OCCURRENCE, DISTRIBUTION, AND FATE OF 4-NONYLPHENOL IN KANSAS DOMESTIC WASTEWATER TREATMENT PLANTS

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ABSTRACT

4-nonylphenol (4-NP), an endocrine disruptor, is one of the major metabolites of nonylphenol polyethoxylates (NPnEOs), the most commonly used non-ionic surfactants that are often discharged into wastewater treatment plants. Our investigation in three Northeastern Kansas wastewater treatment plants has shown that the concentrations of 4-NP in influent ranged from 3.39 to 169 : g/L, while effluent concentrations remained at lower levels. A significant portion of 4-NP was not degraded during the treatment processes but instead was mass transferred to the dewatered biosolids. The concentration of 4-NP in dewatered biosolids reached as high as 300 mg/kg on dry weight basis in one plant. Approximately 60 percent of the 4-NP in the biosolids was further degraded during biosolids composting process. An average dry weight basis concentration of 130 mg/kg for 4-NP was found in biosolids compost product that was eventually disposed of through land application. Given that about seven million tons of biosolids are produced annually in wastewater treatment plants in the United States, several thousand tons of 4-NP could be potentially released to the soil environment through land application of biosolids. Biosolids land application is becoming the most common means of biosolids disposal as other disposal options become cost prohibitive or heavily regulated. The 4-NP released into the soil environment can potentially enter the water environment due to runoff and leaching. Future research on the transport and transformation of 4-NP in biosolids-amended soil and its impact on surface and ground water quality are needed.

INTRODUCTION

Alkylphenol polyethoxylates (APnEOs) represent an important class of non-ionic surfactants that have been widely used as detergents, emulsifiers, wetting agents, and dispersing agents in industrial cleaning products and industrial process aids (Thiele et al., 1997). Each year more than 200,000 tons of APnEOs, almost half of the global annual production, are produced in the United States (U.S. International Trade Commission, 1995). 4-nonylphenol (4-NP), an endocrine disruptor, is one of the major metabolites of APnEO surfactants. The majority of APnEOs is used in aqueous solutions and eventually is discharged through sewer systems into wastewater treatment plants (WWTPs).

Under anaerobic conditions APnEOs can go through step-wise de-ethoxylation, producing intermediate metabolites consisting of APnEO compounds with lower number of ethoxylate groups and alkylphenol mono- and di-ethoxylates (AP1EO and AP2EO) (Maguire, 1999). Under aerobic conditions, both carboxylation and step-wise de-ethoxylation of ethoxy chain of APnEO parent compounds occur, producing alkylphenol mono- and di-ethoxy carboxylic acids (AP1EC and AP2EC) (Ahel, et al., 1994; Maguire, 1999; Di Corcia et al., 2000). Jonkers and coworkers (2001) recently observed concomitant oxidation of the nonyl chain with carboxylation and de-ethoxylation of the ethoxy chain during aerobic biodegradation of NPnEO parent compounds, leading to metabolites having both a carboxylated ethoxylate and an alkyl chain of varying lengths with a carboxyl functional group. It is believed that the intermediate anaerobic and

aerobic metabolites of APnEO parent compounds can be further anaerobically degraded into alkylphenol such as 4-NP and 4-octylphenol (4-OP); however, complete degradation of those metabolites is believed to be difficult (Maguire, 1999).

Research by Ahel and coworkers (1994) has shown that at least 60-65 percent of APnEOs introduced to 11 Swiss WWTPs were discharged into the environment through effluent. They found that about 60 percent of the discharged compounds were NP1EO and NP2EO, while only a minor portion of 4-NP reached receiving waters directly via effluents. The majority of 4-NP was found to be sludge-bound. Investigations mostly conducted in Europe have reported a wide range of concentrations of metabolites of APnEOs in various environmental matrixes, ranging from nondetectable levels to several thousand Fg L^{-1} in WWTP effluents and surface waters and from several mg kg^{-1} up to several thousand mg kg^{-1} in biosolids (Bennie, 1999). Up to several Fg L^{-1} APnEOs metabolites were detected in ground water and even in drinking water. Since APnEOs and their metabolites are not naturally occurring compounds, their widespread presence in many environmental matrixes is believed to be due to domestic and industrial waste discharges (Bennie, 1999; Davi & Gnudi, 1999).

