Controlling a toxic shock of pentachlorophenol (PCP) to anaerobic digestion using activated carbon addition

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5 1 **Controlling a toxic shock of pentachlorophenol (PCP) to anaerobic digestion using**
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7 2 **activated carbon addition**
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31 10 **Abstract**
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35 11 Several powdered and granular activated carbons (PACs and GACs) were tested for
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37 12 adsorption of pentachlorophenol (PCP) in bench-scale anaerobic digestion reactors to
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39 13 control the toxicity of PCP to acetoclastic methanogenesis. Results showed that the
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41 14 adsorption capacities of PAC were reduced by 21-54%, depending on the PAC addition
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43 15 time, in the presence of the methanogenic sludge compared to the controls without
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45 16 sludge. As a preventive measure, PAC at a low dose of 20% (mass ratio to the VSS)
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47 17 added 24 hours prior to, or simultaneously with, the addition of PCP could completely
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49 18 eliminate the toxic effects of PCP. At the same dose, PAC also enabled methanogenesis
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51 19 to recover immediately after the sludge had been exposed to PCP for 24 hours. GAC
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was not effective in enabling the recovery of methanogenesis due to its slow adsorption kinetics; however, at a dose of 80% it could partially ameliorate the toxic shock of PCP.

Keywords

Anaerobic digestion, Pentachlorophenol, Toxicity, Activated Carbon

1. Introduction

Anaerobic treatment of low-strength municipal wastewater has the potential to replace the traditionally energy-intensive activated sludge processes because of the considerable progress made in reducing its hydraulic retention time (Hu and Stuckey, 2006; Stuckey, 2012), thus reducing system size and increasing economic viability. However, accidental or intentional discharge of toxic compounds to sewage, which often cause interruption or even shutdown of sewage treatment plants (Linares et al., 2007; Stabley, 2014; UtilityWeek, 2009), poses a big challenge to the smooth operation of anaerobic processes. Compared to activated sludge, anaerobic digestion processes, especially methanogenesis, are more vulnerable to toxicants. It takes a long time for the process to recover due to the very slow growth rate of anaerobes. Hence, a practical toxic shock control measure is vital for the widespread application of anaerobic treatment in municipal wastewater treatment.

Pentachlorophenol (PCP) is known to be one of the most toxic compounds to methanogenesis, and inhibits methanogens at concentrations as low as 0.5-10 mg/L (Chen et al., 2008). However, after acclimating to PCP for 3 months anaerobic sludge could slowly reduce 5 mg/L PCP to less chlorinated phenols (Mun et al., 2008), and after more than 2 years anaerobic sludge could mineralize ~ 25 mg/L PCP to CH₄ and CO₂ (Mikesell and Boyd, 1986). Anaerobic digestion sludge augmented with PCP-

reducing strains, such as *Desulfitobacterium frappieri* PCP-1, was able to treat PCP with concentrations gradually increased from 0 to ~ 4 mg/L within 70 days without acclimation (Tartakovsky et al., 1999). However, when PCP was loaded into the reactor aggressively with an increment of 25% every 6 hours, methane production was inhibited after the PCP reached ~ 4 mg/ in the reactor, even when the biomass of strain PCP-1 augmented in the reactor was 1/3 of the total biomass (Tartakovsky et al., 1999). These examples clearly demonstrate the vulnerability of anaerobic digestion to a shock load of PCP.

The slow biotransformation of PCP under anaerobic conditions poses another challenge for wastewater treatment plants to meet their effluent quality requirements. Complete mineralization of PCP to CH₄ and CO₂ in two successive packed-bed reactors under methanogenic and iron-reducing conditions, respectively, with a total hydraulic retention time (HRT) of 14.4 days, was previously reported (Li et al., 2010). With a shorter HRT of 24 or 48 hours, > 91% of PCP was reduced, but less chlorinated phenols were observed as intermediates (Damianovic et al., 2013; Wang et al., 2013). Compared to a normal HRT of 6-8 hours for municipal wastewater, the HRT needed for biotransformation of PCP was too long, which would result in PCP leaking into the receiving water body, and may cause an environmental disaster. Thus, the best toxicity control measure would be the one that can not only control the toxicity of PCP to anaerobic sludge, but also reduce the concentrations of PCP in the effluent.

