Fate of selected pharmaceutically active compounds in the integrated fixed film activated sludge process

K. J. Murray, W. J. Parker, L. M. Bragg and M. R. Servos

ABSTRACT

The potential for integrated fixed film activated sludge (IFAS) processes to achieve enhanced transformation of pharmaceuticals relative to conventional activated sludge (CAS) processes was assessed. Previous studies have focused on direct comparisons of parallel reactors with and without fixed film carriers and little information is available on the impacts of how varying operating parameters impact the differences in observed pharmaceutical compound (PC) transformation capabilities between CAS reactors and those equipped with both an activated sludge (AS) and fixed film carriers. The testing was carried out using bench scale sequencing batch reactors fed with authentic municipal wastewater and operated at selected combinations of temperature and solids retention time (SRT). PC transformation efficiencies were assessed in a 2^2 factorial design that employed the IFAS and CAS processes, operated in parallel under identical process conditions. Nitrification rate testing that was conducted to obtain insight into the biomass activity demonstrated that IFAS consistently had improved nitrification kinetics despite lower mixed liquor volatile suspended solids levels thereby demonstrating the contribution of the biofilm to nitrification. Increased transformation of atenolol (ATEN; ranging from 10–60%) and trimethoprim (TRIM; ranging from 30–50%) in the IFAS equipped reactors relative to their respective activated sludge (AS) controls was observed under all experimental conditions. Negligible transformation of carbamazepine was observed in both reactors under all conditions investigated. More than 99% of acetaminophen was transformed in both configurations under all conditions. There was no correspondence between nitrification activity and TRIM removal in the control AS while conditions that stimulated nitrification in the control AS also resulted in enhanced removal of ATEN. The results of this study indicate that the integration of biofilms in AS processes enhances transformation of some PCs.

Key words | biofilm, biotransformation, fixed films, IFAS, integrated fixed film activated sludge, nitrification, pharmaceuticals, wastewater

INTRODUCTION

Treated municipal wastewaters have been reported to be the most significant source of pharmaceuticals into the environment (Ternes & Joss 2006). Hence, methods of improving the transformation potential achievable through wastewater treatment facilities have been previously studied (Batt et al. 2007; Kim et al. 2009; Radjenović et al. 2009; Faláš et al. 2012). Past studies investigating the fate of pharmaceutical compounds (PCs) through biological wastewater treatment processes have suggested that operation at extended solids retention times (SRTs) results in increased removal of PCs (Clara et al. 2005; Leu et al. 2012). The majority of these studies have investigated differences between low SRT activated sludge (AS) processes, typically used for organic reduction only, and processes with higher operating SRT, such as nitrifying conventional activated sludge (Kreuzinger et al. 2004) nutrient removal processes (Rosal et al. 2010) and membrane bioreactors (Radjenović et al. 2009; Maeng et al. 2013). It has been hypothesized that co-metabolism via enzymes with broad specificity may be responsible for the removal of PCs. In support of this theory, many researchers have noted a connection between nitrifying conditions and improved removals of certain PCs.

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Historically, pharmaceutical fate investigations have almost exclusively been based on the AS process and its many configurations. Despite their significant implementation, fixed film processes have received relatively little attention with regards to the fate of PCs. In particular, the integrated fixed film activated sludge (IFAS) process, which is an emerging technology utilized for bioreactor retrofitting to meet increasingly stringent effluent requirements, has received limited attention regarding the transformation potential of PCs (Kim et al. 2009). The limited number of investigations of PC fate in biofilm systems have reported that these processes provided enhanced PC transformation capabilities in comparison to AS systems (Kim et al. 2009; Falás et al. 2012; Margot et al. 2013; Zupanc et al. 2013). It is hypothesized that the extended SRT associated with the biofilm in these systems may be responsible for the enhanced transformation of PCs. There is, however, a lack of information on the relationship between process operating conditions, such as temperature and SRT of the AS, and their impact on the ability of the IFAS process to transform pharmaceuticals when directly compared to a suspended growth-only treatment process.

