density of the solute.

3. Results and Discussion

3.1. Interactions of HOCs with fBC

The maximum single point K_d value was observed for E1 ($2.90 \times 10^6\ L\ kg^{-1}$) with the minimum for BPA ($3.03 \times 10^4\ L\ kg^{-1}$); thus, E1 was found to be adsorbed more strongly onto fBC surface than other HOCs (**Fig. 1a and Table A2**). The adsorption coefficient values followed the order of E1 > E2 ≥ EE2 > E3 ≈ BPA. The PMM model parameters for sorption isotherm of each HOC shows that the Polanyi theory may be useful to describe the sorption of HOCs on fBC, as the Polanyi theory relates to competitive sorption of organic compounds as micropore filling (**Fig. A1 and Table A3**). However, we used single solute to check the viability of PMM model. At zero adsorption potential, any undeformed sorbent should have a limiting volume to the total available pores for adsorption based on Polanyi pore filling model **[16]**. The adsorbed volume capacities of E1, E2, E3, EE2 and BPA, calculated from their mass capacities and respective solid-phase density, were 24.0, 23.0, 15.0, 20.0, and 17.0 $cm^3\ kg^{-1}$, respectively **[2]**. The Dubinin-Astakov micropore surface area of fBC was 520.0 $m^2\ kg^{-1}$ and the limiting micropore volume was 0.24

(cm^3 kg^{-1}) [15]. Thus, the micropore volume of fBC was at least 63-100% less than that of the adsorbed volume capacity of HOCs. This result is contradictory with the pore-filling mechanism for un-deformable sorbents [16-17]. Therefore, pore filling cannot be the dominant mechanism for the sorption of HOCs by fBC. Polanyi theory, however, is applicable for either pore-filling or flat surface adsorption and adsorption parameters correlate with the material surface defects curvatures of carbon materials, which can affect the adsorption significantly. BET surface area of fBC was found to be 1.18 (m^2 g^{-1}) [15]. Thus, we hypothesize that surface adsorption of HOCs (rather than pore filling) is the dominant sorption mechanism and which came from different functional groups of fBC surface. Therefore, several factors need to be considered for surface adsorption of HOCs: (i) potential energy of adsorption sites on solute-coated sorbent surface (i.e. fBC) should be lower and more homogeneous than that on un-coated sorbent surface, (ii) sorbed solute molecules on the sorbent surface should have attractive forces for solute molecules, and (iii) the maximum sorption capacity depends on the sorbent surface area and its relative functional groups [16]. However, the higher solid phase concentration of HOCs could be attributed to the functionalization of biochar resulting in the formation of additional sorption sites.

Freundlich isotherm r_{adj}^2 values of HOCs provided a slightly better fit than PMM isotherm r_{adj}^2 values indicating the role of surface functional groups for multilayer sorption of HOCs and the heterogeneity of the sorbent. Freundlich parameter 'n' values of HOCs ranged from 0.17 to 0.27, and all solute isotherms were nonlinear for all HOCs indicating that favourable for multilayer adsorption and heterogeneous energy distribution of fBC (**Tables A3 and A4**). Hence, the net interactive forces involving the solvent, solute, and the adsorbent are assumed to be responsible for the solute adsorption by sorbent surface activity in addition to pore filling in our fBC [18]. Among different forces of attraction, van der Waals force is primarily considered the dominant force for gas or vapour adsorption onto any hydrophobic adsorbent surface (such as CNTs), which may also be significant for adsorption from the aqueous phase [18]. Considering only van der Waals forces, however, may not be applicable in cases when dipole-dipole, induced-dipole, and

H-bonding or π-π donor-acceptor interactions exist, typically in the aqueous phase, where these forces can be necessary especially for chemicals and adsorbents with specific functional groups. Different possible interactions including H-bonds, hydrophobic effects, π-π bonds, covalent and electrostatic interactions can be observed and are responsible for the adsorption of organic chemicals on the surface of fBC. The strengths of such interactions and their and contribution to the overall sorption are a function of the properties of both organic chemicals and fBC surface [18]. Based on pH effects as shown in **Fig. 1b**, lower sorption of HOCs was expected below the point of zero charge of fBC due to the repulsion of same charged species (i.e. positive fBC surface and protonated phenolic –OH groups of HOCs). Above the point of zero charge of fBC, higher sorption of HOCs is expected due to oppositely charged species (negative fBC surface and neutral or deprotonated HOCs). Aside from electrostatic interactions, EDA interactions, H-bond formation and hydrophobic effects may play vital roles. Hence, at pH below 2.2, lower sorption was found, which was mainly due to EDA and hydrophobic effects. The maximum sorption of HOCs at pH 3.0-3.5 could be due to EDA interactions together with strong H-bond formation, hydrophobic effects and electrostatic interactions. At pH 8.0-9.5, the sorption for each HOC was also slightly higher than pH 3.0-3.5, which might be due to H-bond formations and EDA interactions. Details on the noncovalent forces based on experimental findings are presented in the following subsections.

3.2. Role of H-bond and π-H bond for HOC sorption

pH impacted sorption of all HOCs (**Fig. 1b**), with the most significant effect at pH range 3.0-3.5 and the least significant effect above pH 9.5. We categorized the adsorption data fall into three distinct pH ranges: pH below 2.2, pH 3.0-3.5, and pH 8.0-9.5. In general, the variations of sorption at different pH indicated that H-bond formation was involved.

