# MICROPOLLUTANT BIOTRANSFORMATIONS PERFORMED BY WASTEWATER MICROBIAL COMMUNITIES IN CONVENTIONAL ACTIVATED SLUDGE AND UP-FLOW ANAEROBIC SLUDGE BLANKET PROCESSES

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by

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# ABSTRACT

Biological wastewater treatment processes exhibit variable extents of organic micropollutant (MP) biotransformation. Conventional activated sludge (CAS) systems have been frequently studied, but up-flow anaerobic sludge blankets (UASBs) have received less attention. We hypothesize that the unique wastewater microbial communities in a CAS and UASB will perform different types of MP biotransformations at different rates. To test this hypothesis, we collected influent and effluent samples from a full-scale CAS and a pilot-scale UASB receiving the same influent. We measured each sample by means of high-resolution mass spectrometry to quantify up to 175 MPs in each sample. We measured 39 MPs at concentrations greater than the limit of quantification in both systems. We used a variety of metrics to identify 17 MPs that exhibited better removal in the CAS, 16 MPs that exhibited better removal in the UASB, and 7 MPs that exhibited similar removal in both systems. To validate these findings and to investigate the types of biotransformations performed by each microbial community, we conducted batch experiments in bioreactors seeded with wastewater microbial communities harvested from the CAS and UASB into which we spiked 24 MPs of particular interest (12 MPs that exhibited better removal in the full-scale CAS and 12 MPs that exhibited better removal in the pilot-scale UASB). Temporal sampling revealed MP biotransformations in both experiments, some of which had rates that agreed with our observations made at full- and pilot-scale, and some of which had rates that did not. Nevertheless, analysis of biotransformation products revealed that those formed exclusively in the batch experiments seeded with wastewater microbial communities harvested from the CAS were primarily the result of oxidations (monohydroxylation, ether dealkylation, amine dealkylation). Interestingly, those formed in both types of experiments seeded with wastewater microbial communities harvested from either the CAS or UASB were primarily the

result of redox-independent hydrolyses (ester hydrolysis, amide hydrolysis). Finally, different biotransformation products of androsterone were found in experiments seeded with wastewater microbial communities harvested from the CAS or UASB; the biotransformation product found in the CAS experiment was the result of a monohydroxylation (oxidation) and the biotransformation product found in the UASB experiment was the result of a hydrogenation (reduction). Together, our data provide novel insights on the relative functioning of two wastewater microbial communities operated under different redox conditions. Our study demonstrates the potential of the UASB reactor to biotransform MPs and the relative biotransformation potential of CAS and UASB processes. We also present structures of seven biotransformation products, five of which (transformation products of gabapenitn, diphenhydramine, amphetamine, ethyl-butylacetylamino-propionate, adrenosterone) have not been previously reported in the literature.

# BIOGRAPHICAL SKETCH

M Amalio Martin (originally Michael Amalio Martin) was born on September 15th 1999 in La Jolla California. M was raised by two loving parents, Dr. Darci Strother and Dr. Francisco Martin, both professors in Spanish. Growing up, M faced and overcame many struggles. Despite their struggles M remained curious about the world around them and was determined to learn as much as they could. From a very young age, M knew that they wanted to be a scientist. After encouragement from a few very influential instructors during their time at Mission Hills High School, M entered the world of higher education through California State University Channel Islands where they would go on to attain a Bachelors of Science in Biology, with a minor in Chemistry, all while consistently maintaining a high GPA. During their time at CSU Channel Islands, M aided in a local science fair, visited the Channel Islands themselves, made lifelong friends, and during Covid-19, tutored local youth. These experiences made M appreciate how much they enjoyed sharing what they had learned with the next generation of bright minds. With their sights set on graduate school, M was admitted to Cornell University in 2021. Having never traveled beyond their comfort zone, M was excited and nervous to step into a new chapter of their life. Originally planning to complete a PhD with Dr. Damian Helbling, M determined that there was still so much to see and do in the world, and that while a PhD may have been possible, a Masters degree would permit M the freedom to explore other areas of both science and the world. In the summer of 2022, M discovered that they were experiencing a number of mental and physical health issues relating in part to gender dysphoria and as a result M began to present as non-binary and then as feminine over the next year. As people grow and learn, they can also change and as such I am unsure if I will still be M moving into the future; however I hope that this brief story can inspire bravery and hope for those wrestling with bullying, body dysmorphia, gender

dysphoria, neurodivergence, anxiety, depression, and trauma as I have. While I may not always be M, I will always be myself and I am so excited for whatever may lie ahead of me.