Compared to their parent compounds, degradation products of APnEOs are more toxic and estrogenic. There is an increase in the toxicity of APnEOs with decreasing ethoxy chain length (Yoshimura, 1986; Hall et al., 1989). Research has shown that APnEOs and their metabolites are acutely toxic to fish (LC_{50} = 17 - 3000 Fg L^{-1}), invertebrates (LC_{50} = 20 - 3000 Fg L^{-1}), and algae (LC_{50} = 27 - 2500 Fg L^{-1}) (Servos, 1999). This class of compounds can also bind to estrogen receptors (Routledge & Sumpter, 1996; Cooney, 2000) resulting in the expression of several responses both in vitro and in vivo, including the induction of vitellogenin, a biomarker for estrogen exposure (Tyler et al., 1996). Solé and coworkers (2000) observed a positive correlation between the amount of NP detected in surface waters and vitellogenin induction in male carp. Gray and Metcalf's research (1997) showed that after being exposed to 50 Fg L^{-1} 4-NP for three months, 50 percent of the male Japanese Medaka (*Oryzias Latipes*) fish developed ova-testes. Elevated concentrations of NPnEOs and metabolites, especially 4-NP, in surface water are strongly believed to contribute to the observed widespread sexual disruption in wild populations of river fish throughout the United Kingdom, especially in the rivers that receive large amount of discharges from WWTPs (Jobling et al., 1998).

Although intensive investigations have been conducted in Canada and several European countries (Ahel et al., 1994; Paxéus, 1996; Bennie et al., 1998; Bennie, 1999; Di Corcia et al., 1994 and 2000), only few U. S. reports have documented the occurrence of 4-NP in influents, effluents, and digested sludge of U.S domestic and industrial WWTPs (Naylor et al., 1992; Chalaux et al., 1994; Field and Reed, 1996). The reported concentrations of 4-NP in inflent, efflent, and digested sludges of the investigated U.S. WWTPs range from nondetectable to 13 mg L^{-1}, 2 mg L^{-1}, and 400 mg kg^{-1}, respectively. None of the U.S research has reported comprehensive study on the occurrence, distribution, and fate of 4-NP during treatment processes in WWTPs.

The objectives of this research were to investigate the occurrence and distribution of 4-NP in three Northeast Kansas WWTPs that use different wastewater treatment methods, and to understand its transformation during the treatment processes.

METHODS AND MATERIALS

Selected Wastewater Treatment Plants

The three wastewater treatment plants selected for this study are located along the Kansas River in Kansas. Plant 1 is a 0.75 MGD facility serving a population of 3,700 with no major industries. Plant 2 is a 5.5 MGD facility that serves a town of 50,000 with a few small industries, and Plant 3 is a 12 MGD facility serving a city of 150,000 with several medium scale industries. All three facilities are operated as activated sludge systems. Plant 3 was designed for carbon removal only; Plant 2 is operated for carbon removal and nitrification; and the operation of Plant 1 includes nitrification and denitrification (Figure 1). Effluents from those three plants are discharged into the Kansas River.

Sample Collection Protocols

Grab samples of wastewater and/or mixed liquor were collected in the summer of 2000 at various points within each WWTP (Figure 1) in 1-L amber jars, immediately transported to the lab in coolers packed with ice, filtered with Whatman #41 paper, and stored at 4°C until extraction. For preservation, each filtered water sample was mixed with ~ 4 mL chloroform in order to retard microbial activities. Twenty-liter glass jars were used to collect grab samples of sludge from the aerators, return sludge lines, and the digesters. The sludge samples were then transported immediately back to the lab where excess water was removed via centrifugation. The sludge sediment samples were then frozen at -10°C until analysis.