To ensure that the effect of solution pH on HOC sorption was not a result of changes in the aqueous phase and solute activity coefficients, the solubilities of HOCs, PHEN and DNB were

measured in three different solution pH values (**Table 1**). The fact that no significant changes in solubilities were observed indicated that the changes in sorption with pH resulted from the effects on sorbent-sorbate interactions, not solute-solvent interactions. Previously, Zhu et al. [**19**] studied PHEN interaction mechanisms on soil organic matter and concluded that sorption of PHEN was solely due to π-π interactions by rejecting solute hydrophobicity, H-bonding, solute co-planarity, solute activity coefficient, and mineral surface proportionating. We also observed that sorption of PHEN was high at low pH (**Fig. 2a**) and the solubility of PHEN was not affected by solution pH. One may assume that the variation of K_d values at different pH for sorption of PHEN was related to H-bond formation. However, H-bond and π-H-bond formation at low pH by fBC can be ruled out based on the following assumptions. NON-2 is a compound commonly known as stronger H-acceptor and non π-donor than aromatic hydrocarbons [**19**]. If the hypothesis was correct, plausible explanation of NON-2 sorption (at low pH) is that NON-2-sorption should increase as pH decreases. This is due to H-bond formation by a polar ketonic group of NON-2 with proton groups of sorbent, and π-H-bond formation by NON-2 with the aromatic π-system of sorbent (i.e. graphitic unit of fBC). However, the opposite trend where the sorption of NON-2 on fBC was decreasing with pH decrease was observed (**Fig. A2**). We also carried out the solubility test for NON-2 and found no changes in solubilities at pH 1.8 and pH 3.5 but at pH 8.5 solubility decreased by ~25%. Also, PHEN molecule does not have any oxygenated functional group to form H-bonds with a surface functional group of fBC. However, sorption of NON-2 at different pH was mainly due to hydrophobic effect. Thus, H-bonding and π-H-bonding (by the withdrawal of electron density from the ring by the H-bond) of fBC cannot explane the acid-enhanced sorption of PHEN[19]. Therefore, sorption of PHEN was mainly due to hydrophobic effects and π-π-EDA interactions between PHEN and fBC. On the other side, selected HOCs have at least one hydroxyl group along with other groups connected with arene units in their structure. Consequently, sorption interactions of HOCs will not be similar in all cases as PHEN. For example, a variation of solution pH indicates that H-bond formation is associated with surface functional groups on fBC and HOCs. Hence,

after excluding π-H-bonding, it can be concluded that the pH-dependent sorption of HOCs is undoubtedly due to their H-bonding involvement plus other interactions such as π-π EDA interactions, and hydrophobic interactions. However, the contribution of H-bonding to the overall adsorption of HOCs is unknown.

3.3. Normalization to hydrophobic effects

The role of hydrophobic interactions was premeditated using inert solvent-n-hexadecane. Higher single point adsorption coefficient (K_d) of HOCs attributed to the higher sorption of HOCs by fBC. Thus, the normalization of K_d values by hexadecane-water partition coefficient (K_{HW}) could rule out the hydrophobic effect, and potential adsorption mechanism could be due to π-π interactions. We measured the hexadecane-water partition coefficient (K_{HW}) of HOCs and PHEN at 25 ºC (**Table 1**). The hydrophobicity followed the order of PHEN > E1 > E2 > EE2 > E3 > BPA. The resulting parameters are listed in **Tables 1** and **A4.** The normalized partition coefficient (K_d/K_{HW}) of HOCs showed significantly higher values than PHEN (**Fig. 3a**) indicating the significant role of π-π interactions for HOCs sorption by fBC. Moreover, this relationship was directly related to K_d and K_{HW} of HOCs (**Fig. 3b**).

3.4. Evidence for π-π EDA interactions

3.4.1. Evidence from experimental finding

From literature, it has been reported that the adsorption of chemical contaminants on CMs increases with increasing oxygen-containing functional groups, which is partially attributed to π-π EDA interactions [3,13,20]. Surface carboxylic acid, nitro, and ketonic groups of CMs can act as an electron acceptor to form π-π EDA interactions with aromatic molecules and thereby enhance sorption [21]. On the other hand, graphitic carbon like structure, hydroxyl and amine groups present in different CMs can serve as π-electron-donor site depending on the type of functionalization. The strongest π-π interactions are between oppositely charged arene units, while

12

the weakest one are between like polarized units **[21]**. All HOCs used in this study have at least one hydroxyl group connected with arene unit and we hypothesis that this arene unit could act as the π-electron-donor site due to resonance effects. If the hypothesis is correct, then the presence of a competitor (π-electron acceptor) in the same solution should affect sorption capacity to some extent. Thus, to prove this concept, we provided both π-electron-donor (PHEN) and π-electron-acceptors (DNB and PABA) at different concentrations in each HOC solution at three different pH (pH 1.5-1.8, 3.0-3.5, and 8.5-9.5) individually and observed their effects.

