

Removal of Hormones and Pharmaceuticals in the “Advanced Water Recycling Demonstration Plant” in Queensland, Australia

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Abstract An advanced water recycling demonstration plant was employed to investigate the effectiveness of a number of treatment technologies in the removal of some residuals of commonly prescribed pharmaceuticals as well as natural and synthetic hormones found in sewage.

Analysis of targeted compounds was carried out by solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). Initial tests were undertaken to determine the background concentrations of the analytes during various stages of treatment. Subsequent tests, undertaken by spiking with standard solutions of the target compounds provided further information on the removal efficiencies of some selected treatment modules. The results of the study indicate that while ozonation, microfiltration and nanofiltration were partially effective; treatment by reverse osmosis was the most universally successful in the removal of the target residuals.

While significantly more data is required for a full evaluation, this initial investigation suggests that reverse osmosis may be an effective means of removing a wider range of pharmaceutically active residuals and hormones from treated sewage.

Keywords Microfiltration; nanofiltration; ozonation; reverse osmosis.

Introduction

Pharmaceuticals, hormones and their metabolites are rapidly emerging environmental contaminants of concern and many are only partially removed during conventional sewage treatment processes (Andreozzi *et al.*, 2003; Huggett *et al.*, 2003; Khan and Ongerth, 2003; Koutsouba *et al.*, 2003). Factors contributing to the observed persistence of some compounds in secondary effluents include typically high water solubility and, in some cases, a resistance to aerobic biodegradation.

The presence of pharmaceutical and hormonal residuals in sewage may influence how the water is used, recycled or discharged. Few studies have investigated the effectiveness of advanced water treatment technologies to remove these biologically active compounds. In this study, a unique opportunity allowed researchers to examine the efficacy of a range of advanced processes to remove some pharmaceutical and steroidal estrogen compounds from municipal sewage effluent.

The Queensland Government, as a component of the Queensland Water Recycling Strategy, commissioned the construction of the Advanced Water Recycling Demonstration Plant (AWRDP) (Gibson and Apostolidis, 2001), which is shown in Figure 1.

The plant consists of eight modules, each housing a different water treatment technology. For these studies, the influent to the AWRDP was taken from tertiary treated effluent from the nearby Brendale Wastewater Treatment Plant (Pine Rivers Shire Council).



Figure 1 Photograph of the AWRDP

The modules of the AWRDP included lime clarification (LC), dissolved air flotation (DAF), dual media filtration (DMF), ozonation (O₃), biological activated carbon filtration (BAC), microfiltration (MF), combined reverse-osmosis/nanofiltration (RO/NF) and UV-disinfection. Chlorine can be added if the use of the water requires the presence of a disinfection residual. Individual modules may be brought on-line or bypassed very quickly and simply. A schematic illustration of the AWRDP modules is presented in Figure 2.

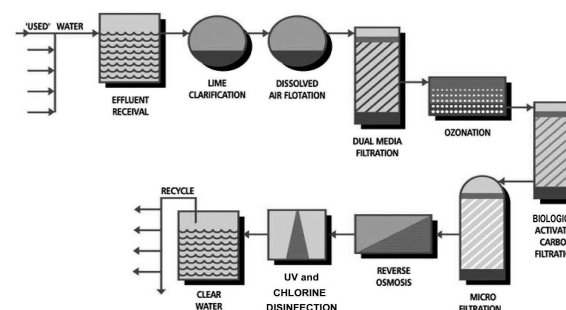


Figure 2 Schematic illustration of QLD AWRDP showing individual modules

Pharmaceutical residues to be investigated were selected according to their likelihood of being detected in the treated sewage (Khan and Ongerth, 2004) and their propensity for simultaneous extraction and analysis. The estrogenic hormones were selected as key estrogenic compounds in sewage. The chemical structures of the target analytes are shown in Figure 3.