Activated carbon has been shown to have very high adsorptive capacities for phenolic compounds, including PCP in pure water (Dabrowski et al., 2005). The adsorption isotherm can be best fitted with the Freundlich model, $Q_e = K_f C_e^{1/n}$ (Dabrowski et al., 2005), where, Q_e (mg/g) is the equilibrium PCP adsorbed on the

carbon, C_e (mg/L) is the equilibrium PCP concentration in aqueous solution, and K_f and $1/n$ are Freundlich isotherm constants. The reported K_f of commercially-available granular activated carbon (GAC) varies from 14.12 (Jung et al., 2001) to 150 (Dabrowski et al., 2005). The absorptivity of activated carbon for PCP was strongly affected by pH, with K_f increasing from 100 at pH 9.0 to 150 at pH 7.0, and 260 at pH 3.0 (Dabrowski et al., 2005). Dissolved oxygen (DO) also significantly increases adsorption, especially the irreversible adsorption of activated carbons for phenolic compounds due to polymerization of these compounds through oxidative coupling reactions (Tessmer et al., 1997; Vidic et al., 1993). This suggests that anaerobic conditions may increase the reversible adsorption of phenolic compounds, and thus favour the long-term application of activated carbon, as the reversible adsorption sites may be regenerated eventually by anaerobic sludge due to biodegradation.

The incorporation of activated carbon into biological processes, either in activated sludge or anaerobic digestion, has been well established (Cecen et al., 2003; Moteleb et al., 2002; Rattier et al., 2012; van der Zee et al., 2003). The major benefits of activated carbon addition include stabilizing the bioprocesses in the presence of toxic shocks, enhancing the removal of the refractory organics, and improving the settling and dewaterability of the activated sludge. Recently, both PAC and GAC were exploited in aerobic and anaerobic membrane bioreactors (MBRs) to reduce fouling and improve effluent quality (Do et al., 2009; Hu and Stuckey, 2007; Kim et al., 2011; Torretta et al., 2013).

However, recovering biological processes from toxic shocks using activated carbon addition has rarely been investigated. To the best of our knowledge, the only reported case compared different recovery strategies in anaerobic reactors facing toxic shocks

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5 91 from phenolic compounds, in which PAC addition was shown to be the best (Wang and
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7 92 Han, 2012). The aim of the present study was to investigate whether activated carbon
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9 93 could be used to recover or prevent methanogenesis from a toxic shock of PCP and keep
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11 94 the effluent quality below the regulatory standards, and whether the time of addition
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13 95 would affect the recovery.
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16 96 **2. Material and Methods**

17 97 **2.1. Activated carbon**

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19 98 The seven varieties of commercially available activated carbon used as adsorbents
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21 99 in this study were donated by Cabot Norit (Netherlands) and Calgon Carbon
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23 100 (Singapore); the properties of the carbons are listed in Table 1, and were used as
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25 101 received. The pore structure and surface area of three selected carbons were measured
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27 102 via mercury intrusion in a Micromeritics AutoPore IV9500 (Norcross, GA , US)
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29 103 porosimeter and a N₂ adsorption isotherm in a Micromeritics TriStar 3000 (Norcross,
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31 104 GA , US) porosimeter.
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34 105 **2.2. Adsorption isotherm**

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36 106 The traditional bottle-point method was used for the adsorption isotherm tests. A
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38 107 solution of 100 µM PCP in 0.025 M NaOH was prepared under a N₂ atmosphere. The
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40 108 pH of solution was adjusted to ~7.2 with HCl, and then 50 mL of PCP solution was
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42 109 pipette into a N₂-preflushed serum bottle. In addition, a highly concentrated stock of
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44 110 PCP (200 mM) in methanol was prepared and 250 µL (or 100 µL) of the stock was
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46 111 spiked into each serum bottle to obtain final PCP concentrations of 1185 µM for PAC
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48 112 (or 563 µM for GAC) in the bottles. Accurately weighed quantities of carbons were
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5 113 added to the bottles which were then sealed with butyl rubber stoppers and aluminum
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7 114 caps and placed on a rotary shaker at 150 rpm and 35 °C.
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10 115 **2.3. Medium**

11 116 The basal medium contained in 1 litre of Milli-Q water: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 4 mg;
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13 117 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mg; K_2HPO_4 , 11 mg; NaCl, 7 mg; trace element solution SL-10
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15 118 (DSMZ medium 320), 1 mL; resazurin, 0.5 mg; NaHCO_3 , 3000 mg; and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 50
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17 119 mg. The basal medium was boiled for 15 minutes and cooled on ice under a 20% CO_2 /
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19 120 80% N_2 or pure N_2 atmosphere. NaHCO_3 and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ were added after boiling and
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21 121 cooling. The acetic acid (Ace) medium was prepared by adding 2 g acetic acid to 1 L of
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23 122 the basal medium after boiling and cooling, and then adjusting the pH to 7.3 with 8 M
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25 123 NaOH.
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31 124 **2.4. Sludge inocula**

