The Influence of Operating Parameters on the Biodegradation of Steroid Estrogens and Alkylphenolic Compounds during Biological Wastewater Treatment Processes

Yoong K.K. Koh[1], Tze Y. Chiu[2] Alan R. Boobis[3], Mark D. Scrimshaw[4], John P. Bagnall[5], Ana Soares[5], Simon Pollard[5], Elise Cartmell[5*] and John N. Lester[5]

[1]Public Utilities Board, Technology and Water Quality Office, 40 Scotts Road #15-01, Environment Building, 228231, Singapore.

[2]Earth Tech Engineering Limited, Wentworth Business Park, Tankersley, Barnsley, S75 3DL, UK.

[3]Faculty of Medicine, Division of Experimental Medicine and Toxicology, Imperial College London, Hammersmith Campus, London, W12 0NN, UK.

[4]Institute for the Environment, Brunel University, Uxbridge, Middlesex, UB8 3PH, UK

[5]Centre for Water Science, School of Applied Sciences, Cranfield University, Bedfordshire, MK43 0AL, UK.

*Corresponding author:

Abstract. This study investigated operational factors influencing the removal of steroid estrogens and alkylphenolic compounds in two sewage treatment works, one a nitrifying/denitrifying activated sludge plant and the other a nitrifying/denitrifying activated sludge plant with phosphorus removal. Removal efficiencies of >90% for steroid estrogens and for longer chain nonylphenol ethoxylates ($NP_{4\text{-}12}EO$) were observed at both works, which had equal sludge ages of 13 days. However, the biological activity in terms of milligrams of estrogen removed per tonne of biomass was found to be 50-60% more efficient in the nitrifying/denitrifying activated sludge works compared to the works which additionally incorporated phosphorus removal. A temperature reduction of 6°C had no impact on the removal of free estrogens, but removal of the conjugated estrone-3-sulphate was reduced by 20%. The apparent biomass sorption (LogKp) values were greater in the nitrifying/denitrifying works than those in the nitrifying/denitrifying works with phosphorus removal for both steroid estrogens and alkylphenolic compounds possibly indicating a different cell surface structure and therefore microbial population. The difference in biological activity (mg $tonne^{-1}$) identified in this study, of up to seven

times, suggests that there is the potential for enhancing the removal of estrogens and alkylphenols if more detailed knowledge of the factors responsible for these differences can be identified and maximised, thus potentially improving the quality of receiving waters.

Introduction

Natural and synthetic estrogens and non-ionic surfactants such as alkylphenol polyethoxylates (APEOs) are endocrine disrupting chemicals (EDCs) that can cause adverse effects on the sexual and reproductive systems in wildlife and fish (*1*,*2*). The effluents discharged from sewage treatment works (STWs) are major sources of these anthropogenic chemicals to the aquatic environment (*3*). In addition, APEOs biodegrade during wastewater treatment to generate the parent alkylphenols (AP), octylphenol (OP) and nonylphenol (NP), the shorter chain mono to triethoxylates (NP_1EO, NP_2EO and NP_3EO) and a range of carboxylated intermediate by-products (*4*,*5*) which are more estrogenic than their parent substances *(5-8*). In the aquatic environment these compounds are amenable to further biotransformation and bioconcentration (*9*) and may potentially bioaccumulate (*10*); as a consequence of this behaviour complex issues for environmental health arise (*2*). While secondary biological treatment of wastewater significantly reduces the concentration of some of these compounds, as presently configured and operated, these processes cannot afford adequate protection of the aquatic environment (*11*). Regulatory authorities are seeking to reduce and ultimately eliminate this problem. In the UK, a £40 million ($75 million) National Demonstration Program (NDP) has been undertaken by the water industry as part of the asset management plan four (AMP4) settlement, initiated by the Environment Agency (EA) of England and Wales to investigate the potential removal of steroid estrogens from final effluents (*12*). An initial report from the study has concluded that STWs, where treatment involved nitrifying activated sludge, were able to remove steroid estrogens more effectively than those with other biological treatment processes (*13*).

The primary objective of conventional wastewater treatment is the removal of carbon and nitrogen and possibly phosphorus (*14*), hence current configurations are not designed or operated to remove EDCs (*15-17*). Tertiary treatment technologies such as granular activated carbon (GAC), advanced oxidation processes (AOPs), and membrane filtration have been suggested to remove these micropollutants (*18*,*19*). However, the presence of

high levels of insoluble and dissolved organic matter may interfere with the adsorption process and could therefore result in lower than anticipated removals when GAC is used (*20*) The same issue also means that empolyment of AOPs may require high doses of oxidants, thus resulting in increased cost (*21*). Undoubtedly, advanced treatment technologies will remove these compounds and ameliorate the impact of EDCs on surface waters, but they will inevitably result in significant financial and environmental costs through increased energy consumption and carbon dioxide emissions (*21*). Environmental sustainability therefore requires the consideration of alternative strategies such as optimisation or modification of existing STWs by determining the operating parameters that govern the removal of these substances within the STW.

Several studies have attempted to link certain operating parameters and the removal of EDCs in STWs. In activated sludge systems, solid retention time (SRT) (*22-25*) and hydraulic retention time (HRT) (*3,26*) have both been proposed as factors which may regulate EDC removal, however, explicit information on their precise mode of effect is lacking. There is also no conclusive evidence on the significance of other variables, such as temperature, partitioning to solids and dissolved oxygen concentrations that would inform decisions on the optimisation of STWs for the removal of these chemicals (*11,27*).

This study was undertaken to determine the role of operating parameters in the removal and biodegradation of selected steroid estrogens, APEOs and their metabolites in two treatment processes: a nitrifying/denitrifying activated sludge plant (N/DN) and a nitrifying/denitrifying activated sludge plant with phosphorus (P) removal (N/DN-P). Such treatment processes are frequently installed at large urban STW where discharges contribute significantly to flows in receiving waters, which are therefore likely to be impacted by discharges of EDCs. The objective of this study was to compare the biological activity of each process, and investigate factors that may influence it, such as organic loading, temperature and dissolved oxygen concentration.

Materials and Methods

Sewage Treatment Works. Samples were collected at appropriate stages of the biological treatment processes (after primary sedimentation) at two full-scale STWs (Figure 1), the characteristics of which are described in Table 1. Both STWs were required to nitrify in order to comply with effluent ammonia requirements and the N/DN-

P works had an additional anaerobic zone for biological P removal. Chemical precipitation (ferrous) was used following secondary treatment to further reduce final effluent phosphorus concentrations in the N/DN-P works.

