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Predominance of char sorption over substrate concentration and soil pH in influencing biodegradation of benzonitrile

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Abstract

Incomplete combustion of field crop residues results in the production of char, a material rich in charcoal-type substances. Consequently, char is an effective adsorbent of organic compounds and when incorporated into soil may adsorb soil-applied pesticides, thereby altering their susceptibility to biodegradation. We investigated the relative importance of char, soil pH and initial substrate concentration in biodegradation of pesticides in soils by measuring the biodegradation of benzonitrile in soil as a function of soil char content (0% and 1% by weight), initial benzonitrile concentration (0.1, 1.06, and 10.2 mg l^{-1}) and soil pH (5.2, 6.9 and 8.5). Preliminary experiments revealed that wheat straw char had a much greater benzonitrile sorption capacity than did soil to which the char was added. The extent of benzonitrile degradation decreased as initial benzonitrile concentration increased in both buffer solution and soil slurry. In contrast, the degradation increased as initial benzonitrile concentration increased in char-amended slurry. In un-amended soil slurry, the benzonitrile degradation was lower at pH 5.2 than at pH 6.9 or 8.5, but in char-amended soil slurry the degradation was not affected by pH, again presumably due to adsorption of benzonitrile by the char. Adsorption by soil char appears to be more important than either initial substrate concentration or soil pH in controlling benzonitrile degradation in char-amended soil slurry. The presence of crop residue-derived chars may alter pesticide degradation patterns normally observed in soils and thus significantly affect their environmental fate.

Introduction

Biodegradation of pesticides in soils is a major process for attenuation of these common soil contaminants. The process is influenced by many factors, among which are soil pH, initial substrate (i.e. pesticide) concentration, and the extent to which the pesticide is sorbed by soils. There has been ample evidence that biodegradation varies greatly with these factors. It has been reported that two isolates for isoproturon degradation had a narrow pH optimum (7–7.5). The bacteria rapidly degraded the herbicide in sterile soil at pH 7.5, but no degradation occurred at pH 6.5 (Bending et al. 2003). Houot et al. (2000) observed that in soils with pH values higher than 6.5 (6.5–8.2), up to 80% of added atrazine was mineralized. When soil pH was lower than 6.0, $<25\%$ of added atrazine was degraded. The initial concentration of a contaminant also influences the biodegradation of that contaminant, with biodegradation occurring rapidly at some concentrations while being substantially inhibited at others (Rubin et al. 1982; Simkins & Alexander 1984; Wang et al. 1984). Contaminants existing in the sorbed phase may be partially available to microorganisms (Feng et al. 2000; Park et al. 2001, 2002), but it is generally acknowledged that contaminants existing in the

aqueous phase are more bioaccessible than those in sorbed phase (Guerin & Boyd 1992; Lahlou & Ortega-Calvo 1999; Ogram et al. 1985). Because aqueous-phase contaminant concentrations in soils are controlled largely by the extent to which contaminants are sorbed by the soils, sorption/desorption is likely to be the most important factor controlling biodegradation of contaminants in soils.

The composition and hence the sorptivity of soils are often altered by agricultural practices. A good example of this is the soil chars that result from field burning of vegetation for such purposes as disposal of post-harvest crop residues, weed control, immediate land clearing, and so forth. The incomplete combustion of organic materials, which is characteristic of such burns, produces a char that usually contains significant amounts of high adsorbing components such as charcoal and black carbon. When incorporated into an agricultural soil, these chars may greatly enhance the sorptive capacity of the soil for pesticides.

The adsorptive properties of soil chars were first proposed by Hilton and Yuen (1963) who reported that the sorption of substituted urea and *S*-triazine herbicides by Hawaiian sugarcane soils which had routinely been burned to remove cane trash remained high after removal of soil organic matter by hydrogen peroxide. Sorption of these herbicides was reduced markedly, however, after igniting the soils. The authors suggested that these observations could be explained by the existence of peroxide-resistant chars that may have been produced during the cane trash burns. Yang and Sheng (2003a) reported that, on a unit mass basis, the diuron adsorptive capacity of a char derived from burning of wheat residue was 400–2500 times greater than that of a soil containing 2.1% organic matter. They also reported that the diuron sorption by a char-amended soil increased with increasing char content. After aging in the soil for up to 12 months, the char remained highly effective in adsorbing diuron, indicating the refractory nature of the char (Yang & Sheng 2003b). Zhang et al. (2004) reported substantially less biodegradation of benzonitrile in char-amended soil than in soil not containing char and attributed this primarily to effective adsorption of benzonitrile by the char, which significantly reduced aqueous-phase concentrations of benzonitrile, and to the fact that desorption from the char was slower than from the soil.