Apparent view of the **Figs 4** and **A3** provided an overall idea of the competitors' effects on HOC sorption. DNB has a higher influence on the reduction of sorption capacity of HOCs than PHEN. This change is high at pH 3.0-3.5 compared to other pH values. For example, in the presence of PHEN, no significant change in the solid-phase sorption capacity of BPA observed. This indicates the minor role of π-electron-donor-donor interactions as arene unit in BPA served as the π-electron-donor site. In contrast, the presence of DNB reduced the solid-phase sorption capacity of E1 and BPA by ~48% and ~25%, respectively, clearly supporting the role of EDA interactions. However, the presence of PHEN also affect sorption capacity to few extent, and this might be due to co-solute effects. Similar strong EDA interactions have also happened for E2, E3 and EE2 (**Fig. A3**). Thus, DNB had a negative effect on the adsorption of HOCs. This result is consistent with the π-π-EDA interactions after normalizing hydrophobic effects as shown in **Fig. 3a**, indicating the protagonist role of the EDA interactions in solution. In addition, we also used another model π-electron-acceptor such as PABA and obtained similar results (**Figs A4 and A5**). Thereby, these findings indicated the protagonist role of EDA interactions in the sorption of EDCs by fBC.

Moreover, a previous study hypothesised that increasing solution pH apparently facilitated deprotonation of the acidic functional groups (-COOH, -OH) of CMs **[3]**. Deprotonated functional groups (e.g. -COO$^-$ and -O$^-$) might modify the hydrophobicity and the net charge on the carbon surface, which can further promote the π-electron-donor ability of the graphene surface (-COO$^-$ is

a strong electron donor), thereby enhancing π-π EDA interactions of two nitroaromatics (e.g. DNB and 1,3,5-trinitrobenzene as π-electron acceptors) **[3]**. They observed that sorption of nitroaromatics to single-walled CNTs increased by 2-3 times with the increase of solution pH. Thus, similar interactions may apply to the system in this study. We observed that sorption of DNB by fBC was slightly increased when pH was increased from 3.0-3.5 to 8.5-9.5, indicating the role of EDA interactions between DNB and deprotonated acid functional group (-COO[-]) of fBC (**Fig. 2b**). All these results indicated that π-π-electron-donor-acceptor interaction was one of the significant forces responsible for the overall HOC-fBC interactions.

3.4.2. UV-Vis spectroscopic evidence

Fig. 5 shows the presence of π-π charge-transfer absorbance band in the UV region for the mixture of DNB (as an acceptor), PHEN (as a donor) and HOCs (such as BPA, E3 and E1 as π-donor). Noteworthy deviation of adsorption intensity was observed for PHEN and DNB solutions (**Fig. 5a**). A similar result was also observed for DNB and E1 or BPA interactions indicating the π-π EDA interactions took place (**Fig. 5b-d**). On the other hand, no interaction (no deviation of adsorption intensity) was observed for the mixture of PHEN and E1/BPA/E3. We also increased the donor-acceptor concentration and similar result observed which indicate the EDA interactions between π-electron-donor site and π-electron-acceptor site of the selected model compounds (**Fig. 5b**). Hence, we predict same interactions in solutions at different pH happened between π-electron donor and π-electron acceptor sites of fBC and π-electron donor site in the target HOCs.

3.4.3. ^{1}H NMR spectroscopic evidence: specific direction of π-π EDA interactions

Previously, Zhu et al.**[19]** and Wijnja et al.**[22]** used ^{1}H NMR spectroscopy to examine π-π interactions in solutions among PHEN, quinones, N-heteroaromatic cations, naphthalene, pentamethylbenzene, 1,3,5-benzenetricarboxylic acid, 1,4,5,8-naphthalenetetracarboxylic acid and pyridine. In this study, we also used ^{1}H NMR tool to demonstrate molecular complexation in solution between π-electron-donor (PHEN) and π-electron-acceptor (DNB) and their effect on π-π interactions in a solution of E1 and BPA (as π-donor due), separately. We assume that π-π

14

interactions might occur between subunits attached to fBC surface due to π-electron-donor (i.e., graphitic carbon, OH, and NH_2) and/or π-electron-acceptor (e.g., COOH, C=O, C-O-PO_3) groups. Our assumption is reasonable because π-π interactions occur, in here, readily in a nonaqueous solvent (methanol-d_4) and π-π interactions have already been demonstrated in solid-liquid-phase systems based on donor-acceptor competitor experimental proof.

In ^{1}H NMR, it has long been known that placing a nucleus above or below the plane of an aromatic structure causes electronic shielding due to "ring current" effects. Moreover, placing a magnetic nucleus along the edge of an aromatic ring results in the opposite ring current effects so-called deshielding effect. Hence, because of the parallel-planner geometry of a π-π complex system, one may easily expect an upfield chemical shift (δ) of protons on one ring induced by the ring current effect of the opposing ring **[3]**. This has been used as a tool to identify π-π interactions in solution for different compouds **[3, 19]**. Similarly, our case is entirely consistent with the formation of π-π complexes as a function of interactions due to observed upfield chemical shift in complex mixtures of π-donor and π-acceptor model compounds.

Therefore, we calculated the observed frequency (Hz) shift for specific proton (in respective carbon of arene unit) in the same orientation for the respective solute. The specific direction of π-π interactions orientation was identified for each solute. Upfield shifts of protons at different positions of each solute occurred for the following combinations: (i). PHEN (positions a, b, c in **Figs 6a & d**) in a mixture solution of PHEN and DNB; (ii). DNB (positions a, b, and c in **Figs 6a & d**) in a mixture solution of PHEN and DNB; (iii). E1 (positions b, and c in **Figs 6b & d**) in the mixture solution of E1 and DNB; (iv). DNB (positions a, b, and c in **Figs 6b & d**) in the mixture solution of E1 and DNB; (v). BPA (positions b, and c in **Figs 6c & d**) in the mixture of BPA and DNB; (vi). DNB (positions a, b, and c in **Figs 6c & d**) in the mixture of BPA and DNB. No upfield frequency changes for respective protons were observed (Hz = 0) from the interactions of E1 and BPA with PHEN (**Fig. 6**).

