Effects of tetracycline and ibuprofen on the relative abundance of microbial eukaryotic and bacterial populations in wastewater treatment

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ABSTRACT

The activated sludge process in a wastewater treatment plant (WWTP) relies on the activity of microbes to reduce the organic and inorganic matter and produce effluent that is safe to discharge into receiving waters. This research examined the effects of the non-steroidal anti-inflammatory drug ibuprofen and the antibiotic tetracycline on the relative abundance and composition of eukaryotes and bacteria in the microbial population present in activated sludge from a WWTP. The current investigation was designed to observe the impact of these contaminants, at low (environmentally relevant concentrations) as well as high concentrations of the drugs. Using 16S and 18S rRNA gene primer sets and quantitative polymerase chain reaction, the abundance of each population was monitored as well as the relative ratio of the two populations under the various conditions. It was found that current environmentally relevant concentrations of ibuprofen (100 ng/mL) stimulated eukaryotic growth but higher concentrations (2,000 ng/mL, 100,000 ng/mL) reduced their numbers significantly especially in the presence of tetracycline. Finally using denaturing gradient gel electrophoresis, some of the more abundant eukaryotes were identified and it was noted that high ibuprofen and tetracycline concentrations favoured the abundance of some genera.

Key words | bacteria, ibuprofen, microbial abundance, protozoan, tetracycline, wastewater treatment

INTRODUCTION

A vital process of wastewater management is the secondary treatment phase, which operates with the use of microorganisms for waste decomposition. Microorganisms are critical for this process to operate effectively and comply with local regulations. Microorganisms, however, are not immune to changes in the environment, and perturbations in the chemical and physical parameters in the activated sludge process may cause shifts in the microbial population composition and structure and subsequently alter performance efficiency (Du et al. 2014). Although the microbes in the wastewater treatment plant (WWTP) include bacteria, protozoa, fungi, algae, worms, insect larvae and snails, bacteria and protozoa are the two populations most noted for their contribution to the treatment of the wastewater. The bacteria are the major group responsible for biodegradation of the organic matter within the influent, while the protozoa are responsible for grazing on planktonic bacteria and clearing the effluent (Pauli et al. 2001; Madoni 2011; Jaranowska et al. 2012).

There are many classes of contaminants that make their way into wastewater influent. One group is the non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen. Ibuprofen is an antipyretic and analgesic drug that is commonly used by children and adults (Bushra & Aslam 2010) and one of the most commonly used pharmaceuticals around the world due to its low cost and effectiveness (Gilbride & Levinson 2008). With sales of this drug expected to increase by 5–6% per year in the USA, the total load of this drug is only expected to rise (Taucer-Kapteijn et al. 2016). In 2014, a Canadian study showed that ibuprofen could be found with 100% frequency in Canadian WWTPs. The concentrations found in the influent water can range from 2.5 μg/L to 106 μg/L, with removal rates of up to 90% (Guerra et al. 2014; Hoque et al. 2014). This is
in line with what is seen in studies from other parts of the world (Stuart et al. 2012; Pasquini et al. 2013b). This suggests that the WWTP bacteria are indeed capable of breaking down large quantities of ibuprofen (Langenhoff et al. 2013). However, due to a constant influx from the sewer systems, concentrations remain high on a day-to-day basis causing the drug to be pseudo-persistent. The high use of the drug, partnered with its ubiquitous nature and prevalence, makes ibuprofen an ideal compound for further research on its effects on the WWTP microbial community. The drug exerts anti-inflammatory and analgesic effects by non-selective inhibition of the cyclooxygenase enzymes COX-1 and COX-2 which are required in the synthesis of prostaglandins in eukaryotes (Bushra & Aslam 2010). Although this drug continues to enter the urban wastewater treatment system in increasing amounts each year, studies on the severity of its effects on non-human eukaryotic organisms is limited (Pasquini et al. 2014). Ibuprofen at concentrations of 1 mg/L have been shown to cause a significant reduction in attachment and hydanth number in the cnidarian, Hydra attenuate (Quinn et al. 2008). Furthermore, concentrations at the ng/L level have also been shown to affect the reproduction and growth of several aquatic biota (Hayashi et al. 2008; Han et al. 2010; Ragunnett et al. 2011). However, there has not been any recorded mode of action against prokaryotes (Pasquini et al. 2013a), although some previous work has shown that ibuprofen at concentrations above 100 μg/L induced a decrease in extrapolymeric substance production in bacteria, which may result in an overall decrease in activity (Pasquini et al. 2015a). Davids et al. (2017) showed that high and very high concentrations (50–5,000 mg/L) of ibuprofen can cause the bacterial community composition to change; however, they used concentrations that are magnitudes higher than concentrations seen in full scale systems.