Sampling Regime. Three separate sampling campaigns were undertaken: at the N/DN works in Summer 2004 and Winter 2006; and finally at the N/DN-P works in Summer 2006. Discrete samples were collected in 2.5 l amber borosilicate glass vessels with Teflon lined caps from 08:00 on a Monday morning through to 12:00 on Friday. The maximum interval between samples was 4-6 hours depending on the retention time of the unit processes, for practical reasons such as health and safety and accessibility to the sampling points. The samples were not preserved as they were extracted onto solid phase extraction (SPE) cartridges on site within 15 minutes of collection. Sampling frequency was such, that in conjunction with the average daily flows, representative mass balances could be calculated. The monitoring programme allowed for coverage of diurnal (day/night) variation, seasonality (winter/summer) and process type (N/DN and N/DN-P). Little or no precipitation (rain or snow) was experienced during any sampling period. Samples were taken from the settled sewage leading to the activated sludge units, the returned activated sludge (RAS), the final effluents and at the N/DN-P works only, the liquors from sludge thickening treatment containing volatile fatty acids (VFAs) (Figure 1).

Analytical Procedure for Steroid Estrogens and Alkylphenolic Compounds. Steroid estrogens and APEOs were determined in the dissolved and adsorbed phases in all samples. Methodology for the determination of the natural and synthetic steroid estrogens: estrone (E1); 17β-estradiol (E2); estriol (E3); sulphate conjugate of estrone (E1-3S); and 17α-ethinylestradiol (EE2) in the dissolved phase (*28*) and on solids (*29*) has previously been reported. Estrogens are predominantly excreted as either glucuronide or sulphate conjugates, although only estrone 3-sulfate (E1-3S) has been detected in UK sewages (*30*), probably as a result of the predominance of this conjugate in urine and the rapid deconjugation of the glucuronides (*31*). Therefore, only this conjugate was analysed. The alkylphenolic compounds: alkylphenols, alkylphenol polyethoxylates (APEO) and alkylphenol ethoxycarboxylates (APEC) in the dissolved phase were determined by the method of Koh et al. (*32*). Methodology for the determination of these compounds on the solid phase, along with full descriptions of all methods and their performance are

provided in the supplementary information. In summary, 1 l sewage samples for both steroid estrogens and APEOs were filtered through glass fibre GF/C filters (VWR International, Leicestershire, UK) prior to solid phase extraction on separate tC18 SPE cartridges. For the dissolved phase, steroid estrogens were extracted from the tC18 cartridges followed by two further sample clean-up stages and quantification using LC/ESI(−)/MS/MS. The alkylphenolic compounds were eluted from the tC18 cartridges and without further clean-up, quantified using LC/MS/MS. The adsorbed and sludge samples for steroid estrogens and APEOs were freeze dried then solvent extracted and subjected to clean-up (three stage for estrogens and single stage for APEO) before quantification by LC/MS/MS.

Mass Balance and Biomass Activity Calculations. Mass balances were completed by multiplying average daily steroid estrogen concentrations (E1, E2, E3, E1-3S and EE2) or alkylphenolic concentrations (NPEO, NPEC and NP) by average daily flows and utilising these values to calculate an average daily flux. The removal efficiencies of the biomass were evaluated by activity i.e. milligram steroid estrogen degraded per tonne of biomass for each estrogen individually and for the sum of all steroid estrogens (∑EST). The degradation was obtained from the flux data, and was the mass of each compound which entered the biological treatment stage and was not accounted for through analysis of effluent or RAS and was assumed to be degraded. For the alkylphenolic compounds activity was calculated for each alkylphenolic compound and for the groups $NP_{1-3}EC$ and $NP_{4-12}EO$. The calculation was based on the mass difference between the settled sewage and the final effluent in milligrams of estrogens or alkylphenolic and dividing it by the mass of the mixed liquor volatile suspended solids (MLVSS) (tonnes).

Results and Discussion

It has been postulated that sludge age, also referred to as solid retention time (SRT), and hydraulic retention time (HRT) are both key factors in the removal of EDCs in biological wastewater treatment processes (*11,27,33-35*). However, Joss et al. (*34*) alluded to the fact that SRT only explained part of the difference in removal efficiency. Sludge loading was suggested as a key parameter due to the potential for competitive substrate inhibition limiting estrogen biodegradation, although to date, no clear relationship has been established, suggesting that other parameters may also be involved. In this study the

works examined had equivalent SRTs and HRTs but varying sludge loadings as measured by the food to microorganism ratio (F:M) (g BOD. g^{-1} MLVSS.d^{-1}) (Table 1).

The Impact of Process Type and Operational Parameters on EDC Degradation. Based on the mass fluxes, degradation of estrogens was 70 - 76% in both the N/DN and N/DN-P works indicating no difference in removal efficiency. The biological degradation efficiencies for NPEOs were lower with 41% and 55% of the flux entering the biological treatment stage degraded in the N/DN and N/DN-P works respectively in 2006 (Figure 2). These NP, NPEC and NPEO data were comparable to that observed by Loyo-Rosales et al. in a nitrifying activated sludge plant (plant 3) (*36*) with 59% of influent ($NP_{0-16}EO$) degraded with production of the $NP_{1-4}EC$.

The total steroid estrogen load (dissolved and adsorbed) in both STWs decreased by almost 1 order of magnitude during treatment from 1806-5508 mg d^{-1} in the settled sewage influents to 117-375 mg d^{-1} in the final effluents. Carballa et al. (*37*) also performed a similar mass balance over the secondary treatment process, albeit for the combination of E1+E2 only, with 497 mg d^{-1} in the settled sewage and 325 mg d^{-1} in the final effluent from a 100,000 population equivalent (PE) STW (*37*). In this present study, the mass of E1+E2 in the settled sewage ranged from 757-3859 mg d^{-1} and in final effluents from 66 mg d^{-1} to 291 mg d^{-1}, indicating that significantly more biodegradation was occurring in the STWs in this study. The negative removal efficiencies for E1 observed by Carballa et al. were suggested to be due to conversion of E2 to E1 which was then more slowly degraded during secondary treatment (*38*). In this present study, higher removals were observed for E1, with degradation occuring during biological treatment in both the N/DN and N/DN-P works. It can only be hypothesized that the lack of E1 degradation in the study by Carballa et al. was potentially due to the low SRTs (*38*). Kreuzinger et al. proposed that higher SRTs (e.g. in works with nitrification) allowed the enrichment of slowly growing bacteria and consequently the establishment of a more diverse biocoenosis with broader physiological capabilities and greater potential for EDC removal compared to STWs with low SRTs (*35*). At the works studied by Kreuzinger et al. the E1+E2+E3 mass balance removals varied from: 16% with a SRTs of <1 day; 66% with a SRT of 9.6 days; 98% with a SRT of 24 days. The removal efficiencies in this study support these findings and were between 78 – 80% in both the N/DN and N/DN-P works for E1+E2+E3 which both had equivalent SRTs of up to 13 days (Table 1).