15

These results show that arene unit of HOCs served as a π-electron-donor unit due to presence of hydroxyl group. Hence, clear π-π EDA interactions observed for DNB and π-electron-donors (i.e. E1 and BPA) in solution and the interactions were specific toward defined protons in each arene unit (i.e. π-π-electron-donor-acceptor system). We predict that the similar interactions also responsible for other HOCs such as E2, EE2 and E3. As fBC consists of both π-electron-donors and π-electron-acceptors groups, thereby, π-π EDA interactions happened at different pH. In addition, the NMR results for different HOCs did not provide evidence of π-H-bonding, as no downfield shifts of protons were observed for different complexes indicating the insignificant involvement of π-H-bonding **[19]**.

3.5. Role of π-donor and π-acceptor domains in fBC structure at different pH

CMs consist of graphitic sheets and different functional groups. For example, CNTs contain more than 95% graphitic carbon. Hence, the hydrophobicity of CNTs is an important factor affecting the sorption of solutes. Functionalization of CMs can introduce hydrophilic moieties to the surface of CMs and thereby affecting the sorption affinity of the contaminants **[23, 24]**. For example, the introduction of O-containing moieties either increases or decreases sorption of organic contaminants and might act as π-electron-donor (e.g. -OH group) and π-electron-acceptor (e.g. -COOH, C=O, -COO-) sites **[14, 25, 26]**. The presence of aromatic amine groups (hetero) in the polyaromatic surface of CMs can act as π-electron-acceptor in forming π^+-π EDA interactions with the π-electron-rich surface of CMs **[2]**. XPS result of our fBC showed that fBC consist of 81.76% C, 13.32% O, 0.8% N and 2.3% P. Hence, fBC surface composed of C=C, –C-OH, -COOH, C=O, heteroaromatic amine and pentavalent tetra coordinated phosphorus (PO_4 i.e. $C-O-PO_3$) groups. These functional groups can serve as either π-electron-donor and π-electron-acceptor or both. Therefore, we can predict π-π interactions based on solution chemistry and effects of competitors (π-donor or acceptor) at different solution pH. Thence, the presence of π-electron-donor and

acceptor compounds in the solution can hamper the sorption significantly, and this result can reveal that the presence and the role of π-donor/acceptor domains in any materials.

In general, π-electron-donor site of any material binds to a π-electron acceptor compound and vice versa. At pH 8.0 to 9.5, fBC surface might serve as the π-electron-acceptor site due to surface ketonic, ester, and hetero-N-cyclic aromatic groups [2]. Also, zeta potential of fBC was found profoundly negative at this pH (-48.0 mV) indicating the presence of the deprotonated carboxylic (-COO⁻, a strong electron-donor), which could serve as hydrophobic moiety and form H-bonds together with EDA interactions. The π-electron-donor ability also came from graphitic carbon; surface –OH (if not dissociate); and –NH$_2$ groups. Therefore, at this pH range, from the comparison of K_d values of PHEN and DNB, it can be concluded that π-electron-donor ability of fBC is stronger than π-electron-acceptor groups (**Fig. 2**). However, both groups are active for EDA interactions. Furthermore, sorption of HOCs did not change significantly in the presence of π-donor PHEN (indicating HOCs served as π-donor groups) and changed a lot in the presence of DNB and PABA (**Figs 4** and **A3-A5**). Therefore, we assume π-electron-donor groups dominants over π-electron-acceptor groups for EDA interactions, but this domination is not strong as like as acid enhanced sorption of HOCs; PHEN; PABA and DNB (at pH 1.5-1.7).

At pH 3.0-3.5, we predict both π-electron-donor and π-electron-acceptor domains of fBC were active to form strong EDA interactions and strong H-bonds. This can be confirmed by the higher sorption of all HOCs (**Fig. 1b**). Thus, maximum pH is from EDA interactions as fBC surface comprises of π-electron-acceptor (e.g. –COOH, C=O, hetro-N-cyclic, C-O-PO$_3$) and π-electron-donor (e.g. graphitic carbon of fBC, –OH, –NH$_2$) groups. Similar interpretation for EDA interactions can be made based on **Fig. 2,** where sorption of PHEN was significance over pH 8.0-9.5. The zeta potential value of fBC at this pH was -18.0 mV and deprotonation of surface –OH groups was insignificant at this pH (due to pK_a sorption at this value of surface –OH group is 8.5-10) indicating the role of H-bonds formations together with EDA interactions [27]. Also, pK_a value of each HOC is more than 10 (**Table 1**). Thus, charge-assisted H-bond formation also played an

imported role for the maximum sorption of HOCs. H-bonds formation can also come from ketonic groups or ester groups of fBC as the maximum sorption of NON-2 (as H-acceptor) was found at pH 3.0-3.5 indicating the role of H-bond formation. These findings indicated the role of both π-electron-donor and acceptor domains of fBC surface for the sorption of HOCs, PHEN and DNB.