The current study investigated the fate of four PCs in parallel bench scale IFAS and AS reactors operated at selected combinations of suspended growth SRT and temperature. Acetaminophen (ACE), atenolol (ATEN), carbamazepine (CBZ) and trimethoprim (TRIM) were analyzed through liquid chromatography tandem mass spectrometry (LC-MS/MS) following solid phase extraction (SPE). These compounds were selected to represent a range of pharmacokinetic properties and on the basis of their reported prevalence in Canadian wastewaters and surface waters. The results of this study are intended to provide additional data on the ability of biofilm processes to transform PCs over a range of operating conditions.

MATERIALS AND METHODS

Experimental design

A comparative investigation was conducted to assess the ability of the AS and IFAS process to transform select pharmaceuticals. This was achieved by operating two bench scale wastewater treatment pilot reactors under similar process conditions. One reactor was operated with only a suspended growth AS biomass (control) and the other was operated with both a biofilm and an AS biomass (IFAS). By operating the two reactors under the same experimental conditions, and at selected combinations of suspended growth SRT and temperature (Figure 1), the effect of the biofilm, as well as SRT and temperature, on PC transformation was examined. In practice, the IFAS process allows the bioreactor to be operated under significantly reduced suspended growth SRTs while still achieving nitrification. However, in this study, both the control and the IFAS configurations were operated with suspended growth SRTs that are typically required to achieve nitrification with AS bioreactors. The IFAS suspended growth SRTs were therefore somewhat greater than the typical design criteria for full scale IFAS process (WEF 2010). However, this approach allowed for a direct comparison of the effects of the biofilm without introducing confounding effects of different suspended growth SRTs. The temperatures evaluated in this study (12 and 18 °C) spanned the range of wastewater temperatures that are typically observed in Southern Ontario.

The testing was conducted using 30 L bench-scale pilot reactors operated as sequencing batch reactors (SBRs). Each of the IFAS SBRs contained 10 L of AC 450 IFAS media (Headworks International, Houston, TX), providing 402 m²/m³ of specific surface area. To provide sufficient freeboard, the conventional SBRs were operated with a total fill volume of 20 L. The volume decanted during each cycle was 13.3 L, with an equivalent volume added to the SBRs during the feed cycle, resulting in a volume of 6.7 L remaining after decant representing the settled and compacted biomass. The conventional SBRs were therefore operated under an hydraulic retention time (HRT) of 9 h.

WEF (2010) suggests that IFAS reactors are typically operated with a media fill fraction between 40 and 60 percent (volume/volume). 10 L of media was initially added to the pilot reactors. However, issues encountered with IFAS media displacement and bulking, the operating volume of the IFAS reactors was increased to 25 L to permit a feed volume of 15 L. This provided a consistent contaminant loading between all pilot reactors. It is reported by the IFAS media supplier that the IFAS media displaces approximately 171 mL per L of media. This results in a displacement of approximately 1.7 L in the IFAS reactors, achieving an effective operating volume of 23.3 L and a slightly increased HRT of 10.8 h.
Authentic municipal wastewater from a large wastewater treatment plant (WWTP) (approximate population serviced: 180,000) that had undergone primary settling was utilized as feed wastewater. Typical characteristics of the feed wastewater are summarized in Table 1.

The SBRs used in this study had been previously employed in prior studies and, therefore, the biomass in the reactors had developed over several years prior to the current study. The SBRs operated on a 6 h treatment cycle which consisted of feed (30 min); react (200 min); waste (5 min); settle (55 min); and decant (70 min). The feed, decant and waste volumes were all conveyed to and from the reactors by means of peristaltic pumps.

All reactors were of glass construction and were equipped with water jackets fed by either heated (20 °C) or cooled (11 °C) water to maintain the target temperature. The pH was maintained between 7.0–7.5 through the addition of sodium bicarbonate via programmable logic controller (PLC) actuated peristaltic pumps. Dissolved oxygen was maintained by means of luminescent dissolved oxygen probes that informed a PLC which controlled the flow of compressed air into the reactors. The control SBRs were operated at a target dissolved oxygen concentration of 2 mg/L and the IFAS SBRs were operated at 4 mg/L based on typical operation conditions suggested by WEF (2010).