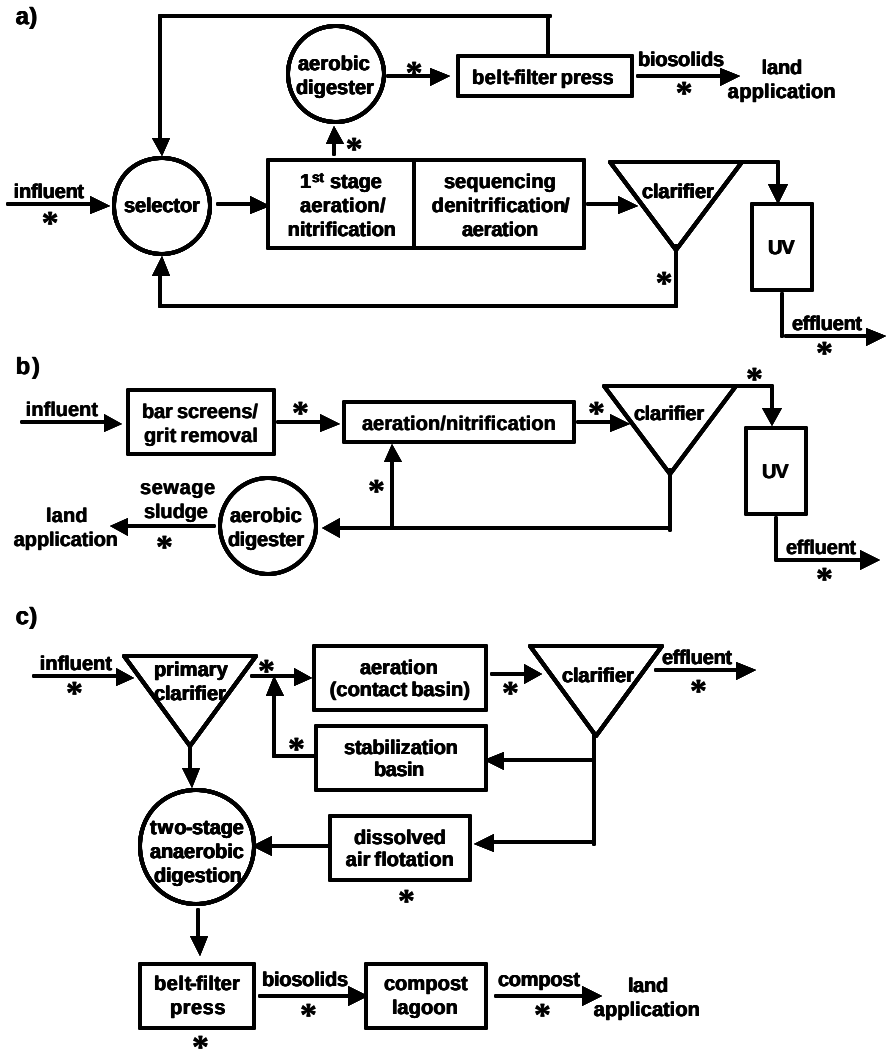

Figure 1. Process schematic of (a) Plant 1 (0.75 MGD), (b) Plant 2 (5.5 MGD), and (c) Plant 3 (12 MGD). Asterisk (*) indicates sampling locations.