The total NPEO load (dissolved and absorbed) in both STWs in 2006 also decreased by nearly 1 order of magnitude from 330486 - 646209 mg d^{-1} in the settled sewage influents to 54910 – 112924 mg d^{-1} in the final effluents (Figure 3). At the N/DN works in 2004, similar reductions in the total NPEO load was observed from 1787390 mg d^{-1} to 83630 mg d^{-1} (*39*) with removal efficiencies of 93 – 96% observed for $NP_{4-12}EO$ (Table 2). Formation of $NP_{2-3}EO$ was observed at both the N/DN and N/DN-P works in 2006 which reduced the overall removal efficiencies to 73 – 91 % for $NP_{1-12}EO$, below the 99.1% and 93.7% removals reported by Loyo-Rosales et al. during summer and winter periods respectively for $NP_{0-16}EO$ (*36*). The increased biodegradation (95%) observed in 2004 (Figure 2) may be a reflection of the higher concentrations of NPEO detected in 2004 compared to 2006 or the higher sewage temperature in the summer of 2004 of 18^{o}C compared to 12^{o}C in the winter of 2006. The low concentrations of total NPEO detected in the return activated sludge (RAS) in 2004 were probably because NPEC compounds were not determined or included in the mass balance. In 2006, the total NPEC mass in the RAS was 71141 mg d^{-1} in the N/DN works which was approximately equivalent to the NP and NPEO concentration of 69082 mg d^{-1}. If the 2006 proportions of NPEC to NP and NPEO were applied across the 2004 mass balance the biodegradation would be reduced to 88% from 95%.

The NPEC compounds comprised about 50% of the total alkylphenolic compounds in the final effluent compared to <5% in the settled sewage, with $NP_{1-3}EC$ exhibiting an increase in concentration from <1 µg l^{-1} in the settled sewage to 1.3 and 2 µg l^{-1} in the N/DN and N/DN-P works respectively..

Evaluation of Biomass Activity as a Determining Factor on EDC Removal. Both the removal efficiencies and final effluent concentrations given in Table 4 at the two STWs were similar. However, the removal efficiencies of the biomass at the STWs were also evaluated by activity i.e. milligram steroid estrogen or NPEO biodegraded per tonne of biomass. The two STWs were equally efficient in removing organics with removal of chemical oxygen demand (COD) being >82% (Table 1), however, the biomass activity was different. The food to micro-organism (F:M) ratio in the N/DN works was twice that of the N/DN-P works with 0.1 g BOD. g^{-1} MLVSS d^{-1} and 0.05 g BOD. g^{-1} MLVSS d^{-1} respectively. The overall biomass activity per tonne of steroid estrogen removed was

highest in the N/DN works in 2004 at 116.7 mg tonne^{-1} and lowest in the N/DN-P works at 39.4 mg tonne^{-1}. This difference in efficiency of the biomass was also observed in relation to the removal of NPEO, with an activity of 11977 mg $NP_{4\text{-}12}EO$ tonne^{-1} in the N/DN works and 4221 mg tonne^{-1} in the N/DN-P works (Table 2). It is apparent that these results do not necessarily support the hypothesis that higher organic loadings, as measured by the F:M ratios, result in the inhibition of steroid estrogen biodegradation (*24*). This is assuming that the influent inert non-degradable material is consistent between the works. Furthermore, although the MLVSS concentrations on both sampling occasions at the N/DN works were much lower than the N/DN-P works, steroid estrogen biodegradation based on removal in mg tonne^{-1} of biomass, was higher for the N/DN works.. It is hypothesized that the biomass in the N/DN works was different to that in the N/DN-P works. The apparent LogKp values also infer possible differences between the biomass at the two works. The generally lower LogKp values determined for all compounds at the N/DN-P works compared with the N/DN works (Table 3), are indicative of different absorption capacities of the biomass, as it is known that some genera of bacteria are far more hydrophobic than others, and that the proportional abundance hydrophobic genera increases with sludge age (Davenport et al., 2000)

The greater biological activity observed at the N/DN works does not support the hypothesis that the varied environmental conditions with respect to redox (aerobic, anoxic and anaerobic) present in the N/DN-P works provides a more diverse bacterial community with more complex biochemistry which can potentially enhance the biodegradation of EDCs (*34*). Therefore, it appears that examining the correlation between SRT and EDC removal efficiencies, although useful in predicting if removals can occur, does not provide a true representation of the biomass activity and propensity for EDC removal.

It has been postulated that increasing the sludge age increases the diversity of the consortia of bacteria present in a treatment plant allowing the growth of EDC degrading organisms (*40*) and that the ability to remove EDCs is assumed to be a property of some of the slower growing organisms that can only colonise the treatment plant at long sludge ages. It is unlikely that the presence of EDCs are specifically selecting for these organisms, as the low concentrations of EDC found in wastewaters could only support a small number of cells. It is more likely that EDC degradation occurs fortuitously in

organisms scavenging a wide range of carbon sources and there is recent evidence that the primary mechanism for EE2 degradation in STWs is more likely to be due to the activity of heterotrophic bacteria than ammonia oxidising bacteria (*41*). Heterotrophic organisms that efficiently scavenge low concentrations of a resource are sometimes referred to under the descriptive population term of "K strategists" (*42*). "K strategists" have a high affinity for resources (i.e. a low Monod half saturation coefficient) and low growth rates (i.e. a low μ_{max}), a property consistent with the long sludge ages required for degradation and utilisation of low concentrations of EDCs.