The reactors were operated at the target suspended growth SRT on the basis of wasting volumes that were established from measurements of the effluent total suspended solids (TSS) and the mixed liquor suspended solids. The procedure was employed to establish the SRT of the suspended growth biomass only and all subsequent references to SRT are for the suspended growth. The overall SRT of the IFAS SBRs was longer than the reported values due to the presence of the biofilm. No attempt was made to quantify the age of the biofilm due to uncertainties associated with quantifying the biomass inventory on the carriers. The fill, react and waste phases all occurred under aerated conditions and therefore the duration of the aerobic period was 235 min, or approximately 65% of each cycle. The remainder of the time the reactors were not aerated to facilitate settling and decanting. It was however anticipated that biological activity would be reduced during this time as most readily degraded material would be removed during the preceding aerobic cycle. Under the target SRT conditions of 7 and 20 days, the aerobic SRTs were estimated to be approximately 4.6 and 11.7 days, respectively.

The performance of each process with respect to chemical oxygen demand (COD) and nitrogen species was assessed to ensure the reactors were operating under steady state conditions at the time of PC sample collection. As the IFAS process is frequently utilized in practice to provide nitrification, the ability of the reactors to achieve nitrification was given particular focus. Batch nitrification testing was conducted to assess the performance of the nitrifiers within each reactor under each experimental condition. The batch specific nitrification rate (SNR) tests assessed nitrification rates through measurements of the total ammonia nitrogen (TAN), NO3-N and NO2-N.

In each SNR test, the reactor feed was dosed with anhydrous ammonium chloride (NH4Cl) to establish an initial TAN concentration of approximately 30 mg/L. Simultaneously, a 50 mL sample was collected for the determination of the mixed liquor volatiles suspended solids (MLVSS) concentration. SNR testing was typically conducted over a period of 2.5 to 3 h with samples collected at intervals of 30 min to 1 h. All collected samples were analyzed for TAN, NO3-N and NO2-N. Linear regression was used to determine the nitrification rate.

This investigation focused on the fate of four PCs, namely:

- the anti-epileptic CBZ;
- the non-steroidal anti-inflammatory drug ACE;
- the beta blocker ATEN; and
- the antibiotic TRIM.

The four PCs investigated were selected on the basis of:

- a range of biotransformation potential previously observed in other investigations;
- a variety of pharmacokinetic properties;
- prevalence in Canadian waters;
- highly limited potential for volatilization within the pilot bioreactors;

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Influent feed characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Average concentration</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO3)</td>
<td>275</td>
</tr>
<tr>
<td>cBOD5 (mg/L)</td>
<td>83</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>248</td>
</tr>
<tr>
<td>TAN (mg/L)</td>
<td>20.9</td>
</tr>
<tr>
<td>TKN (mg/L)</td>
<td>26.8</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>88</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>8.0</td>
</tr>
</tbody>
</table>

cBOD5, 5 day carbonaceous biochemical oxygen demand; COD, chemical oxygen demand; TAN, total ammonia nitrogen; TKN, total Kjeldahl nitrogen; TSS, total suspended solids; TP, total phosphorus.
• low potential for sorption to biomass solids, as demonstrated by previous studies;
• prevalence in prior studies to permit comparisons with published data;
• ability to be quantitated using available analytical equipment utilizing multi-residue methods; and
• availability of labelled standards.

The removal of PCs through wastewater treatment can be attributed to three main fate mechanisms, namely: volatilization, sorption and transformation (Schwarzenbach et al. 2005). Struijs et al. (1991) estimated that for compounds with a Henry’s Law constant less than $1 \times 10^{-4}$ (atm·m$^3$·mol$^{-1}$), volatilization is expected to account for approximately 5% or less of the total removal from a wastewater treatment process. Similarly, Ternes & Joss (2006) report that a Henry’s Law constant greater than $3 \times 10^{-5}$ (Pa·m$^3$·mol$^{-1}$) is required to observe any losses due to air stripping in a bioreactor with fine bubble aeration. Ternes & Joss (2006) also postulate that compounds with a $K_d$ value less than 0.3 L·gSS$^{-1}$ can be expected to experience removal of <10% in a typical municipal sewage treatment plant. Table 2 presents Henry’s Law constants and $K_d$ values for the four PCs investigated under this study, as reported in the literature.