Another contaminant group in wastewater influent is antibiotics such as tetracyclines. Tetracyclines are a group of broad-spectrum antibiotics first discovered in the early 1940s and later approved by the FDA in 1948 (Nelson 2002; Nelson & Levy 2011). Tetracyclines also have sub-classifications such as short acting (i.e. tetracycline), intermediate-acting (i.e. demeclocycline) and long-acting compounds (i.e. minocycline and doxycycline). Specifically, tetracyclines inhibit protein synthesis in bacteria by blocking aminoacyl-tRNA and preventing it from binding to the 30S ribosome (Nelson 2002). Tetracyclines have been commonly used for both human and animal care; however, the majority of its use today is in veterinary medicine and animal husbandry, such as growth promotion and prevention of bacterial infections in livestock (Daughton 2015). Tetracyclines have been detected in various environments such as soil, water, wastewater and activated sludge (Toth et al. 2011; Zhang et al. 2013) with concentrations ranging from a few ng/L to more than 4 μg/L (Ma et al. 2015). To date, laboratory studies have shown that the addition of tetracycline could substantially alter the structure of the bacterial community and increase its diversity (Zhang et al. 2013; Huang et al. 2015) although the reasons behind this effect are not known.

Due to continuous influx, pharmaceuticals are a persistent contaminant in human wastewater, yet studies on the effect of such anthropogenic compounds on the microbial community in WWTPs are very limited. Thus it is important to study the effects of these compounds on the composition and structure of the microbial community in the wastewater treatment processes. Previous work on the effects of sub-inhibitory concentrations of compounds on microbial community structure in natural environments has produced varying results with phospholipid fatty acid analysis. Xi and colleagues (Xi et al. 2015) found no shifts in fatty acid profiles from microbial communities isolated from aquaculture farming containing varying concentrations of 14 widely used antibiotics whereas other studies have noted changes in the microbial community composition from salt marsh sediment (Córdova-Kreylos & Scow 2007), and agricultural soil amended with (Kotzerke et al. 2008) and without manure (Hammesfahr et al. 2008; Yang et al. 2009; Li et al. 2011) when exposed to antibiotics or pharmaceuticals. Moreover, Hammesfahr et al. (2008) noted that sulfadiazine decreased the bacteria:fungi ratio in soils.

Using molecular techniques it has been shown that microbial profiles generated using rRNA gene amplicons and denaturing gradient gel electrophoresis (DGGE) can be used to follow spatial-temporal composition variability of microbial communities in water sources (Muyzer et al. 1993; Gilbride 2015). The primers selected to generate these profiles can specifically follow a specific population or genera in a mixed community.

Overall, pharmaceuticals and antibiotics from households or hospitals are being disposed of or discharged into the environment on a continuous basis via wastewater treatment systems (Emmanuel et al. 2005; Pasquini et al. 2014). Since the wastewater treatment process was never designed to remove these anthropogenic compounds or their metabolites, their residual concentration in wastewater is persistent. Their effects on the structure of the microbial community, an integral part of the secondary process of wastewater systems, are not well documented. Currently, concentration levels of antibiotics and pharmaceuticals are
low and considered to be in the sub-inhibitory concentration range; however, studies examining the effects of drugs on the relationship between the eukaryotic and prokaryotic members of the microbial community in wastewater or the effect of a combination of drugs are very limited.