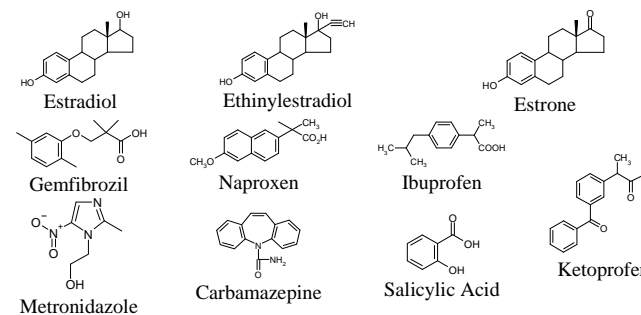


Figure 3 Molecular structures of the estrogenic hormones and pharmaceutically active compounds (PhACs)

Methods

Analysis of Pharmaceuticals

Duplicate 200 ml aliquots of each sample were measured into amber glass bottles. Samples were adjusted to pH 2 with dilute H₂SO₄ and processed immediately after collection. An acetonitrile solution (100 µl) of the surrogates, paracetamol-d₄ (10.11 mg/l), salicylic acid-d₆ (10.24 mg/l) and carbamazepine-d₁₀ (10.09 mg/l), were added to each sample. SPE was performed on preconditioned Envi Chrom P cartridges at a flow rate no greater than 5 ml/min. The SPE tubes were then washed and then dried under nitrogen for at least 3 hours. They were eluted with 2ml of a 50:50 acetonitrile/acetone mixture and evaporated to dryness under a constant stream of N₂ at 30°C. All samples were derivatised by reaction with BSTFA (200 µl) in acetonitrile (200 µl) at 70°C for 1

hour. Samples were allowed to cool to room temperature before adding the internal standard, fluazifop-butyl (12.38 mg/l, 100 µl), and further acetonitrile (200 µl). All mass spectrometric measurements were acquired in selective ion monitoring (SIM) mode on a Hewlett-Packard HP5973 mass-selective detector combined with HP6890 gas chromatograph. Samples were chromatographed using a HP-5MS capillary column (5% Phenyl Methyl Siloxane, 30m x 0.25mm I.D. x 0.25µm film thickness). The carrier gas was helium at a constant flow rate of 1 ml/min. The oven temperature was held at 100°C for 3min after sample injection, and then ramped at 30°C/min to 170°C and then at 1°C/min to 200°C. Finally it was ramped at 30°C/min to 260°C and held for 15 min. A 1ml injection volume was administered by means of a HP6890 series autosampler using hot splitless injection. Ion dwell times were assigned to achieve a scan rate of 3-4 cycles per second within each SIM window. Analytes and their isotopic surrogates were each measured within a single SIM window, as were carbamazepine and phenytoin, which had similar retention times. Quantification was undertaken by calibration with the aid of the internal standard.

Analysis of Hormones

1 L samples were extracted on preconditioned 1g C18 cartridges under vacuum after the addition of estradiol-D4 as surrogate standard. The cartridges were dried under nitrogen and the extracts were eluted with acetonitrile. The eluents were then dried under vacuum. The estrogens were determined by GC-NICI-MS after a two-step derivatization with pentafluorobenzyl bromide followed by *N*-trimethylsilylimidazole. This method was applied for the analysis of 17β-estradiol, estrone and ethinylestradiol in aqueous matrices using the d4-estrone as an internal standard.

Treatment Plant Trials

Initial investigations were carried out to determine “background” concentrations of the target compounds in the effluent from the various stages of the AWRDP. Background analyses were undertaken with the plant in two different configurations. The first configuration did not include the ozonation module. The second run was carried out with all modules including ozonation online. These two configurations were selected to optimise the likelihood of the detection of target compounds in the effluents of the DMF, O₃, BAC, MF and RO/NF modules. All experiments described in this study were conducted with the RO/NF module loaded with both RO and NF subunits (Trisep X20 and XN40 membranes). These subunits were operated in parallel and samples were collected from each simultaneously. In addition to investigating the treatability of contaminants that were measurable in the various module influents, the small scale of the AWRDP permitted the undertaking of spiking experiments. By spiking measurable concentrations of the seven pharmaceutical compounds, it was possible to gain significantly more information on the behaviour of these compounds during the advanced treatment processes. Unfortunately, it was not practical to employ a continuous dosing method during these spiking tests. Therefore, an alternate method was used in which discrete doses were added to a feed reservoir every ten minutes over a one hour period. Two pharmaceutical & hormone spiking experiments were conducted. The first study incorporated both the microfiltration and RO/NF modules. The pharmaceuticals were spiked into the feed tank of the microfiltration module. Samples for analysis were taken from the microfiltration module (feed and permeate) and the RO/NF module (RO permeate, RO retentate, NF permeate, NF retentate). The second spiking experiment incorporated only the RO/NF module. The feed tank to the RO/NF module was spiked. Selected samples were then taken from the RO/NF feed, RO permeate, RO retentate, NF permeate, NF retentate.