The sorption of PHEN, PABA and DNB was increased considerably when the solution pH was low (1.5-1.7) indicating the presence of both π-electron-donor and π-electron-acceptor groups on fBC surface (**Figs 2 and 7**). Also, at pH below 1.8, fBC surface became positive (+4.5 mV), indicating that the surface hydroxyl, carboxyl and amino groups of fBC became protonated (i.e. $COOH_2^+$, $-OH_2^+$, $-NH_3^+$). Therefore the H-bonds formation by fBC at this pH would play a minor role. Graphene surface of fBC could serve as the π-electron-donor site [**28**], and ketonic group, ester groups, $C-O-PO_3$ and hetero-N-cyclic groups of fBC might serve as the π-electron-acceptor groups. Hence, EDA was the main sorption mechanism. Finally, HOC sorption in the presence of PHEN, PABA and DNB (**Figs 4** and **A3-A5**) also indicated the duel behaviours of fBC surfaces at this pH. Hence, at this pH range, both π-electron-donor and π-electron-acceptor groups were significantly active for the sorption of opposite π-electron compounds. The EDA interactions were the strongest interactions among other interactions at this pH range. Therefore, fBC surface consists of different functional groups, and their behaviours change significantly with the change of solution chemistry.

4. Conclusions

The affinity of functinalized biochar toward HOC sorption showed excellent results. The main causes of this affinites are π-π-EDA interactions and hydrogen bond formation together with other sorption mechnisms such as pore filling, electrostatic attractions and hydrophobic effect. Different theoritical, instrumental and experimental results for potential cause of hydrogen bond and π-π-EDA interactions were undertaken. Most importantly, specific direction of π-π-EDA interactions was identified. In addition, π-electron-donor and π-electron-acceptor domains in fBC were

18

responsible for different sorptive mechanism at different solution pH. Therefore, functinalized biochar has the capability for the sorptive removal of a wide range of HOCs from aqueous solutions.

Conflict of Interests

The authors declare no competing financial interest.