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33 125 Anaerobic digestion sludge that had been maintained in a batch reactor with
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35 126 synthetic wastewater and acetic acid (total COD ~ 1 g/L) was used for the study. Just
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37 127 before the assays the volatile suspended solids (VSS) and total suspended solids (TSS)
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39 128 of the sludge were determined. About 250 mL of sludge was centrifuged at 8,000 rpm
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41 129 for 5 min, the supernatant decanted, and the sludge then washed with 250 mL basal
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43 130 medium and resuspended in ~125 mL of basal medium. The sludge was then
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45 131 homogenized via passing it through a 22 gauge needle before use.
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51 132 **2.5. Anaerobic toxicity assay of PCP**

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53 133 The toxicity of PCP to anaerobic sludge was assessed via biogas production of the
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55 134 sludge fed with acetic acid and different concentrations of PCP using the method of
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57 135 Owen et al. (1979). The Ace medium was spiked with different volumes of 2 mM PCP
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(in 0.5 M NaOH) stock solution to result in PCP concentrations of 0.2, 2, 10, 20, 40 and 80 μ M (0.053, 0.53, 2.67, 5.33, 10.66 and 20.32 mg/L). The pH of the media was adjusted to ~ 7.3 with 8M NaOH, and then 45 mL of the medium and 5 mL of the homogenized sludge were pipetted into a 125 mL serum bottle which had been flushed with 20% CO₂ / 80% N₂ for 5 minutes. The bottles (in triplicate) were sealed with butyl rubber stoppers and aluminium caps and incubated at 35 °C and 150 rpm; the final VSS concentration in each bottle was around 1000 mg/L, and a control without PCP was also run. The cumulative biogas volumes were measured at specific intervals with a glass syringe at room temperature, and its composition was analyzed using gas chromatograph (GC).

2.6. Toxic shock recovery test with PACs (SAE2 and WP-AO)

The toxicity assay was also used for the toxicity recovery test. Three different doses of PAC: i.e., 5%, 20% and 80% (mass of PAC / VSS of sludge) were added to sludge with ~15 μ M PCP under different conditions: 1) pre-addition- PAC added 24 hours prior to the addition of PCP; 2) post-addition- PAC added 24 hours after the addition of PCP; and 3) simultaneous addition of PCP and PAC (sim-addition). The tests were carried out in 125 mL serum bottles in triplicate. Each bottle contained 2 g/L of acetic acid of Ace medium, 1 g VSS /L of sludge, 15 μ M of PCP and specific amounts of PAC. The PAC was added from a stock suspension which was prepared by vigorously mixing PAC in the basal medium with a vortex mixer. The total aqueous volume in each bottle was 25 mL, and the PCP concentrations were measured at specific intervals. The biogas production was measured daily with a glass syringe and the methane was analyzed at the end of the experiments.

2.7. Toxic shock recovery test with GAC (NRSC)

The experimental procedures were similar to the test with PAC except that the doses of GAC were increased to 20%, 80% and 320%, and the total aqueous volume in each bottle was increased to 100 mL.

2.8. Analytical methods

Biogas was analyzed using an Agilent 7890A GC equipped with dual TCD channels: one for the separation of O₂, N₂, CH₄ and CO₂ on a MolSieve 5A column using helium as a carrier gas; and the other for the analysis of H₂ on a MolSieve 13x column using argon as a carrier gas.

PCP was analyzed via reverse-phase HPLC (Shimadzu) featuring an Agilent Poroshell 120 EC-C18 (4.6×100 mm, 2.7 µm particle size) column and a PDA detector. The mobile phase consisted of A (0.1% acetic acid) and B (acetonitrile with 0.1% acetic acid added). Ten microlitre (10 µL) of sample was injected for analysis, and the limit of detection (LOD) was 0.2 µM for PCP; PCP concentrations lower than this were analyzed with a triple quadrupole MS equipped with a Z-spray electro-spray interface (LCMS-8030, Shimadzu, Japan). The separation was accomplished using a Synergi 4u Fusion-PR 80 A column (2.0 mm × 150 mm, particle size 4 µm, Phenomenex, Torrance, CA, USA). The Limit Of Detection (LOD) of this method was 0.001 µM (0.266 µg/L) for PCP. All water samples were centrifuged, diluted with an equal volume of methanol and then filtered through 0.45 µm PTFE syringe filters before analyzing on HPLC or LC/MS/MS.