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alongside you. Like I said before, you have me on the edge of my seat to see what comes next for you. All the best!

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# CHAPTER 1 – Background

## 1.1 Introduction to Micropollutants (MPs)

Micropollutants (MPs) are a class of chemical pollutant that have recently become a rising health concern for society as a whole. MPs are defined as anthropogenic chemicals that originate from the use of products by humans.[1] These MPs are often present at incredibly low concentrations, often ranging from microgram per liter to nanogram per liter in concentration. These low concentrations make the removal of MPs from the environment particularly difficult since chemicals at high concentrations are not only more prevalent in their environment, but can also serve as a possible material for cell growth given the right conditions.[2] Additionally, many of the MPs of concern are bioactive molecules that, while beneficial for humans, pose unknown risks and hazards to the environments in which they may eventually reside.[3,4] Several studies have already demonstrated that MPs have infiltrated natural water systems, and in turn have had a negative effect on the natural and human populations that utilize these water systems.[5,6] These negative effects can include endocrine disruption, birth defects, reduced ecosystem diversity, and occasionally algal blooms resulting in eutrophic dead zones. [7–11]

It is due to these negative effects that MPs have been classified as an emerging contaminant. While measures have been instituted by governments around the world to limit the most damaging and noticeable MPs, such as poly chlorinated biphenyls (PCBs)[12], there are still hundreds if not thousands of unstudied anthropogenically sourced chemicals that deposit into natural environments with unknown effects.[9,10] Fortunately, much of the infrastructure developed originally for the removal of bulk organics and bulk nutrients from our water systems in the form of the waste water treatment plant (WWTP) appears to have the ability to interact with and remove some of these MPs.[13–18] This is done through a variety of techniques that will be discussed in

subsequent sections; however, these techniques are not infallible. The WWTP system was not originally created to attack or target MPs and as such many MPs have been observed passing through these systems.[19–21] The MPs may also consist of antibiotics that have passed through the body, unmetabolized. If these pharmaceuticals are able to enter natural environments, then they have the opportunity to culture antibiotic resistance in the microbes they interact with by imposing selection pressures for microbes that are able to resist the antibiotics.[16,22]

Although the risks posed by these chemicals are known, it is also important to acknowledge the utility and necessity of some of these MPs.[23] Without pesticides, we would be unable to feed our ever-growing population. Without pharmaceuticals and antibiotics millions would die of disease. As such, we are unable to consider cessation of MP usage in today's society. We must therefore turn to our WWTPs and strive to make improvements to the systems in place to be able to tackle current and emerging contaminants of concern.[24–26] To this end we investigated the effectiveness of the conventional approach to sludge treatment, the conventional activated sludge basin (CAS), considered to be the conventional method of wastewater treatment via microbial transformation, as well as an emerging technology known as the up-flow anaerobic sludge blanket bioreactor (UASB) to learn more about the processes and possibilities each system may hold for solving this problem, while still maintaining both social and economic feasibility.[24,25,27–39]