Adsorption of pesticides by soil chars may result in biodegradation patterns differing substantially from those observed in soils not containing chars. Furthermore, the impact of chars may be modified by such environmental variables as soil pH and initial substrate concentration. The benzonitrile structural moiety is found in several widely used pesticides (Nawaz et al. 1991, 1992). Therefore, the objectives of this study were to evaluate the effects of substrate concentration and soil pH on benzonitrile degradation in both a char-amended soil and a control soil, and, in a broader context, to investigate the manner in which complex interactions among environmental variables affect pesticide degradation in agricultural soils containing crop residue-derived chars.

Materials and methods

Bacterium and sorbents

A benzonitrile-degrading bacterium previously isolated from an agricultural soil was used in the study. Details on its isolation, identification (*Nocardia* sp.), and characterization have been described in a previous study (Zhang et al. 2004). Its doubling time was 4.5 h. To prepare inocula for the biodegradation experiments, the stock culture of the bacterium was inoculated into 20 ml of a mineral salts medium supplemented with 1 ml of vitamin solution (Stanier et al. 1966; Wolin et al. 1963) and 0.02 ml of neat benzonitrile in a 150-ml Erlenmeyer flask. The flask was incubated at 28 °C for 19 h on a platform shaker rotating at 150 rpm. Two millilitre of the resulting suspension was then transferred into 40 ml of the benzonitrile-containing mineral salts medium and incubated for another 19 h as described above. Cells were harvested by centrifugation at 11,700 × *g* for 15 min, washed twice with sterile phosphate buffer solution (PBS, 0.02 M, pH 6.9) to remove residual benzonitrile, and re-suspended in the same PBS. The cell suspension was kept at room temperature (25 °C) and used within 3 h.

Three sorbents were used in the sorption and biodegradation studies: a soil, a wheat char, and the soil amended with the char (1% w:w). Details on the collection, preparation and characteristics

of the three sorbents have been described previously (Zhang et al. 2004). The soil (fine, smectitic, thermic Albaquultic Hapludalfs) had 2.1% organic matter, a mechanical composition of 17.1% sand, 60.4% silt and 22.5% clay, and a CEC of 8.5 cmol kg^{-1} with a pH of 6.1. Air-dried soil was sieved through a 1-mm sieve. The soil had no record of crop residue burns and was presumed to contain little or no crop residue-derived char. The char, produced by burning dry wheat straw on a stainless steel plate in an open field under natural conditions, had an elemental C content of ~13% and a pH of 10.1 in suspension of 1:5 (char:H_2O). The 1% char-amended soil was prepared by thoroughly mixing the char and the soil at a ratio of 1:99 (w:w). Prior to use, the sorbents were sterilized by γ-irradiation (5 Mrad from a ^{60}Co source). In some experiments, the pH values of sorbent slurries were adjusted to neutral (pH = 6.9) with PBS solution. In other experiments, the pH values of the soil and wheat char in 1:1 (solid:liquid) suspensions were adjusted to 5.2, 6.9, and 8.5 with 1 M H_3PO_4 or KOH. The solids were then dried in an oven at 40 °C, ground, sieved (1-mm), and sterilized. The 1% char-amended soil was prepared by mixing the appropriate proportions of soil and char that had previously been adjusted to the same pH.