2.9. Statistical analysis

Statistical analysis was performed using the software SigmaStat 3.0. A one-way ANOVA test was conducted to evaluate the statistically significant difference in PCP

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5 183 adsorption and biogas production at different PAC doses or addition times. A Holm-
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7 184 Sidak multiple comparison test was used in the post-hoc analysis to determine which
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9 185 groups were significantly different. In the case where only two groups were compared,
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11 186 an independent *t*-test was conducted to evaluate the statistically significant difference
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14 187 between the two groups.

188 3. Results and Discussion

189 3.1. Toxicity of PCP

190 The results of the toxicity test are shown in Figure 1; when the methanogenic
191 sludge was exposed to PCP at concentrations lower than 2 µM the biogas production
192 rates were almost the same as the controls. Statistical analysis also indicated there was
193 no significant difference ($p>0.05$) between them. Biogas production from the sludge fed
194 with 10 µM PCP was much slower, although after 18 days the volume had reached
195 maximum levels. There was a lag phase of 30 days before any gas production could be
196 measured with 20 µM PCP, and PCP concentrations greater than 40 µM completely
197 inhibited methanogenesis, even after 74 days.

198 From the data shown in Figure 1, the maximum rate ratio (MRR), i.e., the ratio of
199 biogas production within the first 5 days at different PCP concentrations relative to the
200 controls were calculated, and the MRR versus PCP concentration plotted. The curve
201 was best fitted ($R^2=0.8516$) to the equation $C = 0.6949MRR^{-1.938}$, where C is the PCP
202 concentration. From the plot, the concentration of PCP causing half biogas production
203 (IC_{50}) was 2.66 µM (0.71 mg/L). This value was at the lower end of the reported range
204 of 0.5-10 mg/L (Chen et al., 2008), which indicated that the sludge used in this study
205 was very vulnerable to PCP shocks. In the following test, a PCP concentration of ~ 15
206 µM was selected as the shock load.

3.2. Adsorption of PCP on activated carbon

The adsorption of PCP on activated carbon (Figure S1 in supplementary data) can be best fitted by the Freundlich isotherm equation $Q_e = K_f C_e^{1/n}$. From the curves the constants were calculated and listed in Table 2 together with some other published results for comparison. The constants for PAC determined in this study are quite similar to those reported by Hu et al. (1998), although the adsorption capacities of GACs in the literature are quite variable, so the results of the present study fall into the middle range of the reported constants.

According to the technical specification provided by the manufacturer, the GAC used in this test had a slightly higher surface area than SAE2. However, the adsorption capacity and kinetics of GAC for PCP were much lower than that of SAE2. Based on the adsorption isotherms, 2 PACs (SAE2 and WP-AO) and 1 GAC (NRSC) were selected for further recovery tests; the three carbons are highlighted in Table 2.

In order to investigate the effects of anaerobic sludge on the adsorption of PCP, an experiment was performed to compare the adsorption kinetics of PCP on SAE2 with and without anaerobic sludge. PCP adsorption onto sludge was minimal compared to PAC, however, sludge diminished the adsorptive capacity of PAC for PCP when mixed together (Figure S2 in the supplementary data). Simultaneous mixing had the least effect; when 50 mg/L PAC was added to the sludge (1 g/L VSS) 21 hours prior to the addition of PCP, the overall adsorption kinetics decreased, but the final equilibrium concentrations were close to those of post-addition. Compared to adsorption without sludge, PCP adsorption with sludge was reduced by 21.0%, 44.4%, and 54.1% for sim-, pre- and post-addition, respectively.

These results suggested that the sludge or extracellular polymeric substances (EPS) or soluble microbial products (SMPs) in the sludge occupied some of the adsorptive sites and interfered with the adsorption of PCP; this interference by EPS with the adsorption of phenolic compounds has been previously reported by Widjaja et al. (2004). They found that EPS in activated sludge greater than 50 kDa reduced the adsorption capacity of PAC for 3,5-dichlorophenol (3,5-DCP) by 66-83% depending on the type of PAC. In addition, the biofilm growing on the carbon surface was also found to reduce the adsorption capacity of PAC (Widjaja et al., 2004). In order to ensure that PCP was effectively adsorbed in the toxic shock control test, 5% of PAC was selected as the lowest dose.