Sample Extraction and Cleanup

The solid phase extraction method previously reported by Lee (1999) was modified slightly for the determination of 4NP in wastewater. Samples were extracted in duplicate with quality control samples and procedural blanks. Commercial C-18 columns (300 mg) were used in conjunction with a J & W Scientific vacuum manifold (Folsom, CA) for the wastewater extractions. Prior to acidifying the 200 mL wastewater samples with HCl (pH < 2), 100 Fg of 4-*t*-butylphenol (sublimed, Sigma Chemical, St. Louis, MO) were added as surrogate. The C-18 columns were preconditioned via gravity flow using sequentially 2 mL acetonitrile, 2 mL methanol, and 2 mL deionized water. Samples were pulled entirely through the columns under vacuum (?10 mL/min). The columns were then dried under vacuum (-380 mm Hg) for five min. The 4-NP was eluted from the column with 2 mL methanol. A quantity of 125 Fg 2,4,6-tribromophenol (Sigma Chemical, St. Louis, MO), as internal standard, was then added to the eluant. Finally, the eluant was evaporated to dryness under N_2 (60°C), redissolved in 500 FL methanol, and stored at -10°C until HPLC analysis. Recovery for 4-NP determined from fortified water samples was 83 ± 12 percent.

Sludge sediment samples were extracted using a soxhlet procedure similar to the one reported by Marcomini et al. (1990). Twenty grams (wet weight) of sludge were extracted for 8 hr with 130 mL hexane. The hexane extracts were then concentrated to ~ 5 mL under a gentle stream of N_2 (60°C) and cleaned up by passing through an aminosilica column pre-wetted with 5 mL

hexane. The 4-NP was eluted from the column with 5 mL hexane-acetone (75:25), evaporated to dryness under a gentle stream of N_2 (60°C), redissolved in 500 µL methanol, and stored at -10°C until HPLC analysis.

Analysis of 4-NP by HPLC and GC-MS

4-NP was analyzed via reverse phase HPLC. Following reverse phase HPLC analysis, the presence of 4-NP was confirmed using GC-MSD. 4-NP purchased from Sigma Chemical (St. Louis, MO) was used as standard. A Hewlett-Packard 1050 HPLC containing a quantinary pump, diode array detector (DAD), and a fluorescence detector (FLD) were used for sample analysis. Injections (5 µL) passed through a 25 µL sample loop and the column was kept at 40°C. The DAD was operated under the following conditions: signal = 277 nm, bandwidth = 40 nm, and reference = 350 nm. Data was collected from the FLD at an excitation λ = 230 nm, emission λ = 301 nm, and pmtgain = 6 or 12. A 124 x 4 mm LiChropher 100-RP-18e (5µm) column (Agilent Technologies, Santa Clarita, CA) was used for the reverse phase HPLC. A methanol: water mixture (8:2) was used as the mobile phase at a flow rate of 1.5 mL/min. The instrument detection limit for 4-NP was 0.09 ng/µL. The method quantification limit for 4-NP was 0.64 µg L^{-1}.

A Hewlett-Packard 6890 Series GC-MSD was used to confirm the presence of 4-NP in the sludge and wastewater extracts. The GC-MSD utilized a 5972 model quadrupole mass selective detector and was operated in the electron ionization mode using helium as the carrier gas (12.9 psi; 1.1 mL/min). A 30 m x 0.25 mm x 0.25 µm HP-5MS (Hewlett-Packard, Santa Clarita, CA) was used under the following conditions (De Voogt et al., 1997). The initial column temperature (100°C) was held for 0.5 min prior to a 10°C/min increase to 320°C that was maintained for five min. Injections (1 µL) were in the splitless mode with the following temperatures: injector 200°C and interface line 250°C. Both published spectra (Stephanou & Giger, 1982) and the 4-NP standard were used in the confirmation of 4-NP.

RESULTS AND DISCUSSION

Figure 2 illustrates the concentrations of 4-NP in wastewater samples collected at various points within each wastewater treatment plant in the summer of 2000. Up to 169 µg L^{-1} of 4-NP was detected in the influent sample of Plant 3, a 12 MGD plant located in a medium-sized city that houses several medium-scale industries. Low levels of 4-NP also appeared at 5.94 µg L^{-1} and 3.39 µg L^{-1} in the influents of Plant 1 and Plant 2, respectively. Different from Plant 3, high levels of NPnEOs input to Plants 1 and 2 are not expected due to the less association of these plants with industries, a major source for NPnEOs. Nonionic nonylphenol polyethoxylate surfactants are known to enter wastewater collection systems as NPnEOs and 4-NP is not a naturally occurring organic compound. The detection of 4-NP in the influents of all three WWTPs suggests a rapid anaerobic transformation of its parent compounds, NPnEOs, in the sanitary sewers due to low dissolved oxygen levels in raw municipal wastewaters.