Biodegradation

To measure the effect of initial substrate concentration on biodegradation, soil (2.803 g, pH 6.9) and char-amended soil (2.831 g, pH 6.9) were equilibrated with PBS (pH 6.9) containing benzonitrile at 0.10, 1.06, or 10.2 mg l^{-1} in Teflon-sealed, 30-ml glass centrifuge tubes at room temperature for 24 h. A benzonitrile solution similarly prepared but containing no sorbent served as the control. Following equilibration, each tube was inoculated with 0.1 ml of the bacterial cell suspension (cell density $\approx 3.6 \times 10^8$ CFU ml^{-1}) and incubated at room temperature. Un-inoculated sorbent slurries and controls (no sorbent) were also prepared in order to determine recoveries and abiotic losses of benzonitrile during the incubation period. At various time intervals between 10 and 240 min, the degradation was stopped by adding 0.2 ml of 0.5% Ag_2SO_4 solution. Immediate termination of benzonitrile degradation by the Ag_2SO_4 solution was verified previously in our laboratory. After centrifugation, a 1.0 ml aliquot of supernatant was taken for analyzing benzonitrile and the remaining supernatant (5.8 ml) was removed. To extract sorbed benzonitrile, the centrifuge tubes were added 10 ml of acetonitrile, rotated for over 12 h, and centrifuged. Supernatants were analyzed for benzonitrile concentrations. Extraction recoveries were 85%, 98% and 95% for soil, char and char-amended soil, respectively. Benzonitrile concentrations were adjusted for these recoveries. Abiotic degradation of benzonitrile during the incubation period was found negligible. All experiments were conducted in duplicate and performed at room temperature. The percent of benzonitrile degraded was calculated from the difference between the initial amount of benzonitrile and that remaining at the time of sampling (the amount in solution plus that sorbed) divided by the initial amount.

To evaluate the effect of pH on biodegradation, experiments were conducted using the same procedures described above, except that the sampling period was extended to up to 660 min and the slurries had pH values of 5.2, 6.9, or 8.5. Each sorbent slurry contained 2.803 g of soil, 0.028 g of char, or 2.831 g of char-amended soil and 8 ml of 1.06 mg l^{-1} benzonitrile solution in PBS having the same pH as that of the sorbent.

Benzonitrile in supernatants and extracts was analyzed by HPLC using a Phenomenex Prodigy C18 column and a Hitachi model L-7450A diode array detector at the UV wavelength of 225 nm (Hitachi, Inc.). The mobile phase was 45:55 CH_3CN:H_2O (v:v) at a flow of 1.75 ml min^{-1}. The sample injection volume was 100 μl. The detection limit for benzonitrile was ~0.5 μg l^{-1}.

Results and discussion

Our previous measurements showed that initial benzonitrile concentration of 1.06 mg l^{-1} was reduced to 0.63, 3.5×10^{-3}, and 3.7×10^{-2} mg l^{-1} by soil, wheat char, and 1% char-amended soil, respectively, after an equilibration period of 24 h. The soil alone sorbed 40.5% of the total benzonitrile, while the char and char-amended soil sorbed >99% and 96.5% of the total benzonitrile, respectively. If it is assumed that the presence of wheat char did not change the tendency of the soil itself to sorb benzonitrile (Zhang et al. 2004),

approximately 90% of the benzonitrile sorbed by the 1% char-amended soil was sorbed by the char and only 10% was sorbed by the soil. The presence of 1% wheat char in the soil not only substantially increased the extent of sorption by the whole soil, resulting in a very low benzonitrile concentration in the solution, but was also the dominant sorbent phase in this mixed-sorbent system.

Substantial reductions in aqueous-phase benzonitrile concentrations in the presence of char would be expected to reduce the biodegradation of benzonitrile, because only the aqueous-phase benzonitrile is readily available to microorganisms. At pH 6.9 and with an initial concentration of 1.06 mg l^{-1}, benzonitrile was completely degraded in soil within 360 min (Figure 1). The degradation in the char and char-amended soil slurries was similar to each other but much slower than that in the soil slurry. After 660 min, only 34% and 40% of the benzonitrile had been degraded in the char slurry and char-amended soil slurry, respectively. Larger degradation occurred in the sorbent slurries with higher initial concentrations of benzonitrile. Reduced biodegradation of benzonitrile in the presence of wheat residue-derived char in soil was likely due to the fact that benzonitrile is readily adsorbed by the char (Zhang et al. 2004).