Results

General Plant Performance in Terms of Bulk Organics Removal

The results of the removal of three bulk organics, total organic carbon (TOC), chemical oxygen demand (COD) and chlorophyll (as a bulk indicator for algae) are presented in Figure 4. The processes are operated in series and hence show a steady increase in water quality as expected.

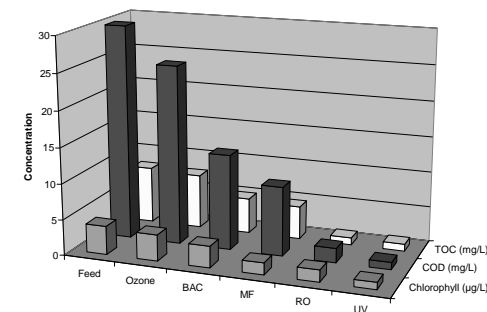


Figure 4 Retention of total organic carbon (TOC), chemical oxygen demand (COD) and chlorophyll by the different AWRDP processes

Background Analysis

The background concentrations of pharmaceutical residuals were measured in the various stages of the AWRDP in two plant configurations.

In the first configuration, the ozone treatment module was offline and samples were collected from the effluents of DMF, BAC, MF and RO/NF modules. Salicylic acid was identified in the effluents of every tested module with DMF (0.33 µg/L), BAC (0.23 µg/L), MF (0.13 µg/L), RO permeate (0.16 µg/L), RO retentate (2.21 µg/L) and NF permeate (0.17 µg/L). Gemfibrozil (0.18 µg/L) was measured in the DMF effluent, however none of the other analytes were measurable in the DMF, BAC, MF, RO permeate or NF permeate samples. However, most were concentrated enough to be measurable in the RO retentate with ibuprofen (0.31 µg/L), gemfibrozil (0.86 µg/L), naproxen (0.70 µg/L), ketoprofen (0.37 µg/L), estrone (0.092 µg/L), estradiol (0.0047 µg/L) and ethinylestradiol (0.0058 µg/L). Metronidazole and carbamazepine were not detectable in any of the samples. Once the ozone module was brought online, further background testing was undertaken on the effluents of the DMF module (immediately before ozone), the O₃ module and the BAC module (immediately after ozone). In these tests, salicylic acid was observed in DMF (0.65 µg/L), O₃ (0.26 µg/L) and BAC (0.34 µg/L) effluents. All other analytes were below the limits of detection.

Spiking Experiments

Concentrations of pharmaceuticals in various stages of the AWRDP during spiking experiments are shown for two plant configurations. The first spiking run was conducted without ozonation online. Six stock solutions, each containing salicylic acid (3.2 mg), ibuprofen (1.6 mg), gemfibrozil (1.2 mg), naproxen (0.8 mg) and ketoprofen (0.8 mg) in 200 ml of distilled water were prepared. These were dosed into the feed tank of the MF module at 10 minute intervals for one hour. Samples were collected after 30 minutes, 60 minutes and 75 minutes plant run time. Samples were collected from the feed water to the RO/NF module, both the permeate and retentate of a NF subunit, and the permeate from a RO subunit. Table 1 shows concentrations of each pharmaceutical residue in each sample.

Table 1 Concentration of pharmaceuticals (µg/l) during Spiking Experiment 1 (ozone offline)

Source	30 minutes				60 minutes				75 minutes			
	RO/NF feed	NF per	NF ret	RO per	RO/NF feed	NF per	NF ret	RO per	RO/NF feed	NF per	NF ret	RO per
Salicylic Acid	2.33	19.89	11.42	0.25	4.79	3.80	10.38	n.d.	n.d.	4.12	5.82	n.d.
Ibuprofen	0.36	0.47	0.72	n.d.	0.68	0.14	1.95	n.d.	0.36	0.22	1.88	n.d.
Gemfibrozil	0.76	0.43	1.08	n.d.	1.91	n.d.	5.31	n.d.	0.41	0.32	4.30	n.d.
Naproxen	0.31	0.39	1.11	n.d.	0.53	n.d.	3.17	n.d.	n.d.	n.d.	2.02	n.d.
Ketoprofen	0.22	n.d.	1.11	n.d.	n.d.	n.d.	2.30	n.d.	n.d.	n.d.	1.99	n.d.

n.d.: not detected.