After the initial treatment stage of sedimentation to remove large particles and aeration/nitrification, the concentration of 4-NP in the wastewater decreased dramatically to 0.68 µg L^{-1}, 0.82 µg L^{-1}, and nondetectable (samples labeled CT and ST) in Plant 1, Plant 2, and Plant 3, respectively (Figure 2). At this stage, a combination of aeration and nitrification gave a lower 4-NP reduction (89 percent and 76 percent in Plants 1 and 2, respectively) compared to the 100 percent 4-NP reduction for Plant 3 with only aeration process. Nitrification might have promoted further transformation of parent compounds to 4-NP in Plant 1 and 2. However, production of 4-NP from NPnEOs is unlikely at this point in Plant 3 because of the aerobic condition. In Plant 3, the level of 4-NP decreased 68 percent to a concentration of 55 µg L^{-1} after sedimentation. Degradation and partition on to the biomass are speculated to be the possible reasons for the high reduction of 4-NP levels in the wastewater samples collected at the initial treatment stage. Due to its lipophilic nature, 4-NP present in the raw wastewater had great potential to partition on to the organic matter in the contact basin for aeration/nitrification. Partition on to the organic matter could potentially retard the biological degradation of 4-NP during the later treatment processes.

In Plant 1, after the 1st stage aeration/nitrification, the sludge sediment was sent to aerobic digester for further treatment and the wastewater was directed to the denitrification/aeration tank. The appearance of 4-NP in wastewater of aerobic digester, return activated sludge, and effluent at levels of 1.15 µg L^{-1}, 1.5 µg L^{-1} and 1.9 µg L^{-1}, respectively, suggests further transformation of NPnEOs to 4-NP and/or possible cation/aeration tank and clarifier. In Plant 2, 0.11 µg L^{-1} and 1.53 µg L^{-1} of 4-NP were also detected in wastewater samples from clarifier and return activated sludge, respectively (Figure 2). The level of 4-NP in effluent of Plant 2 was

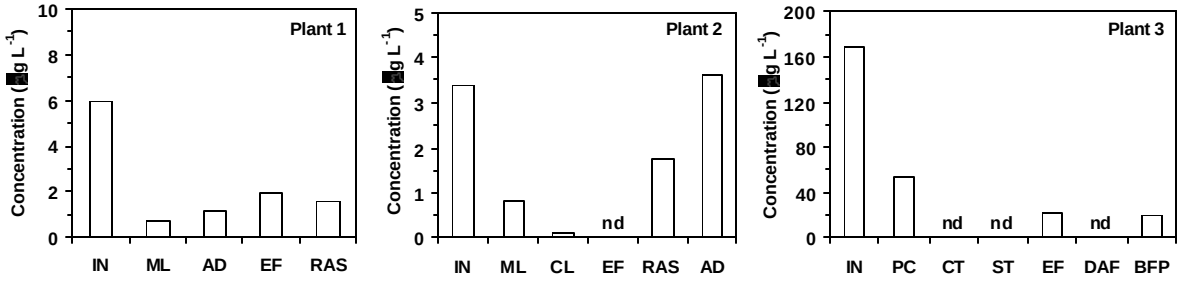

Figure 2. Concentrations of 4-NP in the wastewater samples collected at different treatment stages in three Kansas wastewater treatment plants. (nd, nondetectable). *Plant 1*: IN = influent, ML = mixed liquor from 1st stage aerator, AD = effluent of aerobic digester, EF = effluent, and RAS = return activated sludge from clarifier; *Plant 2*: IN = influent, ML = mixed liquor from aerator, CL = effluent from clarifier, EF = effluent, RAS = return activated sludge from clarifier, and AD = effluent of aerobic digester; *Plant 3*: IN = influent, PC = effluent from primary clarifier, CT = effluent from aeration contact basin, ST = effluent from stabilization tank, EF = effluent, DAF = mixed liquor in dissolved air flotation tank, and BFP = effluent from belt-filter press.