Influence of Temperature on the Biodegradation of Steroid Estrogens. The evaluation of removal based on biomass activity established that there were differences between the N/DN and N/DN-P works, postulated to be due to variations in the establishment of a more diverse biocoenosis. Therefore, because removal efficiencies of organic micropollutants are known to be more sensitive to temperature than removal of biochemical oxygen demand (BOD) and suspended solids (SS) (*43,44*), the effect of temperature was evaluated by undertaking a further study of the N/DN works during the winter for steroid estrogens. Recorded sewage temperatures were 18^{o}C (summer) and 12^{o}C (winter) with corresponding air temperatures of 21^{o}C and 6^{o}C. The 6 ^{o}C reduction in sewage temperature did not have an effect on the removal of free estrogens, which was consistent with other studies which have observed minimal impact of temperature on the removal of unconjugated estrogens (e.g. E2) (*16,45,46*).

However, the 6^{o}C reduction in temperature did effect the removal of the conjugate E1-3S which was ~20% lower in the N/DN works in winter (59%±6) compared to summer (78%±4). Hence it appears that deconjugation was inhibited by the reduction in temperature, rather than the biodegradation of the deconjugated moiety E1. This is consistent with the observation by D'Ascenzo et al. who reported removal of E1-3S at 64% in six activated sludge plants in Rome during the Autumn, although values for the temperature of either sewage or air temperature were reported (*47*). It could be postulated that the low removal of E1-3S was probably due to the low activity of arylsulphatase enzymes (caused by the low temperature) or the absence of bacteria containing these enzymes during the cold season (*48*).

Impact of Nitrification on the Biodegradation of Steroid Estrogens. Nitrification activity has been reported to be correlated with estrogen removal (*49*). Both works in this study fully nitrified and therefore dissolved oxygen (DO) was not thought to directly influence EDC biodegradation in this study. It has been demonstrated that degradation of steroid estrogens is associated with the co-metabolism of the ammonia oxidizing bacteria in nitrifying activated sludge (*50*) which may dominate nitrifying plants such as those sampled in this study. However, there is strong evidence that cometabolic degradation of EE2 by ammonia oxidising bacteria is not an important removal mechanism in STWs (*41*). Therefore, the difference in removal of EDCs between the N/DN and N/DN-P works was probably not due to the biochemical activity of ammonia oxidizing bacteria, but may result from the metabolic activity of heterotrophic organisms able to utilise resources present at low concentrations, such as the "K strategists" (*42*) which remains to be established.

Partitioning of EDCs to Particulate Matter. To determine the significance of sorption in the removal process, the distribution of the estrogens and NPEOs between the solid and liquid phase in the mixed liquor was evaluated by calculating the apparent partition coefficient (Kp). This was undertaken to confirm EDC removal mechanisms (sorption or biodegradation) and to establish if there were any differences between the N/DN and N/DN-P works. The observed LogKp values for E1 and E2 in the N/DN works in both summer (2004) and winter (2006) were above those observed in the N/DN-P works in summer (2006) (Table 3). These LogKp values were in the same range as $LogK_d$ values for E1, E2 and EE2 reported by Carbella et al. (*37*); Joss et al. (*34*) and Ternes et al. (*52*). There is an indication in the data presented in Table 3 that overall for steroid estrogens, with the exception of E1-3S in the summer of 2004, that apparent LogKp values were greater in the N/DN works than those in the N/DN-P works. This is supported by the NPEO data with adsorption to solids also being more important in the N/DN works (42%) compared to the N/DN-P works (28%) (Figure 2). This is reflected in the apparent LogKp with higher values observed for each alkylphenol group for the N/DN biomass (1.4-3.2 l kg^{-1}) compared with (0.05-1.4 l kg^{-1}) the N/DN-P biomass (Table 3).

Results for the two STWs examined in this study demonstrated that in the settled sewage 20-30% of E1, E2 and EE2 were associated with suspended solids, however, for the more hydrophilic E3 and E1-3S this decreased to around 10%. This is in agreement with results

from studies using radiolabelled E2 to determine the fate of estrogens in STWs (*51*) which found that at low concentrations, the majority of the radiolabelled E2 remained in the liquid phase and did not adsorb to the solids. Therefore, biodegradation appears to be the dominant removal pathway for steroid estrogens, as demonstrated in Figure 2, where mass balance calculations indicate that $\geqslant$70% of the total steroid estrogens were biodegraded. In contrast adsorption to solids was a more significant for NPEOs with biodegradation observed at $\geqslant$41%.

The Distribution of EDCs in Settled Sewage. At the N/DN works, which had approximately double the retention time in the sewerage system (13 hours) (based on Water Utility design information), higher concentrations of E3 than E1 were observed in the influent and settled sewage. At the N/DN works, the E3:E1 ratio was 1.33 (2004) and 1.69 (2006) compared to 0.42 for the N/DN-P works influent (Figure 4). Deconjugation of the conjugated estrogens in the time taken for the sewage to reach the works was clearly demonstrated by the detection of the unconjugated estrogens E1, E2, EE2 and E3 in the settled sewage. This finding was expected as it has already been reported that the deconjugation of glucuronide conjugates may occur in the sewerage system, while cleavage of the sulphonated conjugates, which require arylsulphatase for cleavage, will only occur in the STWs as this demands more specialized micro-organisms (*53*,*54*). This observation corroborates that of D'Ascenzo et al. who concluded that unconjugated estrogens and sulphated (not glucuronide) estrogens were the dominant species in the influent of STWs (*47*). This study further confirms the importance of this conjugated hormone since it inevitably contributes to the overall estrogenic burden leading to the release of E1 as a consequence of the hydrolysis of the sulphate conjugate.

The Distribution of EDCs in Final Effluents. The concentration of E1 in the final effluents in this study ranged from 4.3 to 5.5 ng l^{-1} (N/DN 2004/06 and N/DN-P 2006) (Table 4) which is in agreement with concentrations (low nanogram per litre) reported in other countries: Italy 9.3 ng l^{-1} (*53*); the Netherlands 4.5 ng l^{-1} (*54*); and Canada 3 ng l^{-1} (*24*). Recent work from France (*55*) has reported E1 concentrations ranging from <1 up to 75 ng l^{-1} (median 5.3 ng l^{-1}), although concentrations of E2 in the effluent were lower, with a median of 1 ng l^{-1}, in good agreement with values reported in Table 4 (0.4-1.1 ng l^{-1}). The N/DN-P works did not achieve the proposed requirement (EEq<1) for all the

compounds whilst the N/DN works (both seasons) was within the Predicted No-Effect Concentration (PNEC) value for E2 and EE2 . The combined PNEC value of <1 EEq was not achieved at either STWs (Table 4) (*56*).