3.3. Adsorption of PCP in anaerobic reactors by PACs (SAE2 and WP-AO)

Figure 2 shows the change in PCP concentrations over 48 hours after PAC addition. At the three PAC doses tested, PCP decreased immediately after the addition of PAC, and these decreases were especially significant when 20% or 80% PAC was added. Within 30 minutes of addition, the PCP concentrations dropped to ~0.5 μM or ~0.05 μM , at doses of 20% or 80% SAE2, respectively. Similar dramatic drops in PCP concentrations were also observed at the same dose of WP-AO, although the final PCP concentrations were slightly greater than those of SAE2. Statistical analysis indicated that, for both PACs, the adsorption of PCP reached equilibrium within 10 hours after PAC addition.

At the low dose of 5%, the addition time of PAC significantly affected PCP adsorption kinetics and equilibrium concentrations. The results shown in Figure 2 A) for SAE2 were similar to the previously observed results, with pre-addition displaying the least adsorption capacity, which resulted in the PCP concentrations not being

significantly different ($p>0.05$) from the ones without PAC within the first 2 hours. Pre-added WP-AO displayed similar adsorption kinetics, although there were no significant differences between the different addition times at a dose of 5% (Figure 2 D) as indicated by statistical analysis. At a dose of 20% (Figure 2 E), the significantly slower adsorption kinetics ($p<0.05$ for PCP concentrations) was still observed within the first 2 hours of PAC addition, although eventually no significant differences remained. When the WP-AO dose was increased to 80% (Figure 2 F), the addition time of PAC did not make a significant difference ($p>0.05$) to PCP adsorption kinetics and equilibrium concentrations, which were also observed when the SAE2 dose was increased to 20% (Figure 2 B) or 80% (Figure 2 C).

3.4. Toxicity control test with PACs (SAE2 and WP-AO)

Figure 3 shows the ratio of cumulative biogas production of the sludge exposed to 15 μ M of PCP at different doses of PAC. Without the addition of PAC, no significant biogas production was observed after 14 days. When both SAE2 and WP-AO at 20% or 80% were added simultaneously with PCP (Figure 3 A & D), biogas rates were almost the same as the controls for SAE2 ($p>0.05$) or very similar to controls for WP-AO ($p<0.05$), and there was no significant difference ($p>0.05$) in biogas production between 20% and 80% for the same PAC. While simultaneous addition of 5% SAE2 almost achieved the same biogas production rate as those of higher PAC doses ($p>0.05$), addition of 5% WP-AO only resulted in half the rate.

At doses of 20% and 80%, prior addition of both PACs (Figure 3 B & E) completely eliminated the toxic effects of PCP, and the biogas rates were the same as the controls ($p>0.05$). Pre-addition of 5% WP-AO resulted in a similar biogas production rate ($p>0.05$), although with high standard deviations (SDs). However, pre-

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5 278 addition of 5% SAE2 did not significantly improve biogas production, and this
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7 279 observation corresponded well with the highest equilibrium PCP concentrations shown
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9 280 in Figure 2 A.

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12 281 Biogas production recovered soon after the addition of 20% or 80% PACs (Figure
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14 282 3 C & F), even though the sludge had been exposed to PCP for 24 hours. However, the
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16 283 toxic effects of PCP were still evident by the slightly slow biogas production rates
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18 284 compared to the controls. Statistical analysis indicated that there were no significant
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20 285 differences ($p>0.05$) in the final biogas production between the doses of 20% and 80%
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22 286 for both PACs, although the biogas rate with 20% SAE2 was slightly greater than that
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24 287 with 80% SAE2. Post-addition of both PACs at 5% was also able to recover biogas
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26 288 production, but it was much slower, and only reached the maximum levels 8 days after
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28 289 the controls.

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33 290 Previously, in a semi-continuous reactor with an HRT of 3 days, 1g/L of PAC was
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35 291 shown to reduce the recovery time of an anaerobic process from 25 to 9 days (Wang and
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37 292 Han, 2012). However, the dose of PAC relative to VSS was not stated by the authors.
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39 293 The present study clearly shows that at 20% or 80%, both SAE2 and WP-AO can be
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41 294 used to eliminate the toxic effects of PCP to anaerobic sludge. However, the addition of
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43 295 5% PAC showed variable effects, which could be due to the heterogeneity of PAC
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45 296 and the sludge; at this low dose, PCP concentrations decreased slowly and a little
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47 297 difference in PAC and sludge would result in quite different PCP exposures, and thus
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49 298 variable gas production profiles. In all experiments with PCP and SAE2, the methane
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51 299 produced was 82.7 ± 8.7 % of the theoretical value ($395 \text{ mL CH}_4/\text{g COD}$ destroyed at 35
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53 300 $^{\circ}\text{C}$) when gas production ceased. This further confirmed that acetoclastic
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55 301 methanogenesis was not impaired by PCP or PAC.