nondetectable. The level of 4NP in the wastewater from aerobic digester of Plant 2 was detected at 3.62 :g L^{-1}, a concentration level similar to that in the influent of this plant. Significant reduction of water input to the aerobic digesters in both plants might induce a concentration effect and, therefore, contribute to the elevated levels of 4-NP in the aerobic digester wastewater samples.

4-NP was found at 20 :g L^{-1} in the effluent of Plant 3 (Figure 2), a level much higher than that in the effluents of the other two plants. However, compared with the 4-NP level in the influent of this plant, an 86 percent concentration reduction was achieved for the effluent. Activated sludge from the secondary clarifier at Plant 3 is first thickened using dissolved air flotation units and then pumped into the first stage of a two-stage anaerobic digestion process. Primary sludge from primary clarifier is pumped directly into the digesters. The two-stage anaerobic digestion process stabilizes the sludge and collects methane in the floating cover of the 2nd stage digesters. The digested sludge is then pumped to a series of belt-filter presses for dewatering. Figure 2 shows the 4-NP concentrations in the wastewater collected from the dissolved air flotation tank before the anaerobic digestion process and in the wastewater produced from the digested sludge that is thickened on the belt-filter presses after the anaerobic digestion. No 4-NP was detected in the water (DAF) before the anaerobic digestion. However, 20 :g L^{-1} 4-NP appeared in the aqueous phase (BFP) associated with the anaerobically digested sludge. Dissolved air flotation process might have promoted the partition of 4-NP on to the biomass in the system and, therefore, decrease the amount of 4-NP in the wastewater. During the anaerobic digestion process, some of NPnEOs might have been converted to 4-NP, causing an increase of 4-NP concentration in the wastewater produced.

Activated sludge in Plant 1 is also dewatered using a belt-filter press, but as seen in Figure 1, undergoes aerobic digestion instead of anaerobic digestion prior to dewatering. Figure 3 shows the dry-weight basis concentrations of 4-NP in the dewatered biosolids collected from Plants 1 and 3. Biosolids from Plant 1 were found to contain 1.3 mg kg^{-1} of 4-NP, while biosolids from Plant 3 contained as high as 300 mg kg^{-1} of 4-NP, more than 200 times higher than that found in the biosolids from Plant 1 and in the sewage sludge sample (1.24 mg kg^{-1}) from Plant 2. The biosolids produced in Plant 3 are transferred to compost lagoons for composting. More than half of the 4-NP in biosolids was removed during the composting process. The concentration of 4-NP in the composted biosolids was detected at 130 mg kg^{-1}.

Using the parameters listed in Table 1, mass distributions for 4-NP in the three plants were estimated for the day of sample collection (Figure 4). For Plant 1, the amount of 4-NP found in its biosolids was 54 percent of that entered into the plant with influent. The amount of 4-NP that was discharged out of Plant 1 with effluent was 31.9 percent of what was detected in the influent of the plant. Since there was a 13.7 percent discrepancy between the amount of 4-NP that entered the plant and what was found in the effluent and biosolids, it is reasonable to assume that negligible amounts of parent compounds NPnEOs were converted