Acknowledgements

Appendix A: Supporting data

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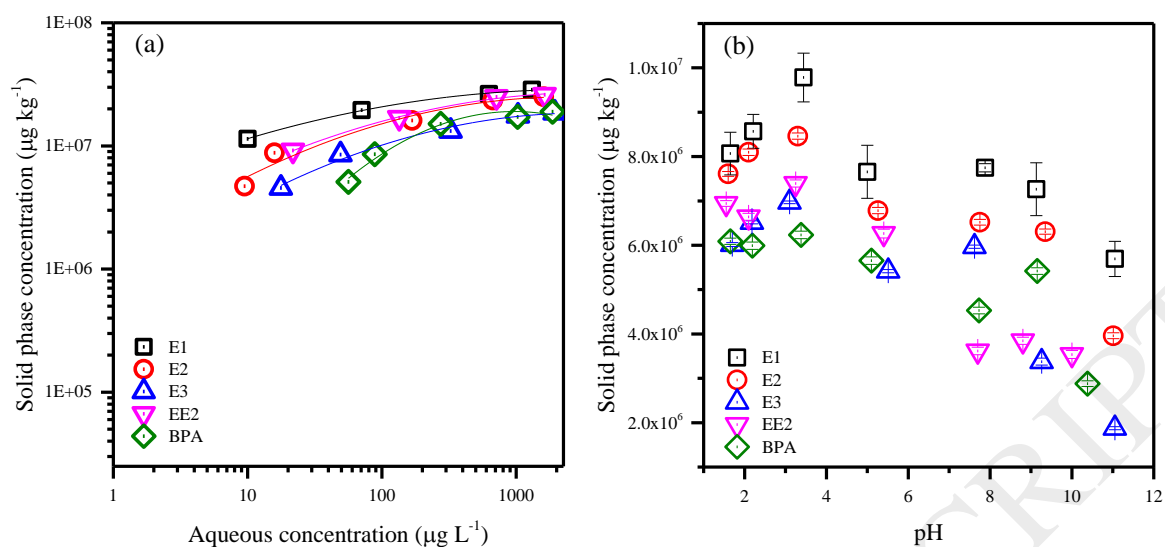
Fig. 1. (a) Adsorption isotherms of estrone (E1), 17*β*-estradiol (E2), estriol (E3), 17*α*-ethynylestradiol (EE2) and bisphenol A (BPA) on fBC at pH 3.0-3.5. Solid lines are the polynomial fitting curves using PMM. (b) Effect of pH on solid phase concentration (μg kg^{-1}) during HOC sorption (initial concentration of each HOC was ~500 μg L^{-1}) by fBC with dosage of 40-60 mg L^{-1}, 25 $^{\circ}$C.
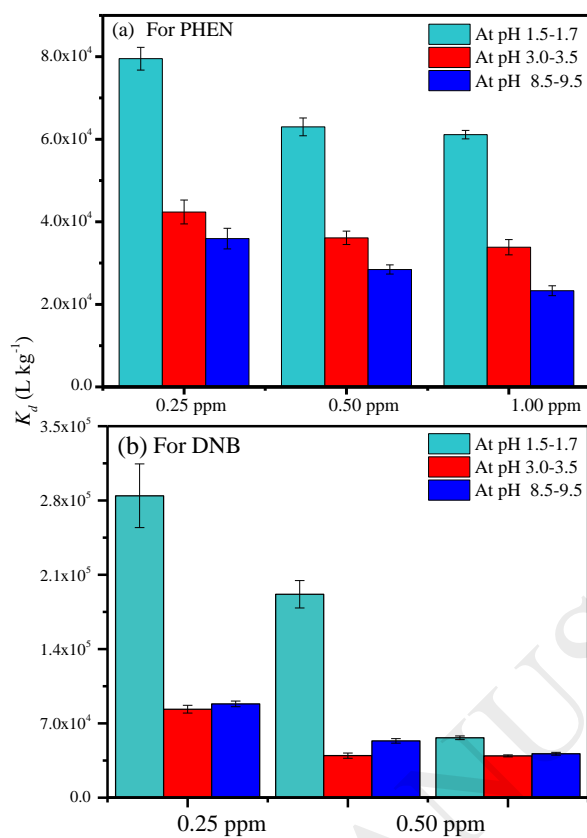
Fig. 2. Individual sorption of different concentrations of (a) phenanthrene (PHEN) and (b) 1,3-dinitrobenzene (DNB) on fBC at different pH, 25 ^{0}C using fBC dosage of 18-25 mg L^{-1} and 40-60 mg L^{-1}, respectively for PHEN and DNB.
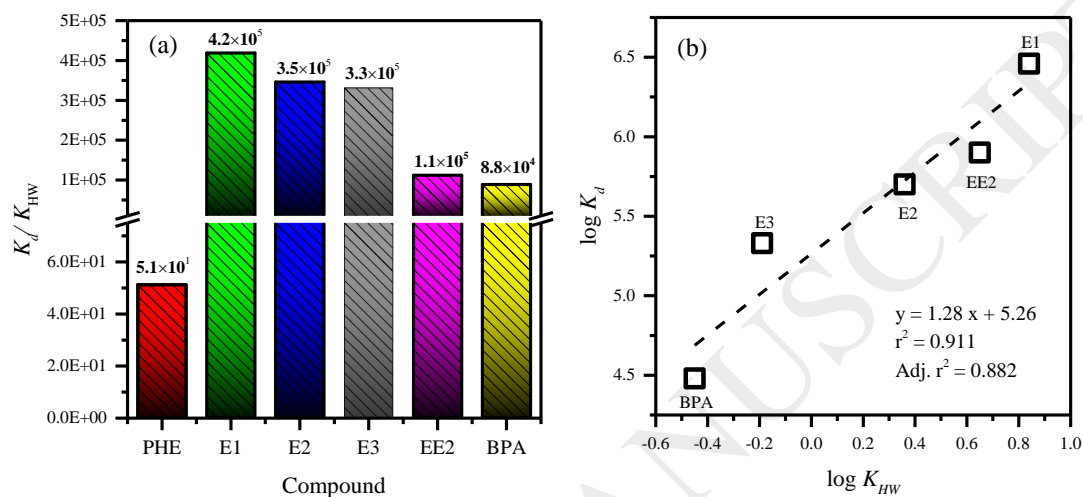
Fig. 3. (a) Comparison of K_d/K_{HW} between PHEN and HOCs such as E1, E2, E3, EE2 and BPA. K_d/K_{HW} was calculated at $C_e = 0.002\ C_s$. The differences among them are consistent with the explanation that π-π bonds played a major role after eliminating hydrophobic effects. The π-π bonds formed among HOCs and fBC were a donor/acceptor system, and much stronger than those between PHEN and fBC. (b) Relationship between log K_{HW} vs log K_d as calculated at $C_e = 0.002\ C_s$.
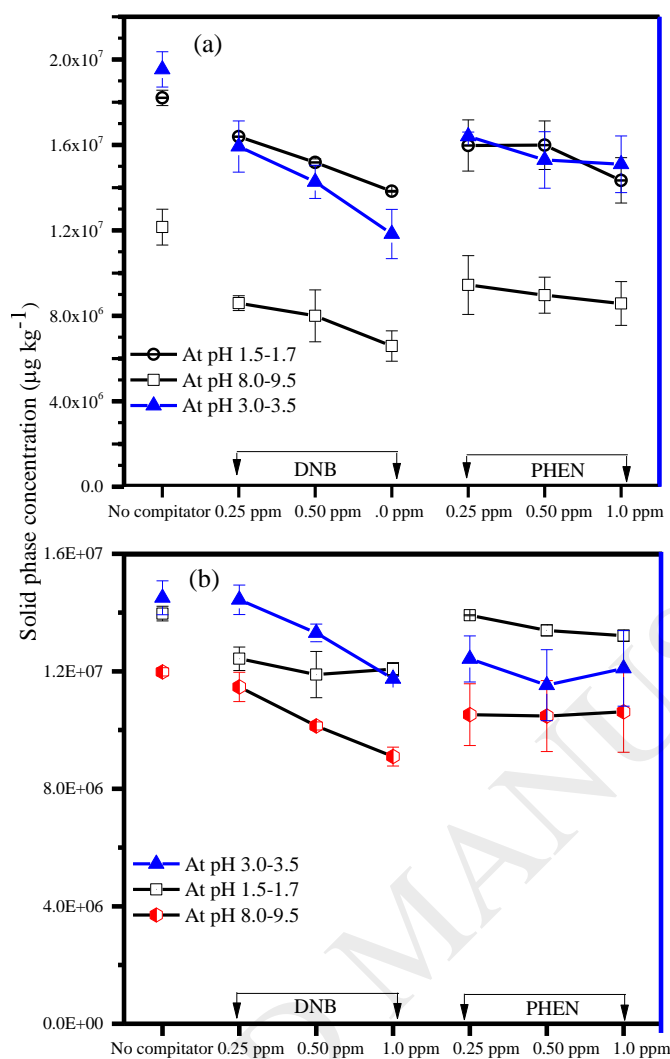
25