Due to the very low potential for volatilization and sorption of the PC compounds selected, it was assumed that any observed loss of PC, as calculated based on comparison of initial and final samples collected from the pilot reactors, was attributed to PC transformation resulting from the wastewater treatment processes employed. PC transformation was assessed by measuring concentrations in the reactor decant (final effluent) relative to those present at the beginning (initial) of a react cycle. Sampling campaigns spanned from Monday to Friday of a week to minimize temporal variability and capture typical conditions. The initial samples were generated each day by blending a sample of the decant from the cycle prior to the target cycle with primary clarifier effluent at a volumetric fraction equivalent to their volumes within the reactor. Decant samples were collected by capturing the SBR decant at the end of the target cycle in a 20 L stainless steel container. All containers used for sample collection were rinsed with methanol once and thrice with deionized water prior to the next sampling event. At the end of each sampling campaign, the 20 L stainless steel containers were soaked in a dilute solution of Contrad-70 (Decon Labs, PA, USA) to ensure no PC carryover occurred between rounds.

All samples were stored in 500 mL amber silanized glass bottles, preserved using ascorbic acid and sodium azide and refrigerated at 4 °C prior to sample preparation and analysis. Ascorbic acid and sodium azide were dosed to achieve in-bottle concentrations of 1 g/L and 50 mg/L, respectively. Small aliquots were collected from the sample bottles prior to the addition of ascorbic acid and sodium azide for the analysis of conventional contaminants.

PC characterization was conducted in three rounds (Table 3); the first round involved a control and IFAS reactor operated at an SRT of 20 d and a temperature of 18 °C. The second round was intended to include both a control and IFAS operated at an SRT of 20 d and a temperature of 12 °C, however, the development of filamentous organisms within the control reactor required that sampling of this reactor be delayed; only samples from the IFAS SBR were obtained at this time. Samples were collected from reactors operating at the remaining conditions during round 3.

Typically, a period equivalent to three SRTs is allowed to reach steady state operating conditions (Sobeck & Higgins 2002). In the current study steady state operation was assessed based on the on-going measurement of conventional parameters as well as meeting the target SRT

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Sampling round</th>
<th>SRT (d)</th>
<th>Temp (°C)</th>
<th>Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>20</td>
<td>18</td>
<td>I20-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C20-18</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>20</td>
<td>12</td>
<td>I20-12</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>20</td>
<td>12</td>
<td>C20-12</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>18</td>
<td></td>
<td>I7-18</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12</td>
<td></td>
<td>I7-12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C7-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C7-12</td>
</tr>
</tbody>
</table>
requirements. However, in all cases, a minimum of two SRTs was provided following operational changes to allow steady state operation to be reached.

Based on the PC concentrations observed in both the initial and final samples, transformation efficiencies were calculated. It should be noted that, due to the configuration and operating nature of the SBR, the initial samples represent a reduced concentration from the reactor influent, due to the dilution effect provided by the volume of mixed liquor remaining in the pilot reactors at the end of decant. In this way, the transformation efficiencies calculated are somewhat lower than would be expected from a plug flow reactor, as would be found in some full scale WWTPs, due to this dilution effect. Statistical analysis utilizing a three factor analysis of variance (ANOVA) approach (\( \alpha = 0.95 \)) was conducted using Minitab 17 software (Minitab Inc., PA, USA). In the ANOVA, factors were considered significant if the calculated p-value was less than 0.05 for that factor. The calculated transformation efficiencies for all PCs were expected to demonstrate some skewing, due to the limitations (truncation of data) imposed by the effluent method quantitation limits (MQLs). While this skewing of transformation efficiency data likely resulted in a slightly non-parametric distribution for some reactors, normality was assumed as the MQLs only affected a limited number of transformation efficiency data.

**Analytical methods**

Samples collected for the purpose of PC fate investigations were analyzed for conventional contaminants to assess the reactor biological performance. The conventional parameters included total and volatile suspended solids (TSS, VSS), total COD (tCOD), soluble COD (sCOD), NH3-N (TAN), NO3-N and NO2-N (effluent samples only). The analysis of all conventional contaminants was performed using the methods described in Standard Methods (APHA 2005). TSS and VSS samples were analyzed using method 2540D and 2540E in Standard Methods (APHA 2005). All remaining chemical parameters were analyzed using HACH Test n’ Tube methods and a HACH DR 2800 spectrophotometer.