Figure 2 shows the biodegradation of benzonitrile at pH 6.9 in PBS, soil, and 1% char-amended soil at initial concentrations of 0.10, 1.06, and 10.2 mg l^{-1}. The fact that both the soil and char-amended soil slurries contained the same amount of soil made it possible to make direct comparisons of biodegradation in these two systems.

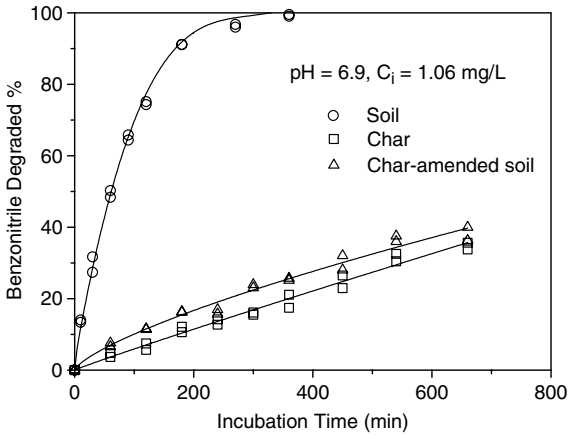

Figure 1. Biodegradation of benzonitrile in slurries of soil, char, and 1% char-amended soil at pH 6.9 and the initial benzonitrile concentration (C_i) of 1.06 mg l^{-1}.

Biodegradation of benzonitrile in PBS served as one type of standard in which influence of sorption was not present. Benzonitrile was readily degraded in PBS; the extent of degradation followed the decreasing order of 0.10 > 1.06 > 10.2 mg l^{-1} (Figure 2a). Higher degradation of pesticides at lower initial concentrations than at higher ones has been reported in the literature (Scow et al. 1986; Simkins & Alexander 1984; Wang et al. 1984). The presence of the soil clearly reduced the degradation of benzonitrile relative to that in PBS, presumably due to sorption by the soil. In the soil slurry, 85%, 62%, and 35% of benzonitrile were degraded after 240 min for initial concentrations of 0.10, 1.06, and 10.2 mg l^{-1}, respectively (Figure 2b). This corresponded to reductions in biodegradation of >44%, 38%, and 30%, respectively, as compared with degradation in PBS. The reduction in degradation decreased (from >44% to 30%) with increasing initial concentration of benzonitrile from 0.10 to 10.2 mg l^{-1}, indicating that sorption to soil reduced the difference in the extent of degradation as affected by initial concentration. However, sorption to soil did not alter the concentration-dependent degradation pattern. That is, the extent of degradation in the soil slurry remained to decrease with increasing initial benzonitrile concentration.

Biodegradation of benzonitrile in char-amended soil proceeded less extensively than in soil without added char, indicating the effect of benzonitrile adsorption to the char (Figure 2c). In contrast to degradation in the un-amended soil slurry, degradation in char-amended soil slurry at an initial concentration of 0.10 mg l^{-1} was not detected; benzonitrile was never detected in the aqueous phase and sorbed phase (i.e., acetonitrile-extractable) benzonitrile concentrations remained constant throughout the entire 240-min incubation period. At an initial benzonitrile concentration of 1.06 mg l^{-1}, only 12% of the benzonitrile had been degraded after 240 min, while the corresponding value for an initial concentration of 10.2 mg l^{-1} was 30%. Assuming that addition of char to soil did not alter the benzonitrile-sorbing properties of the soil, adsorption of benzonitrile by the char reduced degradation by 85%, 50%, and 5% compared to degradation in un-amended soil at initial