The goal of this study is to examine the effects of two well-known drugs, ibuprofen and tetracycline, alone and in combination, on the composition and structure of the microbial community in a secondary treatment system. Specifically, the abundance and diversity of each of the microbial eukaryotic and bacterial populations from a municipal activated sludge community will be monitored using specific rRNA gene primers and DGGE. It is expected that the ibuprofen will affect the eukaryote population while the antibiotic will affect the bacterial community. The ratio of bacterial to eukaryotic microbial abundance will also be examined to understand the effect of the compounds on the community as a whole since the protozoan community relies on the bacterial community as a food source. The two drugs will also be tested in combination since it is expected that drug-mediated interactions may influence population structure and composition within this heterogeneous poly microbial community, which may result in varying consequences for the quality of the effluent.

**MATERIALS AND METHODS**

**Description of WWTP and sample collection**

Effluent was collected from the secondary treatment process of a municipal WWTP in Toronto, Canada. The plant serves a population of approximately 55,000 residents and has a capacity of 34,000 m$^3$ per day. The WWTP employs a primary clarification followed by the conventional activated sludge process for organic compound removal and nitrification. Secondary treatment is followed by the anaerobic digestion of the sludge produced. Average influent concentrations for 2016 were 286 mg/L of total suspended solids (TSS) and 197 mg/L of biological oxygen demand (BOD$_5$), with the treated effluent containing 3 mg/L of TSS and 2 mg/L of BOD$_5$ (Toronto Water 2016).

For this study, grab samples were collected from the aeration tanks in sterile plastic containers and transported to the laboratory for immediate use. The samples were mixed and 500 mL aliquots were placed in each 1 L reactor vessel. The reactors were maintained for 15 days at a temperature range between 20 and 22 °C and a pH between 7 and 7.5 and oxygenated using aquarium bubblers to maintain dissolved oxygen at >2.0 mg/L. It was observed that the color and consistency of the sludge did not change over the duration of the experiment except in those reactors that contained the largest amount of ibuprofen (100,000 ng/mL). It appeared that at this concentration the pharmaceutical caused some foaming in the reactors probably due to the non-medicinal ingredients in the effluent. The non-medicinal ingredients have been listed as acetylated monoglycerides, colloidal silicon dioxide, cornstarch, croscarmellose sodium, methylparaben, microcrystalline cellulose, pharmaceutical glaze, Pharmaceutical ink, povidone, pregelatinized starch, propylparaben, sodium benzoate, sodium lauryl sulfate, stearic acid, sucrose, synthetic iron oxide, titanium dioxide and white wax.

Air was bubbled through a flask containing deionized water to minimize volume loss from the reactors. At days 0, 5, 10 and 15, a 50 mL volume from each reactor was removed for further analysis and replaced with sterile synthetic wastewater (Liao et al. 2003) to supplement the nutrient level but not add in new microbes. The ratio of chemical oxygen demand:N:P in the synthetic wastewater was kept at 100:5:1. The replacement volumes were supplemented with the pharmaceuticals at the appropriate concentrations to prevent dilution of the original concentrations. Any degradation during the experiment was not taken into consideration; however, the reference reactors experienced identical manipulations and therefore differences seen in the test reactors were assumed to represent effects due to the supplemented pharmaceuticals. Removed aliquots were centrifuged at 7,000 rpm for 15 minutes and the pellets were frozen at −20 °C until DNA extraction was carried out.

**Experimental set-up**

Three sets of experiments were carried out. The first two sets contained eight reactors each, where two reactors contained no added pharmaceuticals and were considered the reference reactors. Two reactors were supplemented with 100 ng/mL of ibuprofen, two were supplemented with 50 ng/mL of tetracycline and two were supplemented with both ibuprofen (100 ng/mL) and tetracycline (50 ng/mL). A third set of reactors consisted of 10 reactors, where two reactors were supplemented with 100 ng/mL of ibuprofen, two reactors with 50 ng/mL of tetracycline, two reactors with 2,000 ng/mL of ibuprofen and 50 ng/mL of tetracycline, two reactors with 100,000 ng/mL ibuprofen and 50 ng/mL tetracycline, and two reactors with 100,000 ng/mL ibuprofen and 500 ng/mL tetracycline (Table S1, available with the online version of this paper).
Pharmaceutical stock solutions

A stock solution of tetracycline (50 mg/mL) (Sigma, USA) was made and stored at 4 °C in foil until needed, since daylight can cause breakdown of the chemical structure. Appropriate volumes were added to the reactors to generate final concentrations of 50 ng/mL or 500 ng/mL.