Fig. 4. Sorption performance of (a) E1 and (b) BPA in the absence and presence of competitor such as π-electron-donor PHEN and π-electron-acceptor DNB. Their interactions indicated the role of co-solutes and the role of π-π electron-donor-donor or donor-acceptor multi-system on the sorption performance of HOCs by fBC. Error bar representing the standard deviation. Sorption performance of E2, E3 and EE2 in the absence and presence of π-electron-donor-acceptor system is represented in **Fig. S3**. Each HOC initial concentration was ~1000 μg L^{-1} and fBC dosage was maintained 40-60 mg L^{-1}.
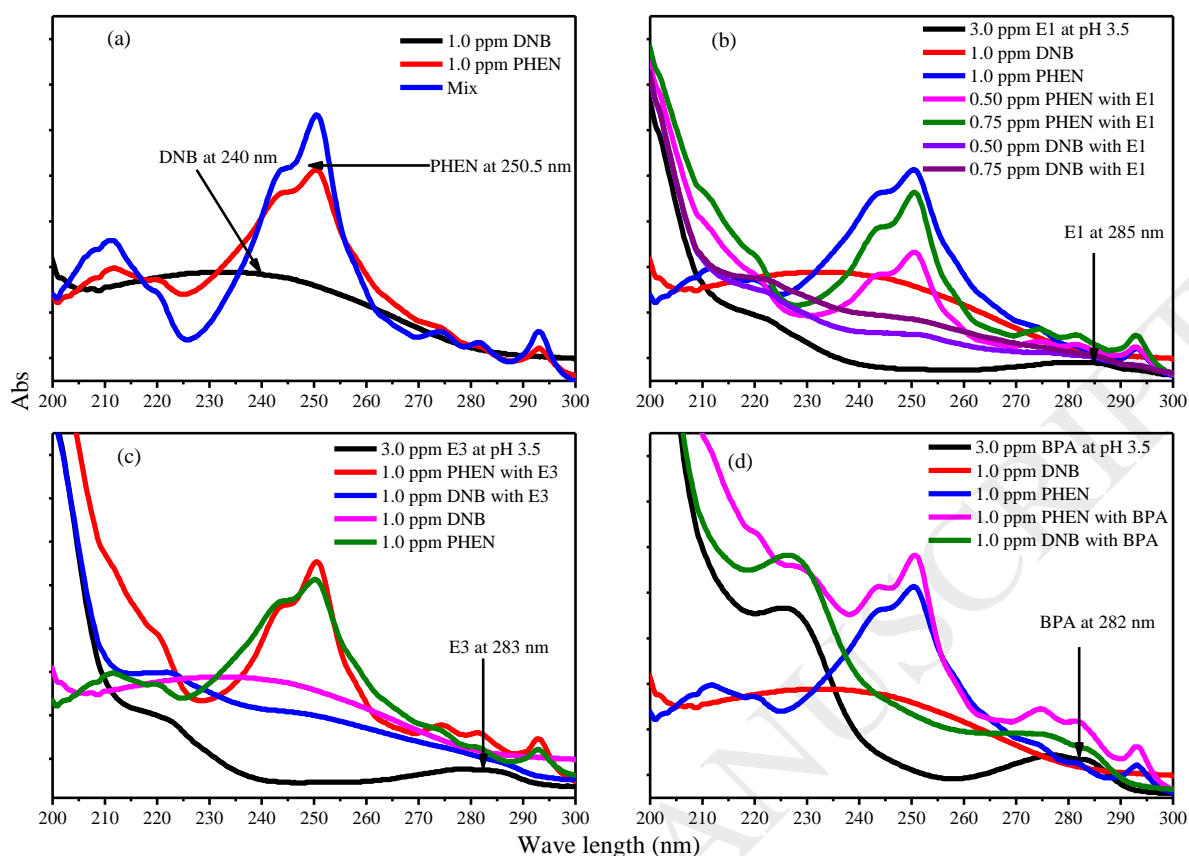
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Fig. 5. UV-Vis spectra difference in the acceptor-donor mixture showing the charge-transfer absorption band of π-π complexes. (a) Donor-PHEN, acceptor DNB and their mixing interaction, (b) Donor-PHEN, acceptor-DNB and HOC-E1 interactions, (c) Donor-PHEN, acceptor-DNB and HOC-E3 interactions, and (d) Donor- PHEN, acceptor-DNB and HOC-BPA interactions clearly indicating the difference of absorption band of π-π complexes.
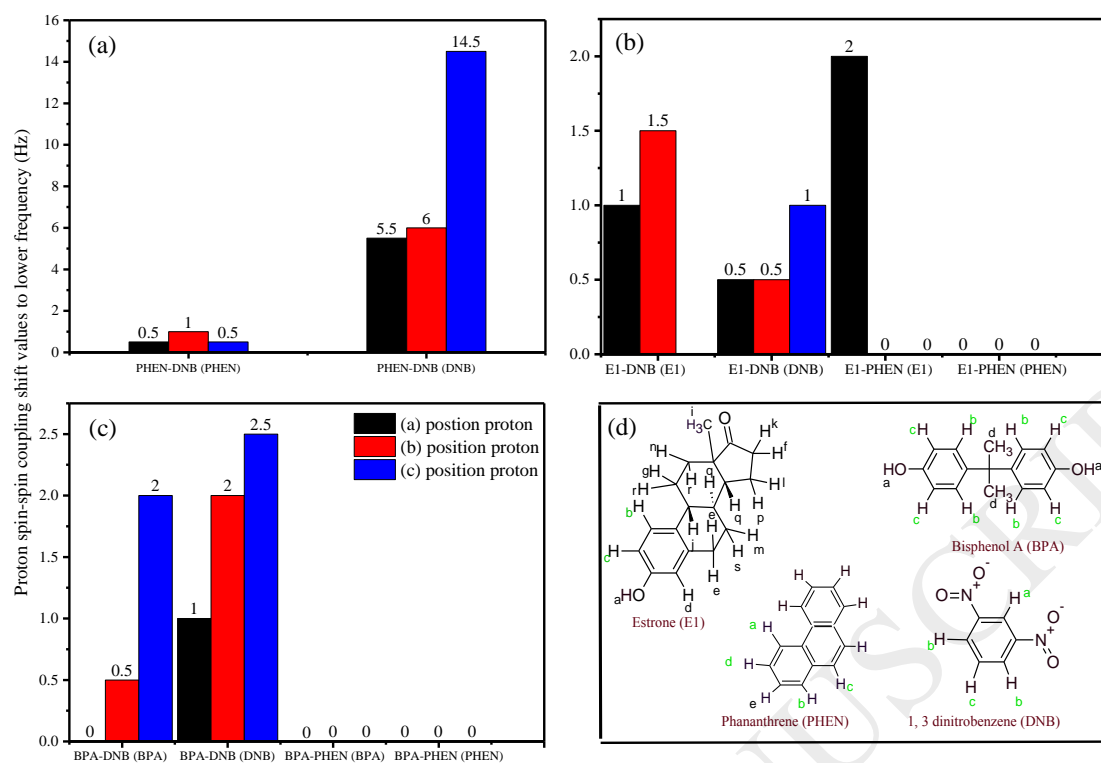
Fig. 6. ^{1}H NMR observed frequency (Hz) shift for different protons in specific carbon bonded protons in E1, BPA, DNB, and PHEN (a-c). ΔHz of proton in each compound with a mixture specified within bracket. Data obtained from a series of mixing solution of solutes and their increased shielding by extra-nuclear electrons (i.e. increasing magnetic field at fixed frequency) from a fixed concentration of solute in methanol-d_4. Observed frequency shift (ΔHz) for each solute in solution was measured by multiplying the chemical shift (δ) of each solute and spectrophotometer frequency (Hz) for different proton positions (marked as green color) as shown in (d). Structural proton positions labelled based on ^{1}H NMR peaks accessible at http://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_result.cgi?STSI=151281072529897. (d) Illustration of proton number in the structure of HOCs, PHEN and DNB.
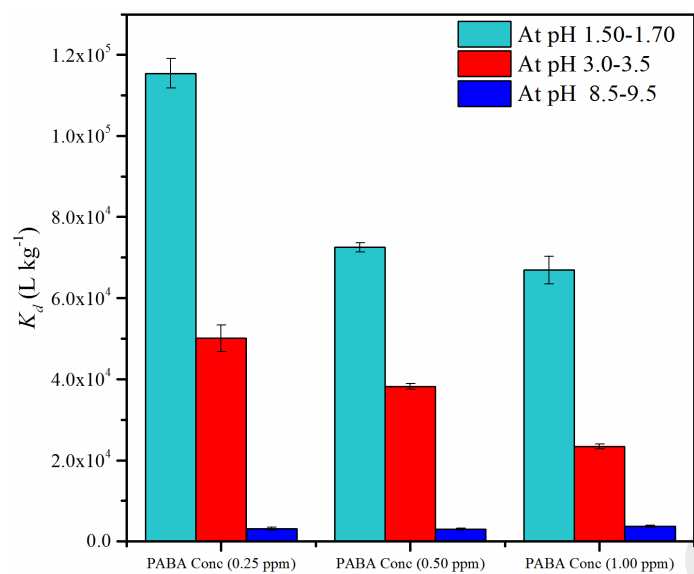
Fig. 7. Sorption of PABA at different concentrations and different pH using fBC dosage of 40-60 mg L^{-1}.