3.5. Adsorption of PCP in anaerobic reactors by GAC (NRSC)

Figure 4 shows the change in PCP concentrations after GAC addition; adsorption of PCP on GAC was much slower compared to PAC. It took about 10 hours to reach equilibrium for PACs even at the low dose of 5%. In contrast, it took about 5 days for equilibrium with NRSC at the high dose of 320% (Figure 4 C), while at 20% it did not reach equilibrium even after 8 days. At 80% (Figure 4 B) PCP concentrations decreased to $< 2 \mu\text{M}$ after 5 days, while at the same dose this occurred within 2 hours for PAC. The PCP concentrations were sampled again on day 15, and the results (not shown here) suggested that adsorption was still continuing.

At the low dose of 20%, pre-addition or simultaneous addition of GAC did not make a significant difference ($p>0.05$) to the adsorption kinetics. However, at 80%, the simultaneous addition of GAC displayed the slowest kinetics; the pre- and post-addition of GAC had similar adsorption kinetics ($p>0.05$).

The rate controlling mechanism of PCP adsorption onto both PAC and GAC was intraparticle diffusion, which comprised pore volume diffusion - diffusion of an adsorbate inside the micro- and meso-pores of an adsorbent and surface diffusion – moving of an adsorbate molecule that had been adsorbed on the surface of an adsorbent from one adsorption site to another site (Leyva-Ramos and Geankoplis, 1994; Leyva-Ramos et al., 2009; Leyva-ramos et al., 2004). Surface diffusion is a temperature-dependent process, which depends on the interaction between the adsorbate and adsorbent molecules; it also increases with surface loading (Do, 1998). For some small molecules, e.g., phenol, the contribution of surface diffusion was high ($>82\%$), which indicated that these molecules diffused quickly in the pore volume (Ocampo-Pérez et al., 2013). However, for big molecules, like PCP, pore volume diffusion would be the rate

limiting step (Leyva-Ramos et al., 2009; Leyva-ramos et al., 2004). The pore volume diffusion rate is directly proportional to the effective pore volume diffusion coefficient, D_{ep} , and inversely proportional to the square of the particle radius (Leyva-Ramos et al., 2009; Leyva-ramos et al., 2004). The effective pore volume diffusion coefficient is defined as $D_{ep} = \frac{\varepsilon_p D_{AW}}{k}$, where ε_p is the void fraction in the carbon particles (porosity), D_{AW} is the molecular diffusion coefficient, and k is the tortuosity factor of the carbon, which is the square of carbon tortuosity τ_p , i.e., the ratio of average pore length to the length of porous medium (Epstein, 1989). Thus, the value of D_{ep} depends on the pore structure and size of the carbon, and also on the size and shape of the adsorbate molecules. The pore size and surface area of the three carbons measured with both a N₂ adsorption isotherm (Table S1 in the supplementary data) and mercury intrusion porosimetry (Table 3) were quite similar. However, the tortuosity factor of NRSC (235.26) was much greater than those of SAE2 (1.76) and WP-AO (1.86). Thus, the major difference in the adsorption rate between GAC and the PACs was due to the considerably greater tortuosity and larger particle size of the GAC.

3.6. Toxic shock control test with GAC (NRSC)

Figure 5 shows the ratio of cumulative biogas production to the controls when the sludge was exposed to 15 μ M PCP at different doses of GAC. Methanogenesis was completely inhibited by PCP during the first 6 days as indicated by statistical analysis ($p < 0.05$ from day 7), even when 80% GAC was added simultaneously with PCP (Figure 5A), but when 20% GAC was added simultaneously inhibition lasted for about 14 days. The significant differences ($p < 0.05$) in biogas production between 20% and 80% GAC were obvious after 15 days.