A stock solution of ibuprofen (1 mg/mL) was made using standard Advil® tablets and stored at 4 °C. Appropriate volumes were added to the reactors to generate final concentrations of 100 ng/mL, 2,000 ng/mL or 100,000 ng/mL.

DNA extraction

Reactor samples were processed for community DNA using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, USA). A NanoDrop spectrophotometer (Thermo Scientific, USA) was used to measure the concentrations of each DNA sample in order to determine the concentrations of DNA to adjust volume quantity accordingly for quantitative polymerase chain reaction (qPCR) reactions.

Quantitative PCR and calculations

Primers for the prokaryotic and eukaryotic 16S and 18S rRNA genes were used to quantify the amount of each population. Primers and the protocol for qPCR were followed according to Lin et al. (2014). The 16S rRNA bacterial genes were amplified using 200 nM of each the forward primer 5′-CTACGGGAGGCAGCAG-3′ and the reverse primer 5′-ATTACCGCGGCTGCTGG-3′ (Muyzer et al. 1993). The qPCR protocol for 16S rRNA prokaryotic gene amplification was as follows: hot start at 95 °C, followed by 25 cycles at 92 °C for 45 s, 55 °C for 45 s, 72 °C for 90 s with a final extension at 72 °C for 7 min. For the 18S rRNA eukaryotic gene, 0.5 μM forward primer 5′-GGCAAGTCTGGTGCCAG-3′ and 0.5 μM reverse primer 5′-ATTACCGCGGCTGCTGG-3′ (Muyzer et al. 1993) were used (Lin et al. 2014). The qPCR protocol for 18S rRNA eukaryotic gene amplification was as follows: 95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s with a final extension at 72 °C for 5 min. These primers were chosen because they produce amplicons equal to or less than 500 bp, which is ideal for qPCR. Fifty nanograms of DNA was added to each reaction to standardize the procedure and the total volume of the reaction was adjusted with Milli-Q water in order to obtain a final reaction volume of 20 μL.

Following the qPCR protocol, the LightCycler software (BioRad Laboratories, Mississauga, ON, Canada) was used to analyze the results and provided information such as amplification curves, melting curves, and Ct values for each sample. The Ct values were obtained and averaged for the same samples and the standard deviations were calculated for the triplicate sets of the Ct values for both the 16S rRNA and 18S rRNA genes. The Ct value of each population was an indication of the absolute abundance of that group.

Community profiles and microbial identification

DGGE was used to analysis the community profiles. The same primers for the bacterial and eukaryotic populations were used as described above except that the forward primer in each case contained a GC clamp (5′-CGC CCG GCC CCG GGC GCC GG GCG GGG CAG GGG GGG GGG GGG GGG GGG GGG GGG –). The gel was performed in a DCode Universal Mutation Detection System (BioRad Laboratories, Mississauga, ON, Canada). PCR products from each scenario were used for DGGE and the gel was run using standard procedures as instructed by the DCode protocol manual (BioRad Laboratories). The denaturing gradient used was 40–70%. The samples were loaded into the gel wells with 30 μL of PCR products and 50 μL (1:1 ratio) of loading dye (2×). The DGGE gels were run for 16 hours at 80 volts with 1× TAE buffer at 60 °C. The gels were stained with SYBR Gold (Thermo Fisher Scientific Invitrogen, USA) for 30 min, then de-stained for 15 min in 1× TAE. The gel was then placed in a UV trans illuminator for viewing. To identify genera represented on the gel, DNA fragments were excised and placed in Eppendorf tubes. To each tube, 30 μL of deionized water was added and left to elute for 3 days at 4 °C. The PCR products were purified using the PCR purification kit (Qiagen, Germany) and the product was sent to ACGT Corp (Toronto, ON, Canada) for DNA sequencing. The sequences retrieved from ACGT Corp were entered into the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990), and the closest identity was determined.