Table 1

Physicochemical Properties of Selected HOCs.

Name	Abbreviation	Chemical formula	pK_a [b]	Solubility [a] (C_s, mg L^{-1})	K_{HW} [a]	$logP$ [b]
Estrone	E1	$C_{18}H_{22}O_2$	10.33	1.17	7.26 ± 0.48	4.03-4.31
17β-Estradiol	E2	$C_{18}H_{24}O_2$	10.33	2.15	2.29 ± 0.21	3.57-3.75
Estriol	E3	$C_{18}H_{24}O_3$	10.33	13.96	0.65 ± 0.02	2.54-2.67
17α-Ethynylestradiol	EE2	$C_{20}H_{24}O_2$	10.33	7.64	4.45 ± 0.12	3.63-3.93
Bisphenol A	BPA	$C_{15}H_{16}O_2$	9.78	313.15	0.34 ± 0.02	3.81-4.04
Phenanthrene	PHE	$C_{14}H_{10}$		1.10 [c]	17.30 ± 1.86	4.46 [c]
1,3-Dinitrobenzene	DNB	$C_6H_4N_2O_4$	13.86 [b]	185 [b]	-	1.49 [c]

[a]: Measured at 25 ºC.

[b]: http://www.t3db.ca/toxins/T3D0782

[c]: https://pubchem.ncbi.nlm.nih.gov