In contrast the final effluent concentrations of NP were below the PNEC value of 330 ng l^{-1} (*57*) with 44 ng l^{-1} and 55 ng l^{-1} in the N/DN and N/DN-P works respectively. This was in agreement with another study where concentrations of 50-300 ng l^{-1} were observed in the final effluent (*58*) but lower than the median value of 1649 ng l^{-1} reported in 2003 from a number of further STWs final effluents (*59*).

The use of tertiary treatment processes (GAC, Ozone, Membrane Filtration) are currently being evaluated to assess their ability to achieve PNEC values (*12*). However, all of these processes come at a high environmental and economic cost (*21*). It would therefore, be highly desirable to operate secondary biological treatment processes to achieve an environmental sustainable solution for EDC removal. The difference in specific biomass activity identified in this study, does suggest that there is the potential for enhancement of EDC removal by biological wastewater treatment. If more detailed knowledge of the factors responsible for these differences can be identified it may allow for maximising .removal during the treatment process.

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Brief

Biodegradation of steroid estrogens and alkylphenolic compounds was examined with >90% removal and 50-60% greater biological activity observed in a

nitrifying/denitrifying activated sludge plant compared to a nitrifying/denitrifying activated sludge plant with phosphorus removal..

Table 1. Overview of the operating parameters of the two sewage treatment works

Operating parameters	N/DN		N/DN-P
	Summer 2004	Winter 2006	Summer 2006
Biological process	Nitrifying/ denitrifying	Nitrifying/ denitrifying	Nitrifying/denitrifying /P-removal
Process technology	Anoxic/Aerobic	Anoxic/Aerobic	Anoxic/Anaerobic/Aerobic
PE	150,000	150,000	250,323
Q activated sludge process (m^3 d^{-1})	12000	17200	44000
HRT θ_τ (h)	13.6 (0.6d)	10.2 (0.4d)	12.1 (0.5d)
SRT θ_c (d)	13	13	9 – 13
DO (g m^{-3})	1.4	3.2	1.8
MLVSS (g m^{-3})	2740	3282	4971
F:M ratio (g BOD. g^{-1} MLVSS.d^{-1})	0.09	0.1	0.05
pH	7-7.5	7-7.6	7.2-7.4
Trade input	<1%	<1%	10%
Ambient (°C)	21	6	27
Sewage (°C)	18	12	22
	Settled Sewage Influent Characteristics		
COD (g m^{-3})	252	286	489
BOD (g m^{-3})	151	141	148
NH_4-N (g m^{-3})	34.5	38	37
NO_3-N (g m^{-3})	3	3.2	2.5
P (g m^{-3})	n/d	n/d	9
TSS (g m^{-3})	266	122	118
	Final Effluent Characteristics		
COD (g m^{-3})	40.1	51	30
BOD (g m^{-3})	4	16	11
NH_4-N (g m^{-3})	<1	0.4	<0.2
NO_3-N (g m^{-3})	28.2	31.9	15
P (g m^{-3})	n/d	n/d	<0.03
TSS (g m^{-3})	8	36	8

KEY: PE – population equivalent; Q - total flow; HRT – hydraulic retention time; SRT – solids retention time; DO – dissolved oxygen; MLVSS – mixed liquor volatile suspended solids; F:M food to micro-organism ratio; COD – chemical oxygen demand; BOD – biological oxygen demand; NH_4-N – ammoniacal nitrogen; NO_3-N – nitrate nitrogen; P – orthophosphate; TSS – total suspended solids. The works MLVSS, COD, BOD, NH_4-N, NO_3-N, P, TSS values are from daily duplicate samples averaged over the 5 day sampling period. Total flow, DO, temperature, pH were daily averages from on-line continuous recorders. The DO set point was 1.5 mg l^{-1} for both works. The variation was ± 1 mg l^{-1} for the N/DN works and ± 3 mg l^{-1} for the N/DN-P works.
n/d = not determined

Table 2. Biomass activity (mg tonne^{-1}) and removal efficiency (%) of steriod estrogens and alkylphenolic compounds in secondary treatment

Steroid estrogens	Biomass activity (mg tonne^{-1}) and removal efficiency (%)[ab]					
	N/DN				N/DN-P	
	2004		2006		2006	
E1	28.8	(89)	34.2	(89)	20.7	(91)
E2	5.4	(94)	11.4	(96)	6.7	(94)
E3	41.7	(99)	62.7	(99)	9.2	(98)
EE2	0.7	(68)	0.8	(65)	0.3	(60)
E1-3S	6.9	(78)	7.6	(59)	2.5	(88)
∑EST	83.5	(93)	116.7	(92)	39.4	(92)
Loading (mg ∑EST m^{-3} d^{-1})	0.25		0.41		0.21	
Alkylphenolic compounds	[c]	[c]				
NP	115	(30)	-19	(-12)	11	(11)
NP_1EC	n/d	n/d	-167	(-136)	-125	(-241)
NP_2EC	n/d	n/d	-611	(-163)	-59	(-52)
NP_3EC	n/d	n/d	-13	(-211)	-10	(-594)
NP_1EO	23	(80)	263	(77)	71	(41)
NP_2EO	497	(68)	-22	(-160)	-4	(-107)
NP_3EO	4021	(85)	-28	(-460)	-5	(-190)
NP_4EO	7368	(92)	180	(78)	103	(86)
NP_5EO	2977	(95)	519	(88)	241	(88)
NP_6EO	6284	(96)	1715	(92)	653	(90)
NP_7EO	9470	(96)	2120	(95)	736	(92)
NP_8EO	14311	(97)	2114	(96)	716	(94)
NP_9EO	13991	(97)	2039	(97)	676	(95)
$NP_{10}EO$	12062	(98)	1609	(97)	534	(96)
$NP_{11}EO$	7065	(98)	1112	(98)	366	(96)
$NP_{12}EO$	6069	(98)	569	(98)	196	(97)
$NP_{1\text{-}3}EC$	n/d	n/d	-791	(-157)	-195	(-110)
$NP_{4\text{-}12}EO$	79597	(96)	11977	(93)	4221	(93)

[a]Biomass activity was calculated by taking the mass difference of the settled sewage and the final effluent in milligrams of estrogens and dividing it by the MLSS concentration in tonne in the secondary tank; [b]Removal % was calculated as $\frac{(M_{in} - (M_{WAS} + M_{out}))}{M_{in}} \times 100\%$ - waste activated sludge (WAS) was estimated to be 2–5% the flow rate of RAS to maintain the SRT of the aeration tank (VFA return is negligible since return flow is circa 1% of main flow).
[c]Values obtained from Koh et al. (2005) (*39*)
Key: ∑EST = sum of steroid estrogens; n/d not determined