Calculation of abundance of each population and the ratio of each population in the overall community

The normalization equation R = 2^(-ΔΔCt) was used to calculate the relative abundance of each population in each condition compared to the reference reactor where ΔΔCt = ΔCt (experimental sample) – ΔCt (reference sample). The
ΔCt (experimental sample) = Ct (experimental at time t) – Ct (reference at time 0) and ΔCt (reference sample) = Ct (reference at time t) – Ct (reference at time 0). In the reactors with no added pharmaceuticals the Ct experimental was identical to Ct reference that resulted in 2^0 which equals 1 and therefore any changes due to parameters other than the pharmaceutical were normalized to 1. The experimental samples are then expressed as an increase or decrease relative to the reference. The equation used an efficiency value of 2, which assumed the PCR performance achieved perfect doubling with each amplification cycle.

The interaction between the two populations was represented as a ratio; \( r = \frac{R\text{ (eukaryote at time t)}}{R\text{ (bacteria at time t)}} \) and reflects the relationship between the two populations. Since the normalized Ct values are used in the calculation the ratio of eukaryote to bacteria is 1 even though the eukaryotic organisms are three- to four-fold less in numbers. Therefore changes due to the pharmaceuticals will affect the ratio and the relationship between the two populations. A Student’s T-test was performed on the population abundance and ratios to determine \( p \) values.

RESULTS AND DISCUSSION

Population abundance

Samples from the reactors were collected and the microbial community DNA was extracted. To measure the relative amount of each of the bacterial and eukaryotic populations, qPCR was performed with primer sets that could distinguish between prokaryotic and eukaryotic DNA. The primers used were specific to the 16S rRNA gene sequences in bacteria and the 18S rRNA gene sequences in eukaryotes. Although the primers for the 18S rRNA gene were not specific to protzoa, a previous study by Ovez & Orhon (2005) has shown that the majority of the eukaryotic community in wastewater is composed of protists with concentrations ranging from 500 to 1,000 cell/mL during optimal conditions to over 5,000 cell/mL during bulking conditions (Pogue & Gilbride 2007). Therefore, it was assumed that the 18S rRNA gene signal would mostly represent the protzoan population.

The qPCR experiments were carried out to determine the threshold point (Ct) values since these values can be used as a measure of the abundance of the target sequences in a DNA sample. The values were measured in triplicate for each duplicate reactor sample for both the 16S rRNA gene and 18S rRNA gene. The Ct values for each population in each reactor are shown in Figure 1(a) for day 0 and day 15 for the reference and each experiment reactor set. The abundance of bacteria did not change in the reference reactor; however, there was an increase in the Ct value in reactor sets 2, 3, and 4 signifying that the relative abundance had decreased. These reactors contained 50 ng/mL of tetracycline or 100 ng/mL of ibuprofen alone or a combination of 100 ng/mL of ibuprofen with 50 ng/mL of tetracycline respectively. However when the pharmaceuticals are supplemented at high and very high concentrations the bacterial population appeared to recover. Kraigher et al. (Kraigher & Mandic-Mulec 2011) also noted a reduction in the diversity of the bacterial community of an activated sludge system when exposed to NSAIDs at low concentration even though there is no known mode of action of...
NSAIDs against prokaryotes. These results indicate that anthropogenic compounds may have effects on bacterial growth that have not yet been elucidated. When the pharmaceuticals were supplemented at high concentration the bacterial abundance was not reduced significantly from the reference. Conversely, the abundance of the eukaryotic population increased slightly at the low pharmaceutical levels and then declined significantly at the higher concentrations. To further study the effect of the pharmaceuticals on the microbial abundances, the Ct values were normalized to remove shifts in abundance due to non-selected parameters using the equation $R = 2^{-\Delta\Delta Ct}$. Figure 1(b) shows the relative abundances of each population after that transformation. Reactor sets 2, 3 and 4 show a significant reduction in the relative abundance of the bacteria with a corresponding increase in relative abundance of eukaryotes. Increasing the amount of ibuprofen to either 2,000 ng/mL or 100,000 ng/mL in the presence of tetracycline (reactors 5, 6 and 7) led to a significant decrease in the relative abundance of eukaryotes (Figure 1(b)). Láng & Kohidai (2012) found that ibuprofen affected the proliferation and migration of the freshwater calcite, *Tetrahymena*, and when introduced in mixtures the effects were complex, concentration-dependent, antagonistic types of interactions. In our case, both tetracycline and ibuprofen seemed to decrease the relative abundance of the bacteria population; however, when supplied in a mixture with ibuprofen, the effect was reduced and even eliminated in reactors 6 and 7. The tetracycline concentrations used are well below minimum inhibitory concentrations of 2–16 μg/mL and therefore its presence most likely does not inhibit bacterial population growth. Additionally, previous studies have shown that antibiotics at sub-inhibitory concentration may be used as carbon sources in mixed populations of microbes and therefore could contribute to increased bacterial numbers (Kummerer 2009). Likewise ibuprofen is not known to have any mode of action on the prokaryotic metabolism. The decrease in the relative abundance of the bacteria may be more directly related to the state of the eukaryotic population. Because the abundance of the eukaryotic population had an opposite corresponding increase in relative abundance at the low concentrations of the drugs and a decrease at the high concentration, the interactions of the eukaryotic population with the bacterial population may have contributed to these shifts in abundance since it is known that the protozoans within a wastewater secondary treatment system graze upon the bacteria and consequently reduce their numbers (Pogue & Gilbride 2007).