The second spiking experiment was conducted with the ozone treatment module online. Six stock solutions, each containing salicylic acid (1.3 mg), ibuprofen (2.2 mg), gemfibrozil (1.2 mg), naproxen (0.8 mg) and ketoprofen (1.1 mg) in 200 ml of distilled water were prepared. These were dosed into the feed tank of the RO/NF module at 10 minute intervals for one hour. Samples were collected at 45 minutes and 75 minutes after the beginning of the dosing period. Samples were collected from the feed and permeate water of the MF module, as well as the retentates and permeates of the RO and NF subunits of the RO/NF module. Table 2 shows the concentrations of each pharmaceutical residue in each sample.

Table 2 Concentration of pharmaceuticals ($\mu\text{g/l}$) during Spiking Experiment 2 (ozone online)

Source	45 minutes						75 minutes					
	MF feed	MF	RO ret	RO per	NF ret	NF per	MF feed	MF	RO ret	RO per	NF ret	NF per
Salicylic Acid	6.87	3.07	1.44	n.d.	0.57	n.d.	0.56	n.d.	n.d.	n.d.	n.d.	n.d.
Ibuprofen	1.87	0.79	1.04	n.d.	2.83	0.85	0.50	n.d.	n.d.	n.d.	0.96	n.d.
Gemfibrozil	2.19	1.53	1.95	n.d.	4.65	0.28	0.70	n.d.	0.15	n.d.	3.36	n.d.
Naproxen	1.65	0.63	1.32	n.d.	2.76	0.49	0.38	n.d.	n.d.	n.d.	0.65	n.d.
Ketoprofen	0.86	0.29	0.82	n.d.	3.09	0.32	n.d.	n.d.	n.d.	n.d.	0.04	n.d.

n.d.: not detected.

The hormone spiking tests were performed at identical intervals with hormone masses of 1.2 mg to allow for losses on the treatment system due to the likely adsorptive interactions as described by Schäfer *et al.* (2003) and Nghiem and Schäfer (2002). The results of these experiments are displayed graphically in Figure 4.

Discussion

These experiments were undertaken to determine the effectiveness of a range of advanced water recycling technologies for the removal of pharmaceuticals from treated sewage effluent. The technologies investigated included ozonation, microfiltration, nanofiltration and reverse-osmosis. The following discussion examines the results of the experiments in relation to the treatment technology and its suitability for use within a treatment train.

Ozonation

An OZAT CFS-2A ozone generator from Ozonia/Switzerland is used in the AWRDP. The generator has a production capacity of 80 g of ozone per hour and requires 2 m³ of air per hour to achieve this. The dosage rate is 17.5 mg O₃/L and the contact time 15 minutes.

Ozonation was only briefly examined in this study. The influent to the ozonation module was the effluent from the dual-media filtration module. By comparing the concentrations of pharmaceuticals in the ozonation influent to the ozonation effluent it was intended to determine the effectiveness of ozonation in the removal of these compounds. Since the influent is already quite highly treated wastewater, only salicylic acid was measurable. The result obtained indicates that salicylic acid was partially degraded from the influent (0.65 $\mu\text{g/l}$) to the effluent (0.26 $\mu\text{g/l}$) during ozonation.

In other recent studies, ozonation has been shown to be variably effective in the abatement of various pharmaceutical and steroidal residues in sewage. Excellent degradation efficiencies have been reported observed for a range of compounds (Adams *et al.*, 2002; Huber *et al.*, 2003; Ternes *et al.*, 2003). However, for some classes of drugs, most satisfactory results have been obtained with increased ozone concentration (Ternes *et al.*, 2002) or the combined application of ozone/hydrogen peroxide (Zwiener and Frimmel, 2000). However, it should be noted that very little is known about the formation of by-products during ozone degradation of organics.

Microfiltration

Initial testing of the wastewater indicated that salicylic acid was partially removed during microfiltration. The previous module (biological activated carbon) exhibited an effluent concentration of 0.23 $\mu\text{g/l}$, while the microfiltration effluent was reduced to 0.13 $\mu\text{g/l}$.

Spiking experiments were undertaken to further investigate the pharmaceutical removal efficiency of microfiltration (Table 2). During the spiking period, all of the analytes were observed in both the feed (influent) to the microfiltration unit and the effluent, however a partial reduction in concentration was evident for all pharmaceuticals (Figure 5). Most of the pharmaceuticals were not measurable in samples collected from the microfiltration unit 25 minutes after the cessation of spiking (Table 2).