Table 3. Apparent biomass sorption coefficient LogKp (l kg^{-1}) for secondary activated sludge

Steroid Estrogen	N/DN 2004 *(this study)*	N/DN 2006 *(this study)*	N/DN-P 2006 *(this study)*	*Carbella et al. 2007 LogKd* (l kg^{-1})	*Joss et al. 2004 LogKd* (l kg^{-1})	*Ternes et al. 2004 LogKd* (l kg^{-1})
E1	2.53	2.40	1.99	2.9	2.95	n/d
E2	2.78	2.67	1.11	4.5	n/d	n/d
E3	2.79	2.35	1.46	n/d	n/d	n/d
EE2	2.93	3.35	2.00	n/d	n/d	2.5
E1-3S	2.05	1.52	1.60	n/d	n/d	n/d
Alkylphenol group						
$NP_{3-12}EO$	n/d	1.6	1.2	n/d	n/d	n/d
$NP_{1-2}EO$	n/d	2.6	0.8	n/d	n/d	n/d
$NP_{1-12}EO$	n/d	1.8	1.2	n/d	n/d	n/d
NP	n/d	3.2	1.4	n/d	n/d	n/d
$NP_{1-3}EC$	n/d	1.4	0.05	n/d	n/d	n/d

Key: n/d not determined

In this study Kp was calculated using average steroid estrogen or APEO return activated sludge (RAS) concentration data in ng l^{-1}. The adsorbed concentration of steroid estrogens and APEOs in RAS was divided by the mixed liquor concentration - MLVSS (mg l^{-1}) to determine ng steroid estrogen kg^{-1} biomass or ng APEO kg^{-1} and then divided by the dissolved concentration of steroid estrogens or APEOS after filtration of RAS to determine the partitioning as $LogKp = \frac{(ng.chemical.sorbed / kg.biomass)}{ng.chemical.dissolved}$

Table 4. Concentrations of estrogens in the final effluents of the investigated works (ng l^{-1}) and range in brackets with their EEq values.

Steroid estrogens	Concentration (ng l^{-1})			PNEC*
	N/DN		N/DN-P	
	2004	2006	2006	
E1	5.1 (2-7.2)	4.3 (3.2-6.2)	5.5 (1.9-9)	3
E2	0.4 (<MDL-0.6)	0.4 (0.2-0.6)	1.1 (<MDL-2.2)	1
E3	0.5 (<MDL-0.8)	0.4 (0.2-0.9)	0.3 (<MDL-1.1)	-
EE2	0.2 (<MDL-1.3)	0.2 (<MDL-0.4)	0.2 (<MDL-1.1)	0.1
E1-3S	3.1 (0.8-4.8)	7.7 (4-12)	0.8 (0.3-1.6)	-
EEq	4.1	3.8	4.9	<1

*Environment Agency (*56*).

$$\text{EEq (ng l}^{-1}) = \frac{[17\alpha - Ethinylestradiol]}{PNEC = 0.1} + \frac{[17\beta - Estradiol]}{PNEC = 1} + \frac{[Estrone]}{PNEC = 3} < 1$$

Key: MDL = Method detection limit 0.1 ng l^{-1} for E1 and E1-3S; 0.2 ng l^{-1} for E2, E3 and EE2

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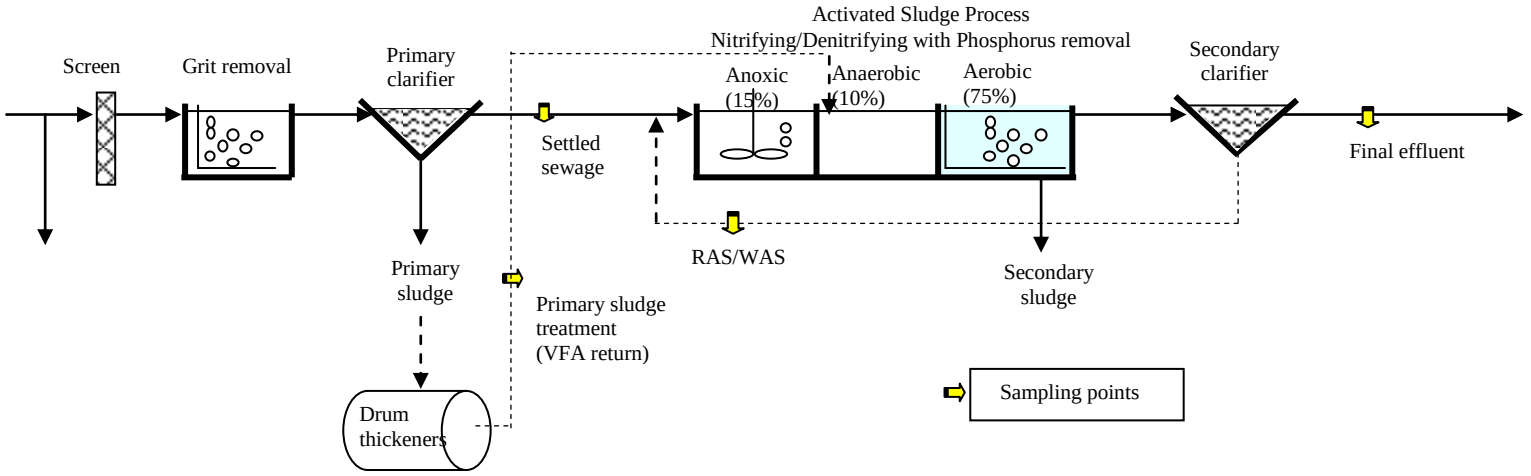
A. Nitrifying/Denitrifying (N/DN) works operating with no internal recycle and a plug flow aerobic zone.



B. Nitrifying/Denitrifying with Phosphorus removal (N/DN-P) works operating with no internal recycle and a plug flow aerobic zone.