The preparation of samples for PC analysis was based on the method of Vanderford & Snyder (2006) and Trenholm et al. (2009) with minor adjustments. Briefly, a sufficient volume of each sample was filtered using a 1 µm nominal glass fibre filter (Pall Corporation) to yield 100 mL of filtrate. Subsequent sample preparation was conducted in sets of 12 with each set containing one method blank (MB) and two matrix spikes (MSs) for quality control purposes. MBs consisted of 100 mL of Milli-Q water and were used to assess analyte carryover associated with sample preparation and analysis. MSs were created using 100 mL of Milli-Q water spiked with 100 µL of a methanol solution containing ATEN, CBZ and TRIM at a concentration of 100 µg/L achieving a concentration of 100 ng/L within the sample. ACE was initially spiked at a concentration of 100 ng/L for the first round of samples processed, but due to signal issues was increased to 10 µg/L for all subsequent samples to more closely match concentrations expected in the primary effluent. Each sample, with the exception of the blank samples, was spiked with 100 µL of methanol solution containing isotopically labelled standards (ILSs) of ATEN, CBZ and TRIM at a concentration of 100 µg/L achieving a final sample concentration of 100 ng/L. Similarly, an isotopically labelled ACE surrogate was initially spiked at 100 ng/L during the first round of samples processed, but increased to 10 µg/L in rounds 2 and 3 of analysis. ILSs, which included Atenolol-d3, Carbamazepine-d10, Trimethoprim-d5 and N-(4-Hydroxyphenyl)-2,3,5,6-d4 Acetamide (deuterated ACE) were obtained from C/D/N Isotopes (Point-Claire, Quebec).

Due to the low concentrations present within the samples as well as the presence of co-eluting compounds, SPE was utilized. Oasis HLB 500 mg 6 cc cartridges (Waters, Mississauga, Ontario) were utilized for extraction as described in Vanderford & Snyder (2006). Automatic SPE was performed using a Dionex Autotrace 280 SPE instrument. Eluted samples were evaporated to dryness under either a gentle stream of nitrogen or using a Dionex Rocket vacuum evaporator. The first round of samples analyzed were reconstituted using 2.5 mL of acetonitrile. However, this low concentration factor (40×) yielded poor results and subsequent analytical phases utilized 500 µL of methanol for reconstitution, resulting in a concentration factor of 200 times. All samples were stored at −20 °C until analysis was performed.

The analysis of all samples for PCs was conducted using LC-MS/MS with electrospray ionization in positive mode (ESI+). Both a commercial laboratory (ACE, ATEN) and a research laboratory at the University of Waterloo (CBZ, TRIM) were used for the analysis of round 1 samples while the second and third rounds of analysis were conducted entirely at the University of Waterloo. The analytical method employed by the commercial laboratory was proprietary in nature, but was based on the EPA 1694 method (Englert 2007) and used an high performance liquid chromatography (HPLC), operated in electrospray ionization positive (ESI+) mode, coupled with an AB Sciex QTRAP 5500 MS for sample analysis. Round 1
Sampling included the collection of 12 samples characterizing seven measurements: three individual; three duplicate; and one triplicate. After review of the analytical data, it was decided to increase the number of samples collected for rounds 2 and 3 to 18 (six measurements collected in triplicate), to reduce data uncertainty. The University of Waterloo laboratory utilized internally developed methods (Arlos et al. 2014). Methods employed to establish recovery and MQLs are presented in detail in Murray (2014).

RESULTS

Measurements of COD were employed to compare the SBR process configurations and operating conditions with respect to removal of organic matter. The total and soluble COD concentrations present in the effluents were relatively elevated when compared to typical treated municipal wastewater (Envirosim Associates 2011) and it was concluded that the sewage contained an elevated concentration of non-biodegradable soluble COD (nbsCOD). ANOVA testing indicated no significant differences in decant COD concentrations between test conditions and hence it was concluded that all reactors provided a similar level of organic removal. The variability in effluent tCOD values was attributed to varying contributions of particulate COD to the effluent tCOD and may have resulted from inefficient settling in the bench scale set-ups.