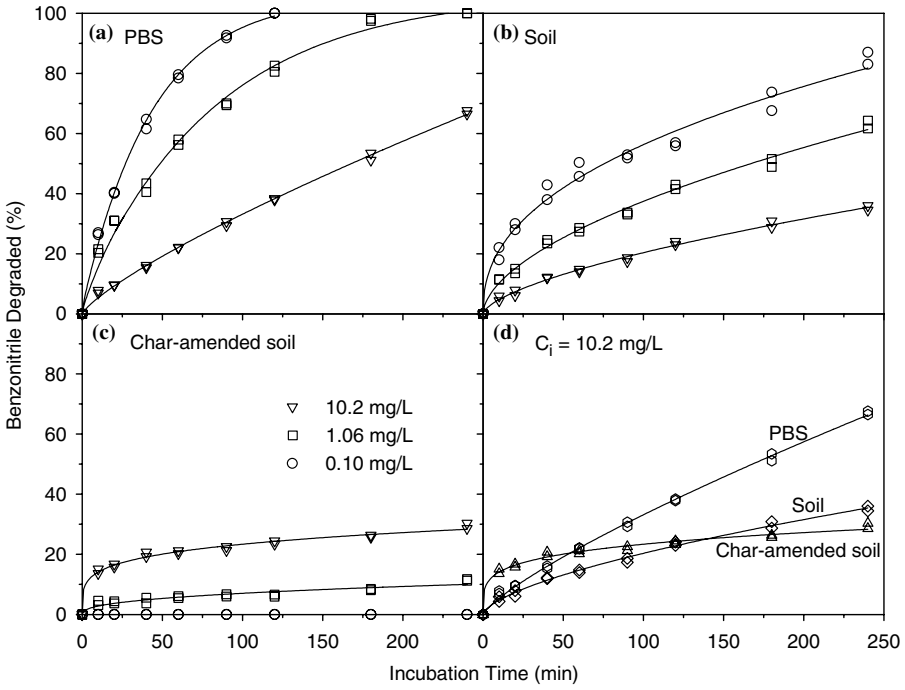

Figure 2. Biodegradation of benzonitrile as influenced by initial benzonitrile concentration (C_i) at pH 6.9 in (a) phosphate buffer solution (PBS), (b) soil slurry, and (c) 1% char-amended-soil slurry. (d) Comparison of degradation among PBS, soil slurry and char-amended soil slurry at C_i of 10.2 mg l^{-1}. Legends are the same for the same C_i in all panels except that they are filled with dots in Panel (B) and with + in Panel (C).

concentrations of 0.10, 1.06, and 10.2 mg l^{-1}, respectively. These results indicate that in the presence of char the higher the initial concentration of benzonitrile was, the lower the reduction in benzonitrile degradation was. The differential reductions, due apparently to the predominant char sorption of benzonitrile, resulted in a reversal in the concentration-dependent degradation pattern in the char-amended soil slurry as compared to PBS and the soil slurry. In the char-amended soil slurry, the extent of degradation increased with increasing initial benzonitrile concentration. These results suggest that highly effective adsorption to char had a much larger influence on the degradation at lower concentrations than at higher concentrations.

Examination of Figure 2d shows that when the initial benzonitrile concentration was 10.2 mg l^{-1}, more benzonitrile was degraded in the char-amended soil slurry than in the un-amended soil slurry during the first 130 min of incubation. Student's *t*-test at the 95% level of confidence indicated that the degradation in the char-amended soil slurry was statistically higher than that in the un-amended soil slurry during the first 90 min. This was not the case when the initial concentration was 1.06 or 0.10 mg l^{-1}. Degradation in the char-amended soil slurry at this concentration (10.2 mg l^{-1}) was even statistically greater than that in PBS during the first 40 min of incubation. Elemental analysis of the char indicated a composition of 14.3% carbon, 0.64% nitrogen, 1.46% phosphorous, 21.0% potassium, 3.36% calcium, 0.89% magnesium, 0.63% sulfur, along with small quantities of other elements (sodium, iron, manganese, zinc, copper, boron, and aluminum). When in their available (soluble) forms, most of these elements are essential for bacterial growth. Further analysis showed that the concentrations of some major elements (e.g. P and K) in the extract of the char-amended soil were much higher than those in the extract of the un-amended soil. However, the cell density was not expected to increase significantly because the time period of observed biodegradation enhancement was much shorter than the doubling time of the bacterium. We also determined the Michaelis–Menton constant of this benzonitrile-degrading organism to be

0.26 mg l^{-1}. The value of this constant was much smaller than the measured equilibrium concentration of benzonitrile (2.90 mg l^{-1}) that was produced when the initial benzonitrile concentration was 10.2 mg l^{-1}, indicating that the substrate availability was not a limiting factor. It is thus speculated that the char supplied essential nutrients that stimulated the activity of the benzonitrile-degrading bacteria at sufficiently high benzonitrile concentrations. The stimulatory effects of nutrients on biodegradation have been reported (Swindoll et al. 1988; Wang et al. 1984; Weir et al. 1995).