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5 349 Prior addition of GAC reduced the inhibitory effects of PCP to the lowest level
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7 350 (Figure 5 B), and there were no lag phases for the pre-addition of 20% or 80% GAC,
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9 351 although the gas production rates were slower than the controls. There was no delay
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11 352 with the 80% GAC reaching the maximum biogas volumes compared to the controls,
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13 353 but the total produced was only about 80% of the controls.
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17 354 Post-addition of GAC did not lead to biogas production recovery immediately.
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19 355 When 80% GAC was added 24 hours after PCP addition (Figure 5 C), inhibition of
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21 356 methanogenesis still lasted for 9 days as indicated by statistical analysis, while at the
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23 357 very high dose of 320%, inhibition still lasted for 7 days.
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27 358 It is clear in this case that the control biogas production was different from the PAC
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29 359 tests. Production did not reach a maximum until after 18 days of incubation, while it
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31 360 was 6 days for the tests with PAC. A different sludge source was used for the GAC test,
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33 361 and thus the low methanogenic activity of this sludge could be the reason why the gas
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35 362 production was slower than in the control.
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39 363 Previously, different carriers including GAC, porous glass, quartz sand, pumice
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41 364 and anthracite were tested in anaerobic fluidized-bed reactors for their ability to protect
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43 365 the biofilm from a toxic shock of 2,4,6-trichlorophenol (2,4,6-TCP) (Petrozzi et al.,
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45 366 1993). These authors observed that all these carriers could protect the biofilm from the
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47 367 toxic shock to some extent, however, in the completely inhibitory range, none of them
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49 368 could prevent the toxic shock. The authors also found that the recovery of the processes
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51 369 was not affected by the type of carriers. The results of the present study also suggest
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53 370 that GAC was not effective in controlling the toxicity of PCP due to its slow adsorption
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55 371 kinetics.
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3.7. Exposure-response model for the toxicity of PCP to methanogenic sludge

The different biogas production profiles between the addition of PAC and GAC were primarily due to the slow adsorption of PCP by GAC, which resulted in different time exposures of the biomass to PCP. The exposure-response relationship is widely used in toxicological assessment, but rarely used in the assessment of toxicity to biological processes. The toxicity of a chemical to biological processes is affected by the chemical and biomass concentrations, and exposure time; the commonly used IC_{50} does not provide information about either. In this study, an exposure-response model was developed for the toxicity of PCP to methanogenic processes.

The exposure of PCP (E , mg PCP-h/g VSS), i.e., the areas covered under the PCP concentration curves, in each PAC addition experiment were determined graphically from the plots of PCP change with time (for PAC) or from the integration of the fitted curves (for GAC). For the controls without activated carbon, the initial PCP concentrations were used as the constant exposure concentrations. In addition, the time it took for the biogas production to reach half of its maximal levels (T_{50}) was used to quantify the toxic effect of each different exposure experiment. The response or effect of the exposure was defined as $R = 1 - T_{50}^T / T_{50}^C$, where T_{50}^T and T_{50}^C were the T_{50} of the test (T) and control (C-no PCP) experiments, respectively. The T_{50} was also the time for determining the exposure. In experiments with PAC, the T_{50}^T could be higher than T_{50}^C , which indicated there were no toxic effects; in which case the R was set to zero. The exposure-response results were plotted in Figure 6, and the curve was best fitted ($R^2=0.87$) with the equation $R = \frac{0.948 E}{115.168 + E}$. This exposure-response curve can be used to estimate the recovery time when the methanogenic sludge was exposed to certain doses of PCP. It can also be used as a toxicity control or recovery guideline.

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5 396 The present study was carried out in serum bottles, however, this technique could
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7 397 easily be implemented in a full-scale anaerobic digestion (AD) system. When an AD
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9 398 system is experiencing a toxic shock of PCP, a specific amount of PAC could be added
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11 399 just before the toxic shock (depending on the warnings received, eg sensors) to
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13 400 eliminate the toxicity of PCP. Alternatively, when there are already indications of
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15 401 toxicity in a reactor (i.e. buildup of VFAs, decreasing gas production) PAC can be
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17 402 added immediately to the digester to ameliorate the shock. Finally, even when the toxic
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19 403 shock is not detected until one day later, PAC can still be added to recover anaerobic
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21 404 digestion. However, if the exposure to PCP is too long according to the exposure-
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23 405 response model, then it would be wiser to replace the sludge with a healthy seed than to
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25 406 wait for the sludge to recover.
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30 407 **4. Conclusions**

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33 408 PAC added at a low dose of 20% (mass ratio to VSS) to a methanogenic culture 24
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35 409 hours before, or simultaneously with, a shock load of PCP could effectively eliminate
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37 410 the toxic effects of PCP to acetoclastic methanogenesis as a preventative measure. At
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39 411 this dose it also enables methanogenesis to recover immediately when PAC was added
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41 412 24 hours after the toxic shock. GAC at a high dose of 80% could also reduce the toxic
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43 413 effects as a preventative measure. However, due to its slow adsorption, GAC was not
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45 414 effective in helping the sludge to recover from the toxic shock.
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13 421 **References**
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5 531 **Figure Captions**

6
7 532 Figure 1 Effects of PCP on biogas production of methanogenic sludge. The error bars
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9 533 represent one standard deviation of triplicate experimental results.