**Interactions between the bacterial and eukaryotic populations within wastewater communities**

To assess whether the pharmaceuticals affect the overall interaction of the populations within the community, a ratio of 18S rRNA gene abundance over the 16S rRNA gene abundance was calculated using the ratio equation, $r = R(\text{eukaryote at time t})/R(\text{bacteria at time t})$. The reference reactor ratio of eukaryotes to bacteria (E:B) was calculated as 1. This ratio does not represent a ratio of the actual numbers of each population since there are approximately 10,000× more bacteria than eukaryotes per millilitre in the wastewater treatment process (Madoni 2011), instead the 1:1 ratio represents that normal relative abundance state of the populations. The simple ratio of E:B was used to represent the microbial community for each sample, where a higher ratio would suggest that the community had shifted in favour of the eukaryotic population and a lower ratio would suggest that the community dynamics had shifted in favour of the bacterial population. Figure 2 shows the ratios. At the lower concentrations there was a shift in the ratios of the two populations in relation to each other whereas at very high ibuprofen concentrations the eukaryotic population was affected as shown by the decrease in the ratio at ibuprofen concentrations of 2,000 ng/mL and 100,000 ng/mL. Increasing the tetracycline concentration to 500 ng/mL did not augment the effect of the ibuprofen.

![Figure 2](http://iwaponline.com/wst/article-pdf/2017/2/430/216883/wst2017020430.pdf)
Population diversity

To examine the composition of the populations in the presence of the pharmaceuticals, community profiles from each reactor were generated by amplifying either the 16S or 18S rRNA gene for the bacterial and eukaryotic populations, respectively, and running the amplicons on a DGGE gel. The images in Figure 3 show the profiles from reference and experimental conditions for both the bacterial and eukaryotic populations. At the low concentrations of either drug, neither population showed a change in their structure (data not shown); however, when the drugs were added in combination, and at the higher concentrations, some shifts in the diversity were observed.