While no benzonitrile was detected in solution at any time during incubation of the char-amended soil with solution having an initial benzonitrile concentration of 0.10 mg l^{-1}, an undetectably small quantity of benzonitrile presumably did exist in the aqueous phase. Lack of benzonitrile biodegradation in char-amended soil for initial benzonitrile concentration of 0.10 mg l^{-1} indicates that the benzonitrile-degrading bacteria were not functioning at such a low aqueous-phase concentration. It has been reported that a *Pseudomonas* sp. strain rapidly mineralized 18 ng of glucose per ml but exhibited little activity on 18 pg ml^{-1} (Boethling & Alexander 1979). Threshold concentrations below which there is little degradation for some organic compounds have been reported (Boethling & Alexander 1979; Rubin et al. 1982). We were unable to obtain the threshold concentration for benzonitrile. However, our results suggest that an initial concentration of 0.10 mg l^{-1} in the char-amended soil slurry resulted in an aqueous-phase benzonitrile concentration lower than the threshold. As many pesticides are present at very low concentrations in soils, the presence of chars may result in aqueous-phase concentrations that are below biodegradation threshold levels, which would effectively inhibit the degradation of these pesticides in soil.

Sorption of benzonitrile by the un-amended soil, the char, and the char-amended soil was not affected by pH (data not shown). This was probably due to the fact that the benzonitrile molecule is not charged. The biodegradation of benzonitrile in the un-amended soil, the char, and the char-amended soil at three pH values is shown in Figure 3. As before, use of the same quantity of soil in both the un-amended soil and char-amended soil slurries, and of the same quantity of char in both the char and char-amended soil slurries allowed direct comparison of degradation among the three sorbents. In the un-amended soil slurry, degradation proceeded much more slowly at pH 5.2 than at either 6.9 or 8.5, suggesting that neutral to moderately alkaline conditions favored degradation. The degradation at these two higher pH values appeared to follow first-order kinetics. On the other hand, degradation at pH 5.2 seemed to follow zero-order kinetics. After 180 min, aqueous-phase benzonitrile concentrations in the soil slurries at pH values 5.2, 6.9 and 8.5 were 0.32, 0.04 and 0.06 mg l^{-1}, respectively, but after 400 min the benzonitrile concentrations were approximately the same. Thus, in un-amended soil

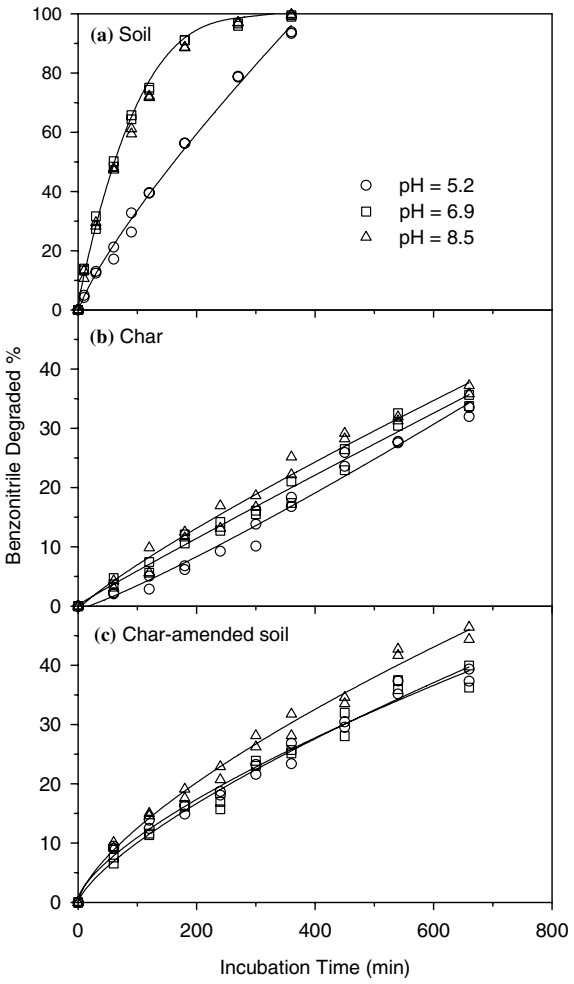

Figure 3. Biodegradation of benzonitrile as influenced by pH at the initial benzonitrile concentration of 1.06 mg l^{-1} in (a) soil slurry, (b) char slurry, and (c) 1% char-amended soil slurry. Legends are the same for all panels.

slurries pH affected the rate of benzonitrile degradation, but not the final proportion of benzonitrile degraded.