### 1.2 Micropollutant Removal in Conventional Activated Sludge (CAS) Processes

MPs are removed in the CAS process via two primary methods of removal. These are chemical transformation and sorption to biological material.[40] However before we dive into how these two removal techniques work, it is important to discuss the concept behind how a CAS reactor functions.[41] In this thesis, we are focusing on the aeration basin present within the Ithaca Area Waste Water Treatment Plant (IAWWTP). This aeration basin is the second step in a three-

step process consisting of a primary settler, the aeration basin, and then a secondary settler. The primary and secondary settlers are utilized to allow larger organic matter to aggregate and sink to the bottom of the basin, in order to have the liquid supernatant be able to pass onto the next step of processing. These three steps are not the only ones present at the IAWWTP, however they are a primary means of organic pollutant removal, and this can include MPs.[21] The CAS is operated as three continuous flow stirred tank reactors (CFSTRs) in series by having a constant flow of influent from the primary settler in the plant (see schematic in **Figure 1.1**). The constant flow of influent is stirred by bubbling oxygen into the bottom of the basin, resulting in not only mixing, but also providing oxygen for aerobic microbes to utilize for redox reactions responsible for possible pollutant degradation.[42] Because these tanks are being constantly stirred, microbes that thrive in this environment are often planktonic and will only survive, grow, and perform chemical removal at the correct levels of dissolved oxygen.[43] This oxygen demand presently accounts for approximately 3% of the United States' yearly energy budget[44], and it selects against microbes that are adherent/stationary as well as anaerobic microbes, resulting in a seemingly consistent spread in terms of microbial diversity. Recent studies have shown that despite a consistent operation of a CAS, the microbial community can vary temporally. This temporal variation may account for the difference in MP transformation observed across multiple studies.[45]

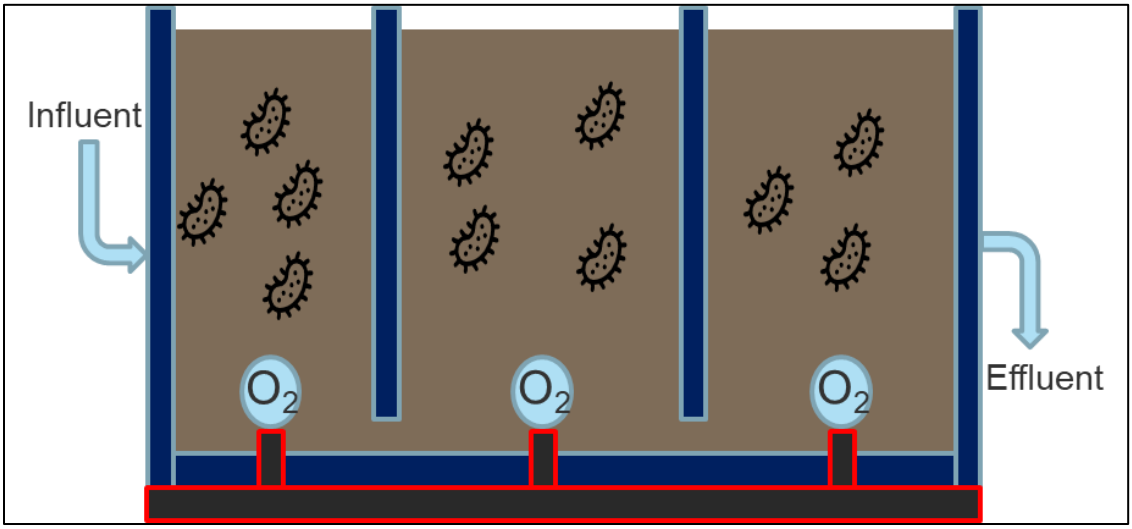


**Figure 1.1**: A graphical summary of the Conventional Activated Sludge (CAS) system as it was operated at the Ithaca Area Wastewater Treatment Plant (IAWWTP)