Bacterial population profiles (Figure 3(a)) are shown for the reference reactor (lane 1) and two reactors containing the pharmaceuticals at 100,000 ng/mL ibuprofen and 50 ng/mL of tetracycline (lane 2) and 500 ng/mL of tetracycline (lane 3). The bacterial population from all the reactors was diverse as shown by the appearance of more than 35 bands in each lane; however, the location and intensity of some bands in the reactors with and without added pharmaceuticals show the variability in composition of the most prominent species of bacteria in the community. Fourteen bands were extracted and sequenced, and the identities of some of the bacterial members representing the major bands were determined (Table 1). Uncultured Polaromonas sp. (band 1), several beta-proteobacteria sp. (bands 4, 10, 12) and some unnamed uncultured bacterium (bands 5, 11, 13, 14) were present in both reference and the experimental reactors amended with tetracycline and ibuprofen although the difference in intensity of some of the bands between reference and treatment suggests the population was influenced by the addition of the drugs. Furthermore, an uncultured Raoultella sp. (band 2), a beta-proteobacteria (band 8) and an unnamed uncultured bacterium (band 7) were seen to disappear after incubation with pharmaceuticals while an uncultured Dokdonella (band 9) started to appear after the samples were treated. The DGGE technique is limited by the ability to detect less abundant species at quantities that can be viewed on the gel and therefore minor species that are affected by the pharmaceutical would not be viewed on the DGGE gel. Additionally, the effects of some compounds may be masked by the robustness of mixed populations in activated sludge that are able to rely on redundancy in the population to maintain function and even diversity.

Likewise, Figure 3(b) shows the profiles of the eukaryotic population in the reference (lane 3) and experimental reactors with 100,000 ibuprofen and 50 ng/mL of tetracycline (lane 2) or 500 ng/mL of tetracycline (lane 1). Overall the eukaryotic population showed less diversity than the bacterial population since fewer genera (fewer bands) are visualized on the DGGE gel. In this case, the shift in the population composition was more pronounced between reactors that contained no pharmaceutical and those that did. Although the diversity of the eukaryotes was not significantly affected (approximately 16–20 bands could be distinguished in each lane regardless of treatment) the composition of the community showed a shift. Twelve bands from the gel were extracted and sequenced (Table 2). The pharmaceuticals appeared to affect both the presence and abundance of the eukaryotic members. First of all, Cryptomycota sp. (Bands 1, 9, 11), Arcella vulgaris (band 4), Arcella hemispherica, (band 5), an uncultured eukaryotic (band 6), and Cercozoa (band 7) were found in all reactors although not always at the same abundance. Slavina appendiculata or Paranais litoralis (bands 2 and 3), Arcella vulgaris (band 4), Arcella hemispherica, (band 5), Cercozoa (band 7) and Slavina...
appendiculata or Paranais litoralis or Nais communis (band 12) were more dominant in the reference reactor; in fact, band 3 appeared to be absent from the reactors with 100,000 ng/mL of ibuprofen. On the other hand, Lamproderma sp. (band 8) or an uncultured eukaryote (bands 8 and 10) were only seen in the experimental reactor where band 1 (also bands 9, 11) (Crytomycota sp.) was also more prominent. Most of the bands were identified as protozoans but because the 18S rRNA gene primers did not discern between the types of eukaryotes, several protists, amoebas, a worm and an uncultured eukaryote were among those identified.

Overall, the low percentage similarities found between the database and the sequenced rRNA gene amplicons

<p>| Table 1 | Bacterial genera found in the communities exposed to tetracycline and ibuprofen |</p>
<table>
<thead>
<tr>
<th>Band</th>
<th>Identification</th>
<th>Accession number</th>
<th>Percent similarity</th>
<th>Present in reference community</th>
<th>Present in experimental community</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polaromonas sp.</td>
<td>JQ290954.1</td>
<td>98%</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Raoultella sp.</td>
<td>EU919218.1</td>
<td>96%</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Uncultured bacterium</td>
<td>FJ406564.1</td>
<td>77%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>β-Proteobacterium</td>
<td>HQ663343.1</td>
<td>76%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Uncultured bacterium</td>
<td>KF080219.1</td>
<td>80%</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Xanthomonas sacchari</td>
<td>KY393338.1</td>
<td>95%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Uncultured bacterium</td>
<td>JF122187.1</td>
<td>82%</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>β-Proteobacterium</td>
<td>HQ663343.1</td>
<td>81%</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>Dokdonella</td>
<td>JF808755.1</td>
<td>98%</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>β-Proteobacterium</td>
<td>HQ663343.1</td>
<td>85%</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Uncultured bacterium</td>
<td>AM932231.1</td>
<td>87%</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>12</td>
<td>β-Proteobacterium</td>
<td>HQ663343.1</td>
<td>87%</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Uncultured bacterium</td>
<td>JQ300389.1</td>
<td>73%</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>14</td>
<td>Uncultured bacterium</td>
<td>JX715941.1</td>
<td>78%</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

-, not present; +, present; band intensity is faint; ++, present; band is apparent; ++++, present, band intensity is strong; +++, present, band intensity is very strong.