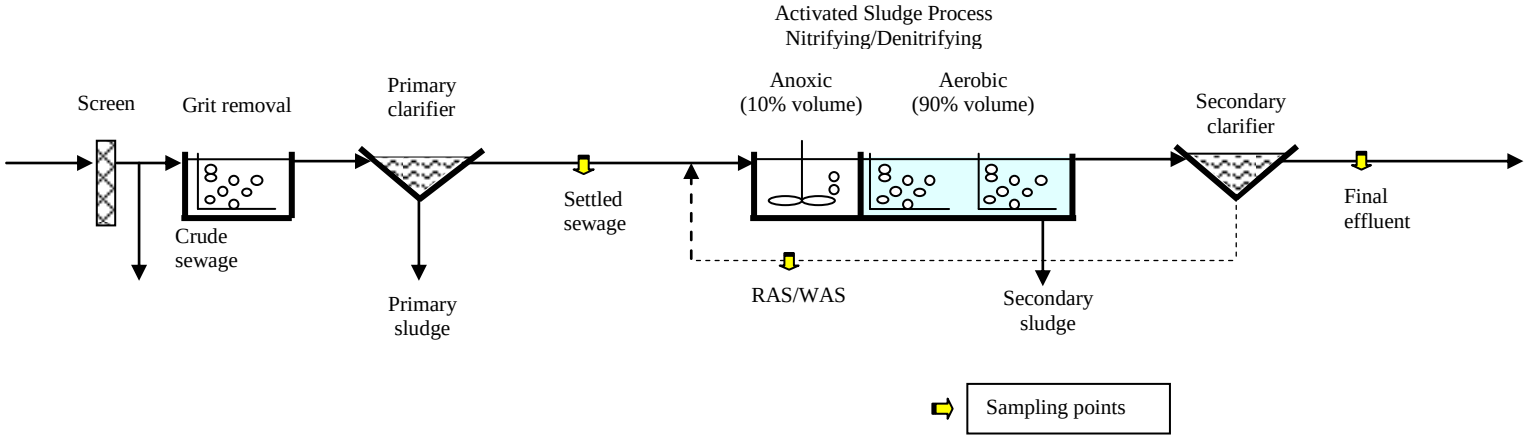


Figure 1. Schematic diagrams of the two activated sludge sewage treatment works sampled – A nitrifying/denitrifying (N/DN) and B nitrifying/denitrifying with phosphorus removal (N/DN-P)

Steroid estrogens | **Alkylphenols**

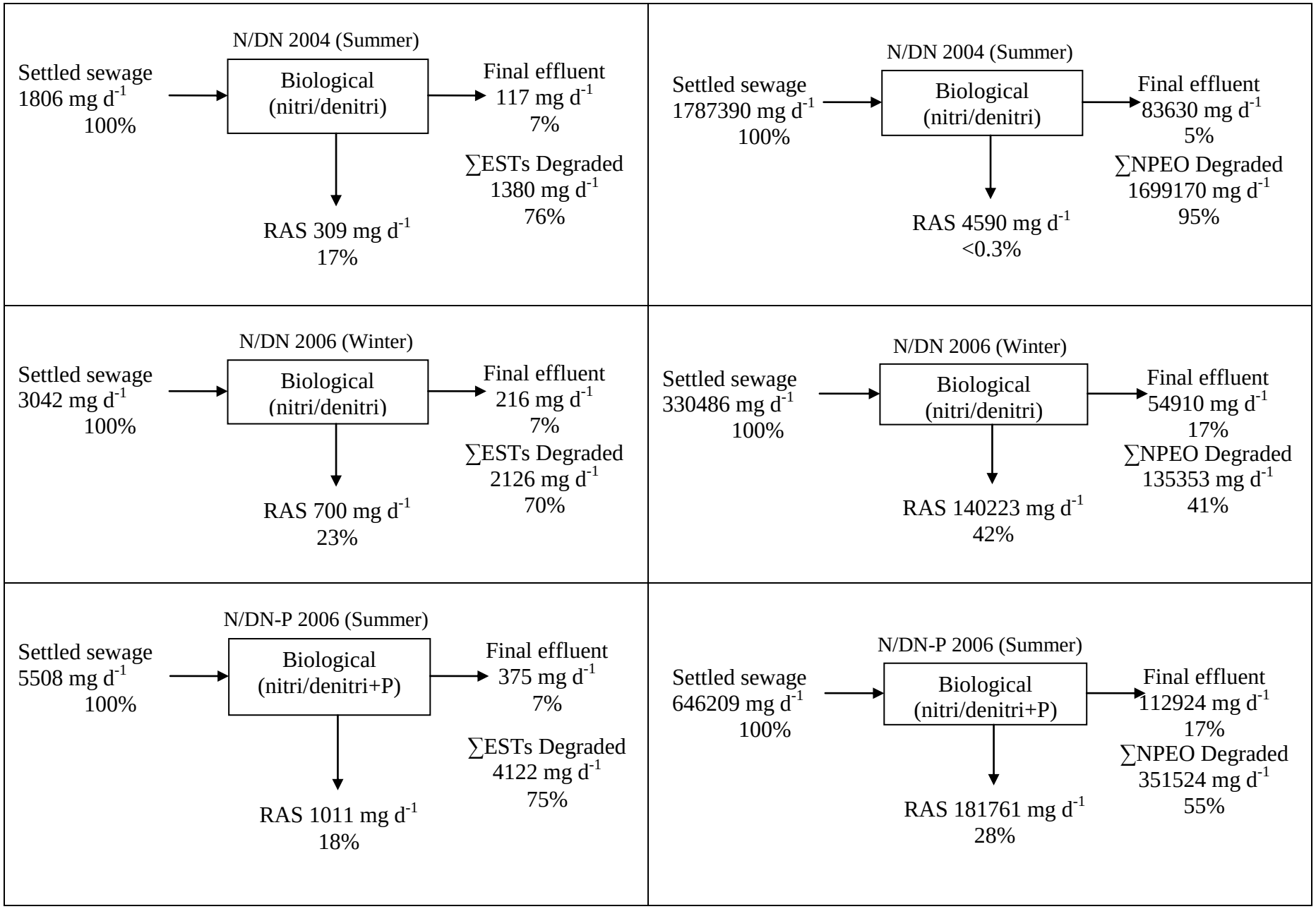


Figure 2. Mass balance of the total steroid estrogens and of alkyl phenolic compounds NPEO, NPEC and NP in the sewage treatment works. The degraded component has been determined from settled sewage – (RAS + final effluent). The N/DN data for alkylphenolic compounds in 2004 are from Koh et al. (*50*) and do not include NPEC.