Previous studies have associated enhanced PC transformation with nitrification and hence the nitrification performance of the reactors was characterized. The IFAS reactors achieved full nitrification under all experimental conditions (decant TAN concentrations less than 0.3 mg/L). By comparison, the control reactors provided full nitrification under all but the low temperature, low SRT condition (C7-12) (mean final concentration = 11.4 mg/L, $\sigma = 4.75$ mg/L). The effluent TAN concentrations within the decant samples collected from the seven conditions that provided nitrification (I20-18, C20-18, I20-12, C20-12, I7-18, C7-18, I7-12) were found not to be statistically different at a confidence limit of 95%, suggesting these reactors provided similar nitrification performance.

Nitrification rate testing was conducted to further assess the activity of the nitrifying biomass in the reactors (Table 4). Table 4 indicates good correspondence between the TAN removal and NOx production rates in all cases, thereby providing confidence in the rate measurements. Further, the results of the nitrification rate testing were consistent with the effluent TAN concentrations observed from the various pilot reactors in that substantial nitrification rates were observed under all conditions except the C7-12 test. When compared with the effluent data, which demonstrated no statistically significant differences in final effluent TAN results between all reactors except C7-12, the nitrification rates (TAN removal rates and NOx production rates) in the IFAS reactors were statistically higher than the controls except for the 7 day SRT, 18oC conditions. The differences in nitrification rates between the IFAS and controls were, however, modest. The nitrification results will be subsequently discussed in context with the PC results.

MLVSS concentrations were measured in the reactors as a means of assessing the suspended growth biomass present under each of the experimental conditions (Figure 2). The MLVSS concentrations were consistently lower in the IFAS reactors (30 to 60%), with the exception of the 7 day SRT 12oC condition. This was attributed to the presence of the biofilm within the IFAS media and the limited substrate availability within the suspended growth phase. Hence, the IFAS reactors provided conventional removal performance that matched that of the paired controls while operating with significantly reduced suspended growth biomass concentrations.

The accuracy and precision of the PC analytical methods were assessed through a review of the MS and MQL values. Table 5 presents the measured recovery of MSs as well as the estimated MQL values for each parameter under each round of sampling. Round 1 MS recoveries were noted to be poor for CBZ (195%, $\sigma = 52\%$) and ATEN (54%, $\sigma = 21\%$) and this was attributed to the sample preparation methodology employed by the commercial laboratory. Rounds 2 and 3 MS recoveries for all parameters demonstrated significant improvements following a modification of the sample preparation procedure. The MQL values were lower for the decant samples in comparison to the initial samples and this was attributed to reduced matrix effects in the final

### Table 4: Results of nitrification rate tests

<table>
<thead>
<tr>
<th>Reactor</th>
<th>SRT (d)</th>
<th>Temp. (°C)</th>
<th>TAN removal rate (g N/d)</th>
<th>NOx production rate (g N/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAS</td>
<td>20</td>
<td>12</td>
<td>3.10 (0.33)</td>
<td>2.48 (0.14)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>5.36 (0.20)</td>
<td>6.46 (0.30)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12</td>
<td>3.26 (0.51)</td>
<td>2.96 (0.18)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>3.54 (0.13)</td>
<td>4.60 (0.17)</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>12</td>
<td>2.84 (0.12)</td>
<td>2.14 (0.27)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>3.34 (0.27)</td>
<td>3.38 (0.46)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12</td>
<td>0.39 (0.11)</td>
<td>0.14 (0.009)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>3.64 (0.11)</td>
<td>3.23 (0.39)</td>
</tr>
</tbody>
</table>

Values shown are mean values, values in parentheses represent standard deviations.
samples. The MQL values were generally higher than those typically reported in the literature, however, the methodology employed to estimate these values was considered to be more conservative than typical methods such as standard addition. Further discussion of the MQL values reported and a comparison to literature values are presented in Murray (2014). The elevated MQL values may have reduced the ability to discriminate between the IFAS and control reactors for ACE that was highly removed in both reactors such that the final values were consistently below the MQL.