However, due to the consistency of the composition of influent, the present microbial community is generally sufficient to remove most of the pollutants of interest. It does this via chemical transformation or sorption to biological materials.[13,16,18,21,46] Starting with sorption, this removal does not change the MPs chemical structure at all, however it does remove it from the aqueous phase. This is because, due to the structure and size of several organic compounds, they may possess lipophilic or hydrophobic properties, causing them to aggregate to one another or any other biological material that is present. This is often expressed in the MPs' octanol water partitioning coefficient ($K_{ow}$) and can also be expressed as the organic carbon water partitioning coefficient ($K_{oc}$) which can be utilized to calculate the sorption coefficient ($K_d$). Additionally, it is well understood that sorption removal processes will occur individually from biotransformation reactions, as the rate of sorption is considered to be near instantaneous when compared to biotransformation rate kinetics.[47] As the reactor is being stirred and mixed, the mixing permits higher chances of chemical collision with microbial organic matter. This matter can consist of

extracellular polymeric substances (EPS), extracellular proteins, or cell membrane lipids, lipophilic chemicals will prefer to enter the organic phase of the reactor by adhering to any open space on microbial surfaces instead of staying suspended in water.[40,48–50] When the effluent from the CAS enters the secondary settler, these microbes as well as whatever is present on their membranes will be removed by having the microbes settle to the bottom of the tank.

The secondary method of action is removal of chemicals and MPs via chemical reaction.[51,52] This can occur when the correct conditions are met for a microbe to utilize a functional group present on the chemical of interest as a step along its metabolic pathway. This can include searching for a usable electron donor or acceptor, looking for metals to form the backbone of essential enzymes, as well as many other possible reactions, all depending on the type of microbe present in the system. Previous studies have shown that across biodegradation and cometabolism models, the level of sorption and the amount of dissolved MP had an impact on the remediation of the MP.[47] By providing the microbes in the CAS reactor with oxygen, we are providing them with the highest level of chemical energy found on the redox ladder in the natural environment. This high energy molecule permits them to complete these reactions faster, and more consistently. Often times, microbes may only utilize a portion of any particular chemical, as their niche in that community is specialized around that one functional group of interest. This may, in turn, leave other functional group sites open to attack from other microbes. If these microbes are specialized to utilize this new group, the cycle will repeat itself until the chemical is mineralized to only its two simplest forms, $CO_2$ and $H_2O$. This is what is referred to as co-metabolism, if multiple microbes are utilizing the same molecule in different stages as a method of energy or material gain, resulting in its degradation.[51–54] It is important to note that due to current regulations and screening methods, once the initial metabolic pathway is utilized, the chemical is considered

as “removed” due to its original form no longer being present.[20] This does not however, indicate that the new molecule is any more or less of a threat to the natural environment as its predecessor was. [20]As such an ideal CAS system would mineralize all compounds it encountered, in order to release only $CO_2$ and $H_2O$. MP removal is not ideal, and the process of MP removal in a CAS system has been well documented around the world for decades.[14] The sum of these studies indicate that while some MPs may be well transformed in a CAS, many will still only be transformed to varying extents depending on system specific conditions, and will never reach mineralization given only CAS processing.

### 1.3 Micropollutant Removal in Up-flow Anaerobic Sludge Blanket (UASB) Processes

MPs are removed by similar mechanisms as the CAS in the UASB, however the system itself operates in a different manner.[31,37–39,55–57] Unlike the CAS, the UASB does not utilize oxygen to encourage microbial degradation of MPs. Instead it fosters a more diverse microbial ecosystem by encouraging the formation of granular colonies of microbes, adhered together via EPS.[36,57] These colonies form a differential redox ladder within themselves, as well as provide a consistent surface for growth of stationary microbes nestled within environmentally resistant microbes on the outer layers of the granule.[58,59] These granules are enforced by a feast and famine feeding regime, where influent is only provided occasionally, but in high amounts, thus encouraging extra creation of EPS as a storage material for when the microbes are no longer able to utilize all the energy stored in the influent rush.[57] This storage EPS doubles as the “glue” that holds the granules together. Additionally, granules are most effective when they form spherical colonies, and while a perfect sphere of microbes is not necessarily attainable, we select for correctly sized and shaped colonies by utilizing high shear force to push any planktonic or inefficient granules to the top of the reactor along with a rush of influent. Both the feast-famine regime and the high shear force are

accomplished by utilizing a tipping bucket influent feeding method. Although it may seem rudimentary, the slow buildup of influent and sudden rush into the system not only propagates healthy granules, but it also requires less energy to facilitate than the energy required to pump $O_2$ into the CAS. This rush is also what gives the UASB the up-flow portion of its name, as it surges all microbes and liquid to the top of the reactor resulting in the effluent leaving from a higher position and exiting through a tube at the top instead of entering into another adjacent reactor. The general concept is depicted in **Figure 1.2**:

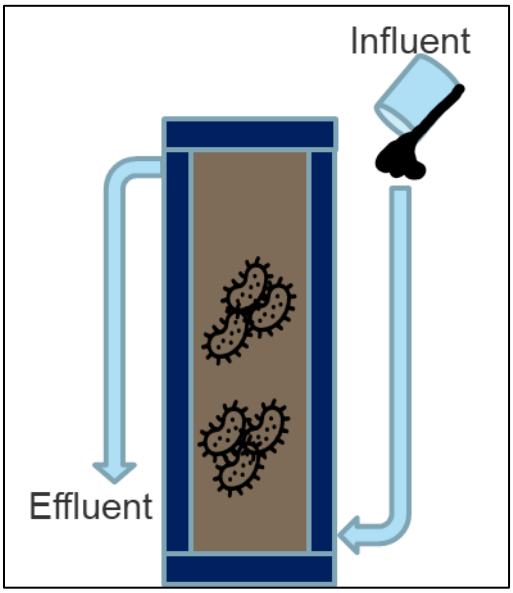


**Figure 1.2**: A graphical summary of the Up-flow Anaerobic Sludge Blanket (UASB) system as it was operated at the Ithaca Area Wastewater Treatment Plant (IAWWTP)

While the system we studied included the tipping bucket method of influent introduction, it is also pertinent to note that UASBs can exist without the addition of this motif. A constant flow UASB is also capable of generating granular sludge and performing pollutant treatment.

There are a few factors to note when we are discussing the differences and similarities between the UASB and the CAS systems. Primarily, the UASB utilized in this study is a prototype

in pilot scale located at the IAWWTP. As such, results of the study may vary if a larger or more established reactor were to be considered. We monitored its influent flow utilizing a peristaltic pump being fed raw wastewater from the plant. This means that while the CAS had a primary settler as its influent source, the raw wastewater UASB influent is likely to contain much more biomass than the CAS influent. Additionally, any MPs that may have been removed in primary settling for the CAS reactor, may be present in the UASB as no prior removal was conducted. Finally, the UASB reactor has an incredibly high biomass density when compared to the CAS, and this may alter MP removal by sorption and co-metabolism.

Although the two main pathways for MP removal remain the same as in the CAS, those being sorption to biological material and co-metabolic degradation, the unique structure of the UASB microbial community can affect the way these two pathways occur.[40,48–54] The biomass density was observed to be approximately 30 times higher in the UASB than in the CAS, and because there is no prior removal via sorption and settling via a primary settler, it was expected that removal by sorption would play a much larger role in MP disappearance. Other studies have also noted that MP removal in the UASB is heavily correlated to sorption to sludge. This indicates that the pH of the system may determine the charged state of MPs, and thus will determine the amount of sorption occurring in a given system.[47,60] Additionally, we observed that due to gravity's effect on the granules, the concentration of granules increased at the base of the reactor and gradually decreased as we sampled other locations along the reactor. There was never any point where the biomass concentration in the UASB was less than or equal to the biomass concentration in the CAS. This means that MPs are expected to sorb and perhaps stay in the reactor for longer periods of time if they are routinely settling to the base of the reactor. As for co-metabolic degradation, as previously mentioned the UASB is utilized to culture unique microbial

communities called granules. These granules are self-adherent, meaning that they do not require any recycling of carriers.[61] As the granule forms, it creates a micro-redox gradient across its radius. The outermost section of the granule is most exposed to incoming influent and as such we expect microbes that are capable of surviving with minimal oxygen, as well as high environmental contact to be present on the outer layer. The outermost layer of microbes are predicted to survive primarily utilizing hydrolysis and fermentation as metabolic biotransformation pathways. As we move deeper into the core of the granule, microbes located here will experience less shear force and as such are expected to be not only suitable for slow, adhered growth, but also permit the growth of microbes that have little to no tolerance for oxygen. These anaerobic microbes are expected to be methanogens that produce the methane gas that can be utilized to recycle the energy of the system. This micro-redox gradient is expected to allow for a higher amount of biodiversity to flourish in the reactor, and along with it, we expect to observe a wider range of community function as more niches allow for a wider net to be cast when attempting to cultivate chemical removal.[62]