<p>| Table 2 | Eukaryotic genera found in the communities exposed to tetracycline and ibuprofen |</p>
<table>
<thead>
<tr>
<th>Band</th>
<th>Identification</th>
<th>Accession number</th>
<th>Percent similarity</th>
<th>Present in reference community</th>
<th>Present in experimental community</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 9, 11</td>
<td>Uncultured Cryptomycota sp.</td>
<td>JN612972.1</td>
<td>90–93%</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Slavina appendiculata</td>
<td>GQ355434.1</td>
<td>99%</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Paranais litoris</td>
<td>KY633359.1</td>
<td>99%</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Slavina appendiculata</td>
<td>GQ355434.1</td>
<td>99%</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Paranais litoris</td>
<td>KY633359.1</td>
<td>99%</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Nais communis</td>
<td>KY633358.1</td>
<td>90%</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Arcella vulgaris</td>
<td>HMB53761.1</td>
<td>95%</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Arcella hemispherica</td>
<td>EU273445.1</td>
<td>98%</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Unculture eukaryote</td>
<td>FJ490225.1</td>
<td>92%</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>10</td>
<td>Cercozoa sp.</td>
<td>HQ007043.1</td>
<td>95%</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>11</td>
<td>Uncultured Cryptomycota</td>
<td>JN612972.1</td>
<td>92%</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>12</td>
<td>Uncultured eukaryote</td>
<td>AB902006.1</td>
<td>95%</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>13</td>
<td>Specaria josinae</td>
<td>KY633361.1</td>
<td>98%</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Slavina appendiculata</td>
<td>KY633353.1</td>
<td>97%</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Paranais litoris</td>
<td>KY633359.1</td>
<td>97%</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>Nais communis</td>
<td>JN612972.1</td>
<td>97%</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

-, not present; +, present; band intensity is faint; ++, present; band is apparent; ++++, present, band intensity is strong; +++, present, band intensity is very strong.
from this system indicated that many microorganisms in the wastewater treatment system have yet to be fully identified or included in the database. However, all the BLAST searches of the sequences verified that the closest identity to the sequences from our system were to microorganisms that had been previously isolated or identified from aquatic environments.

Microbial communities in wastewater are of the utmost importance because of their functional abilities to degrade various contaminants such as organic, inorganic and xenobiotic compounds. The increasing flux of various pharmaceutical compounds into the wastewater deems it necessary to have complete studies on the inhibitory effects of these compounds on the microbial community. Our study shows that the presence of environmental concentrations of tetracycline and ibuprofen affected the individual abundance and diversity of the populations. However, even though the relative abundances in those reactors were close to those found in the reference reactor, the bacterial levels of ibuprofen showed repression of the eukaryotic population was found to be even more significant. Low concentrations reduced the bacterial numbers while very high concentrations of either drug caused a change in the balance between the eukaryotic and bacterial populations. Since the microbial community is an important parameter in biological treatment, monitoring changes in the community abundance and composition may be important for predicting pharmaceutical levels in wastewater.

CONCLUSION

Microbial communities in wastewater treatment systems are exposed to xenobiotic waste on a continuous basis. This study examined the effect of two pharmaceuticals, ibuprofen and tetracycline, on the relative abundance and composition of the bacterial and eukaryotic populations from a municipal wastewater treatment activated sludge treatment system. Environmentally relevant concentrations of either drug caused a change in the balance between the two populations and high levels of the pharmaceuticals affected the composition of the populations. Since the microbial community is an important parameter in biological treatment, monitoring changes in the community abundance and composition may be important for predicting pharmaceutical levels in wastewater.

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REFERENCES


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