The ranges of concentrations of the target PCs in the initial samples are summarized in Table 6. From Table 6 it can be seen that there was considerable variability in the initial concentrations of PCs. CBZ and ATEN were found to have statistically different values between sampling rounds. The concentrations of all compounds were, however, within the range of values reported in the literature (Table 6). For the purposes of this study, removal efficiencies were calculated on the basis of the measured concentrations at the beginning and end of each test cycle and, hence, the impact of the inherent variability in initial concentrations on the calculated removals was minimized.

Acetaminophen was found to be transformed in all reactors, under all experimental conditions at efficiencies greater
than 99%. Majewsky et al. (2011) reported transformation efficiencies of approximately 100% for acetaminophen, regardless of the operating SRT and the activities of autotrophs and heterotrophs. Kreuzinger et al. (2004) reported similar results for acetaminophen for a range of SRTs. The consistently high removal efficiencies precluded differentiation between reactor configurations or elucidation of temperature effects. The ACE results confirmed that the systems operated in this study were demonstrating a capacity for biodegradation of PCs that was consistent with that reported elsewhere.

In contrast with acetaminophen, the CBZ concentrations measured in the paired initial and decant samples were not significantly different. The transformation efficiencies in the IFAS and control reactors were clustered between 0 and −10% across all conditions. Hence, it was concluded that there was no substantive transformation of CBZ in either reactor type over all conditions investigated. Similar results have been reported in previous studies (Kreuzinger et al. 2004; Clara et al. 2005; Radjenović et al. 2009), including those employing both AS and fixed film process (Faláš et al. 2013). In these studies, only very minor (<15%), or in some cases negative, removals were reported regardless of the biological treatment process or operating conditions employed. An ANOVA analysis suggested that SRT, temperature or process configuration (i.e. presence of the IFAS associated biofilms) did not significantly affect transformation efficiency of CBZ. The lack of change in CBZ concentrations between the reactors were consistent with literature and also demonstrate that the analytical methodologies were reliable and reproducible.

The transformation efficiencies observed for TRIM in this study ranged from −10 to 70%. In some cases, the transformation efficiencies may have exceeded 70%, however, this could not be reported with certainty due to MQL limitations. This range of transformation efficiencies was consistent with results reported in the literature (Batt et al. 2007; Göbel et al. 2007; Rosal et al. 2010). In the referenced studies, removals were reported to range from 5% to over 90% in a variety of processes.

Preliminary analysis of the data suggested differences in TRIM behaviour between treatment conditions. Hence, box and whisker plots were created to compare TRIM transformation between the IFAS and control reactors (Figure 3). From these plots, it can be seen that consistent improvements in TRIM transformation efficiency were observed with the IFAS reactors for all four experimental conditions when compared with their respective controls. TRIM demonstrated the most significant transformation efficiency improvement (50%) for I20-18. It was noted that all other conditions resulted in similar improvements in transformation efficiency (30 to 37%) in the IFAS as compared to the control SBRs. This finding is consistent with the results of Faláš et al. (2013), that showed reactors operated with both fixed film carriers and a suspended growth phase demonstrated significantly increased degradation of TRIM when compared to a reactor operated with only a suspended growth phase.

It was demonstrated through ANOVA that SRT, temperature and process configuration had statistically significant effects on the transformation efficiencies of TRIM. Significant two-way and three-way interactions were noted for SRT*Temperature and SRT*Temperature*IFAS which suggested that these factors combined to result in improved transformation efficiencies. The general linear model used to describe TRIM transformation was noted to result in an R² value of 87%. This demonstrated that the three factors considered in the analysis contributed a majority of the observed variability of the data. Collectively, the results of the ANOVA tests and the box and whisker plots demonstrate the improved removals of TRIM in the IFAS process at elevated SRT and temperature (I20-18).
ATEN transformation efficiencies in this study were found to range between 0 and >90%. This range of transformation efficiencies was consistent with previous results reported in the literature (Maurer et al. 2007; Radjenovic et al. 2007; Radjenovic et al. 2009; Rosal et al. 2010) where removals have been reported to range from <10% to approximately 80% in a variety of processes.