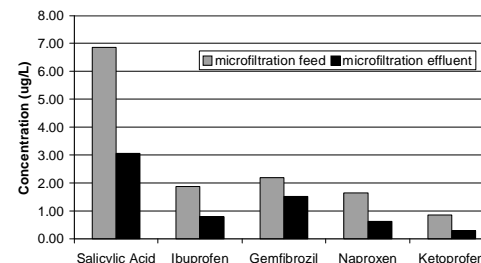


Figure 5 Removal of pharmaceuticals by microfiltration during Spiking Experiment 1.

The observed concentration reductions could be attributable to adsorption on the membrane rather than removal by size exclusion. This possibility could potentially be confirmed by the examination of removal over extended periods of operation, in which time the adsorptive capacity of the membrane for a particular compound may reach saturation. Previous studies have indicated that organics may be adsorbed, on initial spiking, to the microfiltration membrane. Once the influent concentration drops, the compounds then desorb from the membrane (Chang *et al.*, 2002). This process may have contributed to the observations described here.

Another possible explanation is that the compounds may have been removed by size exclusion of larger particles on which the compounds may have been adsorbed. The accumulation of retained components in front of the membrane might also lead to a significant shift in separation behaviour, where the cake layer, rather than the membrane, determines the actual retention.

Nanofiltration

Spiking tests undertaken for the nanofiltration units gave some erratic results, where the membrane permeate concentration was greater than the measured concentration in the feed to the module (Table 1, salicylic acid, ibuprofen, naproxen). This was likely to be partially the result of variations caused by the stepwise (non-continuous) dosing regime and the time delay between sample collections, as well as the proximity to the detection limit. The retentate concentrations showed a definite increase which is a reflection of some degree of retention.

Overall the retention of pharmaceuticals and hormones was lower than for RO which was expected from earlier laboratory trials where eight different nanofiltration and reverse osmosis membranes were compared. It should be noted that the nanofiltration membrane installed in this plant (XN40) has generally a very low retention of organic contaminants as confirmed in laboratory tests (Schäfer *et al.* 2003). Whether nanofiltration will be sufficient for the removal of such contaminants will depend on the levels to be achieved and the intended application of the treated water. It can be expected that retention for individual compounds varies strongly with the membrane type. This can be attributed to the similarity in membrane 'pore' size and the size of the contaminants.

The hormone retention by the membrane processes is shown in Figure 6. Feed concentrations are in a similar range as hormone concentrations found to date in Australian wastewaters (results not published). The permeates are very low confirming small scale results (Schäfer et al. 2003) where NF concentrations are slightly higher than RO permeates. Of interest, again, is the high concentration of all hormones in the RO concentrate.

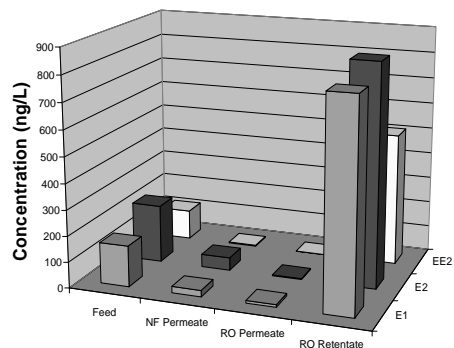


Figure 6 Hormone concentrations in NF and RO feed, permeate and concentrate

Reverse Osmosis

The results of the spiking tests undertaken in this study indicate that the reverse osmosis process was the most effective in the removal of all of the tested pharmaceutical compounds (Table 1 and Table 2). Only in one case was any of the pharmaceuticals identified in the reverse osmosis permeate (salicylic acid, 0.25 µg/l) whereas they were regularly observed in the feed (Table 1) and the retentates (Table 2) at significant concentrations. Hormones appear to be more difficult to remove with traces of those compounds found in the RO permeates. This is in accordance with small laboratory-scale tests. The retention is highly membrane type dependent and further individual membrane testing will be required to fully characterise the retention mechanisms.

The results obtained for the reverse osmosis analysis comply with other recently reported studies, in which pharmaceuticals have been almost universally removed from contaminated waters (Adams *et al.*, 2002; Drewes *et al.*, 2002; Heberer *et al.*, 2002).

Conclusions

Of the advanced wastewater recycling technologies considered in this study, reverse-osmosis was able to demonstrate the most significant and consistent removal of the investigated pharmaceutical compounds and substantial removal of hormones. As such, reverse osmosis shows promising potential for the effective routine removal of a wider range of pharmaceutical residues from treated wastewaters, although some nanofiltration membranes can also achieve substantial removal. The results of this study will provide valuable information for the development of guidelines that will assist water authorities to carry out water recycling projects.

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