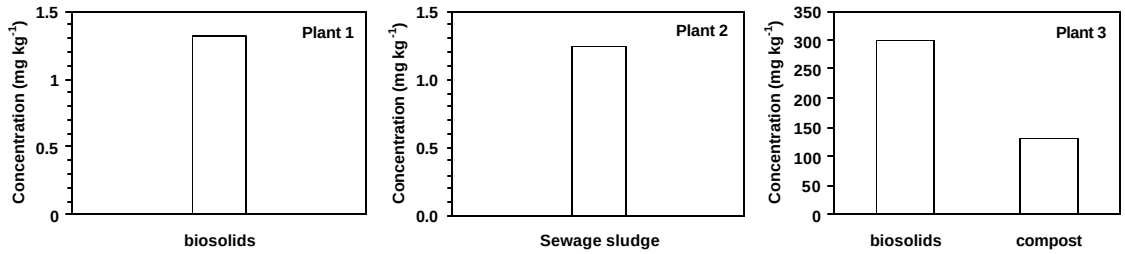

Figure 3. Concentrations of 4-NP in the dewatered biosolids, sewage sludge, and compost collected from the three plants. Detection limit for biosolids = 0.05 mg/kg.

Table 1. Total volume of raw wastewater and production of biosolids in the three plants.

Plant	Raw wastewater input (L day^{-1})	Biosolids/Sludge produced (kg day^{-1})
1	2.45×10^4	60
2	2.84×10^5	350
3	2.00×10^7	10,000

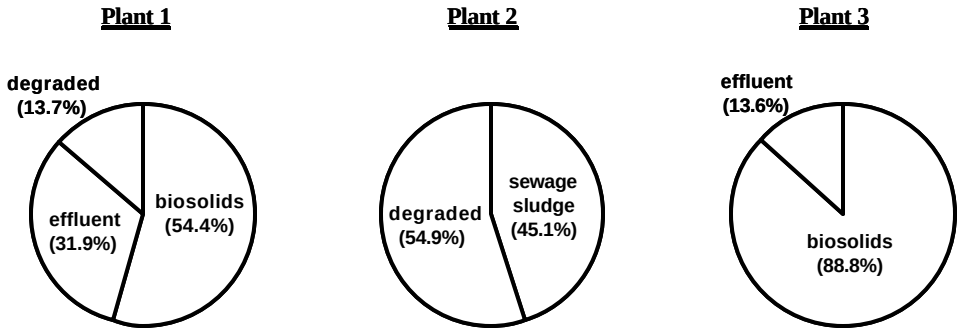

Figure 4. Daily mass distributions of 4-NP in the three plants.

to 4-NP during the treatment processes in Plant 1. The discrepancy was likely due to degradation of 4-NP. Higher degradation efficiency (54.9 percent) was estimated for Plant 2 (Figure 4). About 45.1 percent of 4-NP that entered into this plant was partitioned on to the biomass in sewage sludge produced. No detectable level of 4-NP was found in the effluent of Plant 2. The total amount of 4-NP detected in the effluent and biosolids of Plant 3 is about 100 percent of what entered into the plant. Although certain amounts of parent compounds were transformed into 4-NP at the final anaerobic digestion stage, a significant portion (88.8 percent) of 4-NP entered into Plant 3 was not degraded during the treatment processes but instead was mass transferred to the dewatered biosolids. The remaining 13.6 percent was discharged out of this plant through effluent.

WWTPs (Plant 1 and Plant 2) that served communities with no medium or large-scale industries generally contained smaller quantities of 4-NP in their raw wastewater. It seems that the difference in the size of community that is served by the WWTP has less impact on the occurrence of 4-NP than the scale of the industries in the community. 4-NP was detected at a much higher level in the raw wastewater at Plant 3 that served a medium sized city with several medium-scale industries. Significant accumulation of 4-NP in biosolids/sewage sludge was observed for all three WWTPs investigated, no matter how much 4-NP was

found in the raw wastewater of the WWTPs. Given that about seven million tons of biosolids are produced annually in wastewater treatment plants in the United States, several thousand tons of 4-NP could be potentially released to the soil environment through land application of biosolids. Biosolids land application is becoming the most common means of biosolids disposal as other disposal options become cost prohibitive or heavily regulated. The 4-NP released into the soil environment can potentially enter the water environment due to runoff and leaching. Future research on the transport and transformation of 4-NP in biosolids-amended soil and its impact on surface and ground water quality are needed.

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