There was little or no effect of pH on benzonitrile degradation in the char slurries (Figure 3b). From Student's *t*-test, the difference in degradation between the three pH values was not statistically significant. This apparently was a result of the fact that large amounts of benzonitrile were strongly sorbed by the char at all pH values studied. Thus, aqueous-phase concentrations of benzonitrile were low at all pH values. The effect of pH on benzonitrile degradation in the char-amended soil slurries was similar to that observed in the char slurries, i.e., pH had little or no effect (Figure 3c). This result was again supported by Student's *t*-test that the difference in degradation between the three pH values was not significant during the first 500 min. Degradation in both the char and char-amended soil slurries proceeded more slowly than in the un-amended soil slurries at all three pH values. Benzonitrile desorption from char is known to proceed at a slower rate than from soil (Zhang et al. 2004), and the data presented here suggest that this may be true over a wide range of pH. In general, sorption by crop residue-derived char appeared to exert greater control over benzonitrile degradation rates than did soil pH.

Concave-downward patterns between degradation kinetics and soil pH are usually observed with neutral to weakly alkaline conditions favorable for the degradation by bacteria (Leahy & Colwell 1990; Rutgers et al. 1998). We simply measured the degradation of benzonitrile at three pHs within the normal soil pH range, and did not provide information on the optimal pH for the degradation of benzonitrile. Nawaz et al. (1991, 1992) reported that two hydrolytic enzymes, nitrile hydratase and amidase, were responsible for the sequential metabolism of acrylonitrile by microorganisms. A pH of 8.0 favored maximum activities of the enzymes, and the enzyme activities sharply declined with pH values above and below 8.0. Such a pH-dependence was also reported for the degradation of other pesticides (e.g., Bending et al. 2003). The optimal pH values usually observed are between the two pH values used in this study. Nevertheless, it is important to note that the presence of the char diminished the pH effect on benzonitrile degradation.

Conclusions

Addition of wheat residue-derived char to a soil substantially reduced the extent of benzonitrile biodegradation relative to the un-amended soil, presumably due to effective adsorption of benzonitrile by the char. The percent of benzonitrile degraded in PBS at pH 6.9 after 240 min decreased from 100% to 65% with increasing initial benzonitrile concentration from 0.10 to 10.2 mg l^{-1}. In soil slurries, degradation was reduced by 30–44% compared to that in PBS, with the extent of the reduction decreasing as initial benzonitrile concentration increased from 0.10 to 10.2 mg l^{-1}. These reductions were attributed to sorption of benzonitrile by the soil. Sorption of benzonitrile by char caused benzonitrile degradation in char-amended soil to be lower than that in un-amended soil at initial benzonitrile concentrations of 0.10 and 1.06 mg l^{-1}, but at the highest initial benzonitrile concentration of 10.2 mg l^{-1} degradation was initially higher in char-amended soil than in un-amended soil. This may have been due to increased microbial activity resulting from addition of essential nutrients contained in the char. Benzonitrile degradation in un-amended soil was more rapid at pH values of 6.9 and 8.5 than at pH 5.2, but pH had little effect on degradation in char and char-amended soil, presumably due to high-affinity sorption of benzonitrile by char that did not vary with pH. Compared to initial substrate concentration and soil pH, the presence of char played a dominant role in controlling benzonitrile degradation in soil. This study implies that crop residue burns, a common post-harvest agricultural practice, may significantly alter the pesticide biodegradation processes in soil. Bioremediation technologies for pesticide contaminated soils must be thoroughly evaluated to reduce possible adverse effects that crop residue burns may give rise to. Alternative crop residue management may also be considered to mitigate the pesticide contamination and to maintain the quality of agricultural soils.

Acknowledgements

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