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11 534 Figure 2 PCP concentration change after addition of A) and D) 5%, B) and E) 20%, and
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13 535 C) and F) 80% of PAC (SAE2 on the left and WP-AO on the right). A control (No
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15 536 PAC) with just sludge and PCP was included. The error bars represent one
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17 537 standard deviation of triplicate measurements.

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19 538 Figure 3 Cumulative biogas production relative to controls for sludge incubated with 15
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21 539 μ M PCP at different doses of PAC (SAE2 on the left and WP-AO on the right)
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23 540 added A) and D) simultaneously with, B) and E) prior to, and C) and F) after the
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25 541 addition of PCP. The control had no PCP in it. The error bars represent one
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27 542 standard deviation of triplicate measurements.

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29 543 Figure 4 PCP concentration change after addition of A) 20%, B) 80% and C) 320% of
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31 544 GAC. A control (No GAC) with only sludge and PCP was included. The error bars
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33 545 represent one standard deviation of triplicate measurements.

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35 546 Figure 5 Cumulative biogas production relative to controls for sludge incubated with 15
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37 547 μ M PCP at different doses of GAC added A) simultaneously with, B) 24 hours
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39 548 prior to, and C) 24 hours after the addition of PCP. The error bars represent one
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41 549 standard deviation of triplicate measurements.

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43 550 Figure 6 Exposure-response relationship of methanogenic sludge exposed to PCP.
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Table 1 Specifications and characteristics of activated carbon

Carbon	B.E.T surface area (m ² /g)	Iodine number	Methylene blue adsorption (g/100 g)	Apparent density (kg/m ³)	Particle size	Remarks
Hydroadarco C (HYDR)	600	550	10	510	d ₅₀ = 25 µm d ₉₉ = 150 µm	PAC
SAE 2	925	850	12	450	d ₅₀ = 22 µm d ₉₇ = 150 µm	PAC
SAE Super (SAE-S)	1150	1050	24	425	d ₅₀ = 15 µm d ₉₇ = 150 µm	PAC
NRS EA 0.5-1.5 (NRSC)	950	850	N.A.	410	d ₉₈ = 0.5 mm	Reactivated GAC
Filtrisorb 300 D (F300D)	N.A.	Min. 900	N.A.	470	d ₄ = 0.6 mm d ₈₅ = 2.36 mm d _{eff} = 0.8-1.0 mm*	GAC
F300D React 180 (F300D-R)	N.A.	Min. 800	N.A.	600	d ₅ = 0.425 mm d ₈₅ = 2.36 mm	Reactivated GAC
WP-AO	N.A.	Min 800	N.A.	N.A.	d ₆₀₋₇₀ = 44 µm d ₈₀ = 75 µm	PAC

* d_{eff}, the effective size of activated carbon. N.A., not available.

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Table 2 Freundlich isotherm constants of activated carbons for PCP adsorption

Activated carbon	1/n	K_f	R^2	Carbon type	References
Hy-C	0.24	98.5	0.9839	PAC	This research
SAE2	0.19	245.6	0.9734	PAC	This research
SAES	0.18	231.6	0.9591	PAC	This research
WP-AO	0.29	181.8	0.9035	PAC	This research
NRS	0.24	95.6	0.7579	GAC	This research
F300D	0.48	89.2	0.9065	GAC	This research
F300DR	0.55	79.3	0.9811	GAC	This research
WAKO	0.313	309.0		PAC	(Hu et al., 1998)
R1	0.309	28.84		GAC	(Jung et al., 2001)
R2	0.372	26.00		GAC	(Jung et al., 2001)
R0.8	0.319	31.33		GAC	(Jung et al., 2001)
CG	0.469	14.12		GAC	(Jung et al., 2001)
F300	0.174	189.6		GAC	(Mollah and Robinson, 1996)

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7 **Table 3 Pore characteristics of selected activated carbon measured by mercury intrusion porosimetry**

	SAE2	WP-AO	NRSC
Average pore diameter (4V/A) (nm)	116.5	135.7	135.3
Porosity (%)	58.67	53.56	58.30
Total pore area (m ² /g)	56.376	37.017	44.347
Tortuosity factor	1.76	1.86	235.26

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Electronic Annex

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