Box and whisker plots were created to provide a visual indication of the ATEN transformations occurring within the IFAS and control reactors under the four experimental conditions (Figure 4). From these plots, it can be seen that IFAS demonstrated improved ATEN transformation efficiencies under all four experimental conditions with the most significant improvement in transformation efficiency observed in the I7-12 condition. ANOVA testing confirmed that SRT, temperature and process configuration had statistically significant influences on the transformation efficiencies achieved for ATEN. Significant two-way and three-way interactions were noted for SRT*IFAS and temperature*IFAS as well as SRT*Temperature*IFAS which suggests that these factors likely combined to result in improved transformation efficiencies. The general linear model used to describe ATEN transformation was noted to result in an R² value of 93% suggesting that the experimental factors explained a majority of the variability of the ATEN responses. The impacts of temperature on ATEN transformation were consistent with that reported by Castiglioni et al. (2006) for Italian WWTPs where, under winter conditions, ATEN transformation efficiencies ranged from 0 to 21%, whereas, under summer conditions, transformations efficiencies increased to 36–76%.

The potential interaction between groups of bacteria that are responsible for removal of conventional pollutants (i.e. nitrifying bacteria and heterotrophs) and PCs has been the subject of prior research. For example, Eichhorn et al. (2005) postulated that increased transformation rates could be attributed to the activity of the ammonia monooxygenase enzyme, produced by ammonia oxidizing bacteria (AOBs), as it has been shown to facilitate transformation of a variety of compounds with aromatic rings. The involvement of
AOBs was demonstrated through experiments using AOB inhibitors in which significantly reduced transformations occurred as a result of their addition. A relationship between the activity of nitrifying bacteria and ATEN removal has been reported (Sathyamoorthy et al. 2013) and Casas et al. (2015) suggested that increased ATEN removals may be attributed to co-metabolism, as the transformations observed coincided with nitrification. However, a linkage between ATEN removal and the activity of heterotrophs has also been reported (Sathyamoorthy et al. 2013). Studies of the role of groups of organisms such as AOB in PC removal have often been confounded by other factors such as SRT and the use of inhibitors that may impact multiple groups of organisms. The test plan employed in the current study provided the opportunity to further investigate the role of nitrifying bacteria in PC removal.

As previously discussed, reactor C7-12 did not demonstrate significant nitrification. This was anticipated as the low temperature and reduced SRT were expected to result in nitrifier washout. By contrast, the C7-18 reactor demonstrated substantial nitrification and this was attributed to the increased temperature that allowed sufficiently high growth rates of nitrifying organisms to avoid washout. From Figure 3 it can be observed that compared to C7-12 the stimulation of nitrification in C7-18 did not correspond to an increase in TRIM removal as both reactors achieved mean TRIM transformation efficiencies that were below 10%. Hence, it was inferred that there was no correspondence between nitrification activity and TRIM removal. By contrast, Figure 4 reveals that C7-18 had considerably higher removal of ATEN when compared to C7-12. Hence, there was a correspondence between onset of nitrification and ATEN removal. These results do not, however, imply causation as the higher temperature in C7-18, likely also stimulated the growth of other organism groups, such as heterotrophs that may have been active in ATEN removal. The results suggest that the impact of conditions which influence growth of specific organism types on removal of PCs is compound specific.
CONCLUSIONS

The impact of reactor configuration (IFAS versus AS), temperature and SRT on conventional pollutant and PC removal was investigated. COD removal was statistically similar in the IFAS and control reactors across all four experimental conditions. Full nitrification was observed for all reactors with the exception of the control SBR operated at a 7 day SRT and 12°C condition. The IFAS SBRs were found to demonstrate improved nitrification kinetics when compared to their respective controls operated under the same experimental conditions.

Acetaminophen was transformed at efficiencies greater than 99% under all conditions in both reactor configurations and, therefore, the performance of the reactors for this compound could not be differentiated. CBZ was found to not have been transformed under any conditions investigated. TRIM and ATEN demonstrated improved transformation efficiencies under all conditions within the IFAS reactors as compared to the controls. The presence of IFAS media, SRT and temperature were all found to significantly affect removals of TRIM and ATEN. There was no correspondence between nitrification activity and TRIM removal in the control SBRs while conditions that stimulated nitrification in the control SBRs also resulted in enhanced removal of ATEN. The results suggest that the incorporation of biofilms into AS processes can enhance the transformation of selected PCs.

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