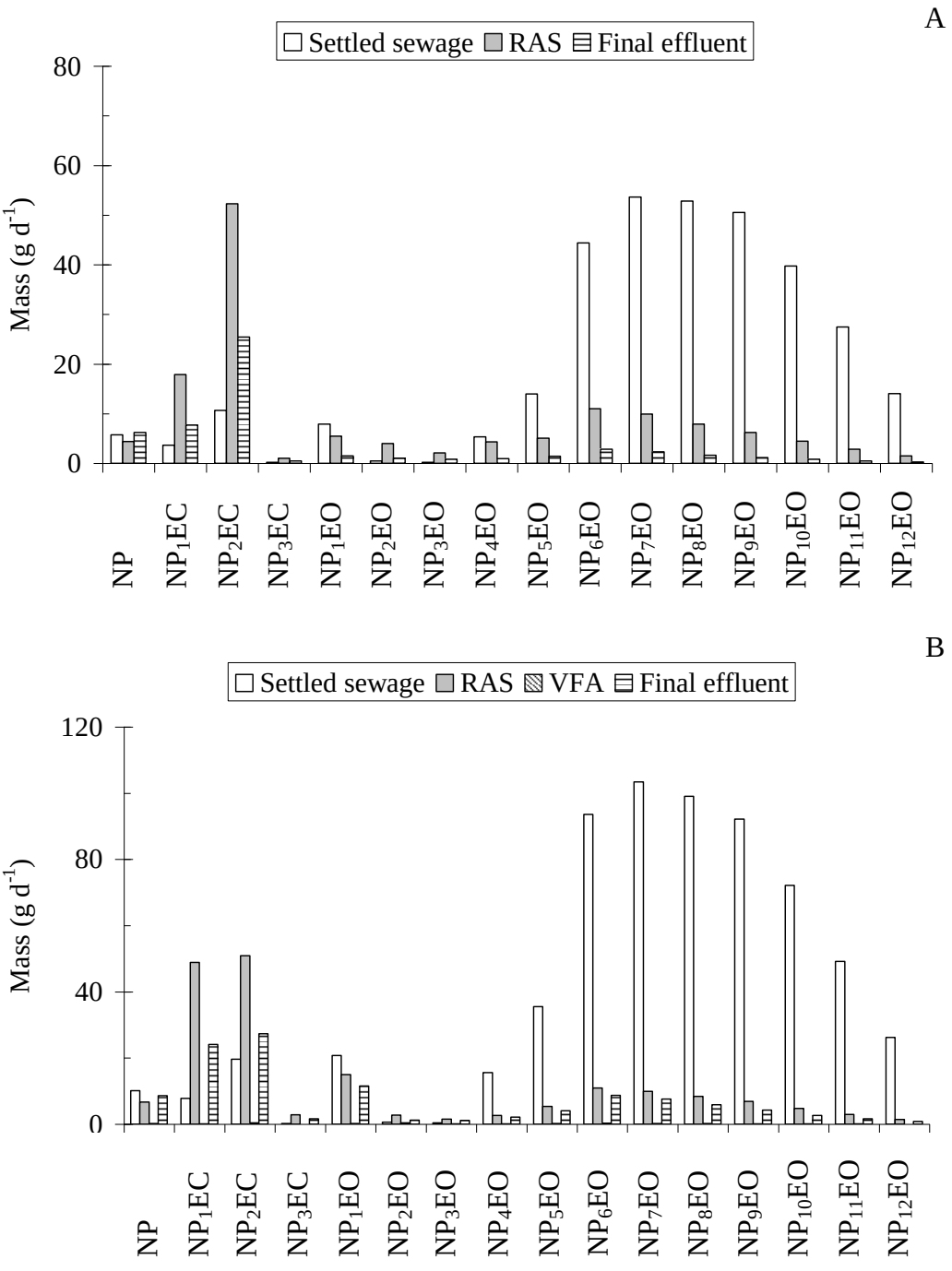


Figure 3. Mass fluxes of NPEO in N/DN (A) (2006) and N/DN-P (B) (2006).

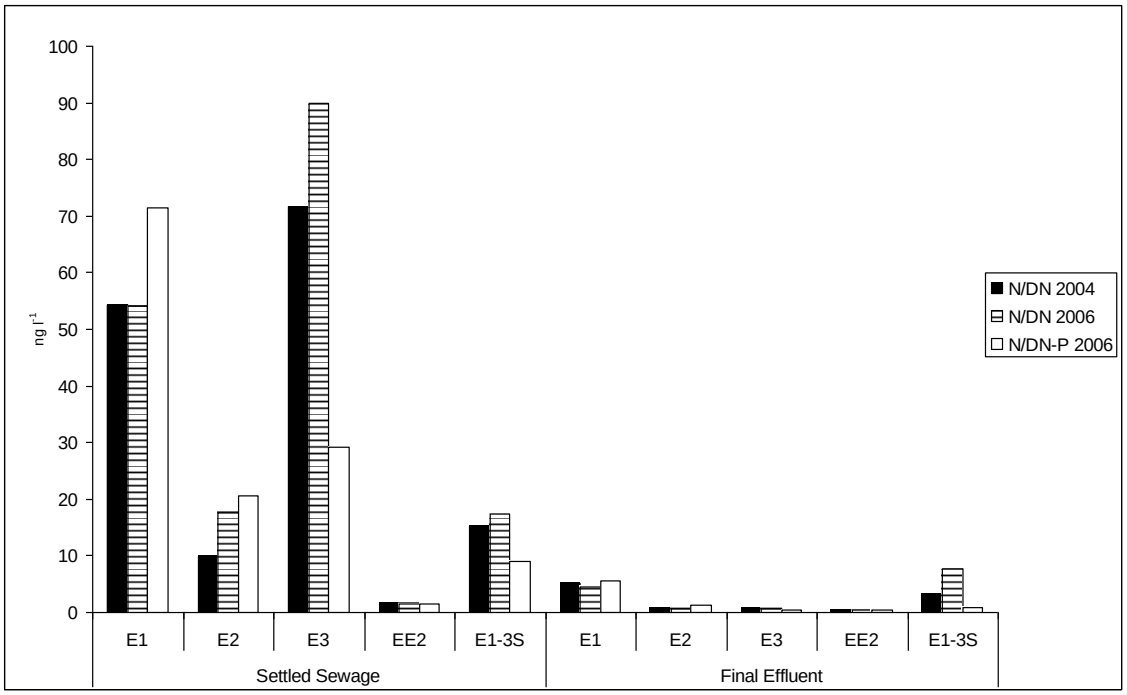


Figure 4. Concentrations of total steroid estrogens (dissolved and adsorbed) in the sewage treatment works