Finally, the UASB provides several benefits as well as a few known drawbacks. The primary benefit is that unlike the CAS that utilizes energy to support its microbes via $O_2$ bubbling, the UASB utilizes minimal energy to pump influent into the system that then requires few outside resources.[63] Additionally, the anaerobic nature of the UASB cultures microbes that utilize metabolic pathways that result in methanogenesis.[64] The UASB has been shown to consistently produce methane gas as part of its operation which could in turn be utilized as a power source to offset any energy costs the system may require. When considering challenges facing underserved communities, this system has the capability to self-regulate, and produce natural gas to assist in maintaining power.[64,65] Unlike a CAS system, it does not require high volumes of concrete and complicated hydraulics to be constructed, and therefore it is much more affordable for developing

communities. One drawback this system presents, is that due to its anaerobic nature, and the unconventional flow pattern of influent, the UASB takes longer to operate, and it can only be operated efficiently in locations where it is naturally warm.[63–65] We calculated the hydraulic retention time (HRT) to be approximately 9.5 hours in the UASB and only 8 in the CAS. At a full scale this HRT may increase for the UASB, depending on volume of influent. The anaerobic nature of the system is both a benefit and a drawback as it allows for a higher level of biodiversity to flourish without needing to compete with the more energetically favorable aerobic microbes, however due to the low energy provided by anaerobic chemicals, chemical reactions are expected to occur more slowly.[65] While the CAS has been historically used to remove bulk loads of most nutrients and pollutants, the UASBs unique anaerobic environment prevents it from removing ammonia and phosphorus as they both require oxidation reactions to remove. Lastly, because the UASB is often implemented at the beginning of water treatment, as is the case in this study, the system is often found to have an increased chemical oxygen demand (COD) and cannot always remove pollutants to plant standards. This issue can be resolved by implementing an additional polishing step to capture nutrients and pollutants that increase COD. By polishing the effluent before it moves on in the processing pathway, the effluent can be utilized more frequently. Ultimately the UASB has its benefits and drawbacks, however we are interested in studying how it removes MPs when compared to the CAS system, and in order to do this we equated them as equals and focused primarily on the function of each microbial community while noting the inherent differences of both. Far fewer studies have been conducted exploring the MP removal potential of a UASB reactor.[60,66] Therefore it is mostly unclear not only how MPs will transform, but also if MP biotransformation occurs. If MP biotransformation does occur in a UASB reactor, more studies surrounding the types of biotransformation's should be conducted.

### 1.4 Micropollutant Removal in Other Types of Biological Processes

MPs are an emerging and complicated problem. The only thing we know for certain is that there is no "one-size-fits-all" solution to the issue, and as such we recognize that other biological systems for treating MPs exist and can be just as effective as the CAS or UASB.[13] Some of the alternatives include the usage of specifically cultured bacteria to treat point sources of MP contamination, algae secondary treatment, the usage of white rot fungi, and the addition of carriers to CAS treatment to bolster possible biodiversity without needing to retrofit our current systems.[13,61,67,68] Additionally, any of the techniques mentioned above are most effective when used in conjunction with one another, a hybrid approach allows for the increased interactions with MPs and chemicals of interest.[13]

A few emergent techniques include the usage of both algae and white rot fungi, a non-taxonomic grouping of various fungal species that utilize wide ranged generalist enzymes to degrade substances in their surroundings. Algal bioremediation has shown promise in removing certain toxins of concern, taking the MPs into their system as a portion of the materials they utilize for growth, and not with any sort of specificity towards classes of MPs, all while undergoing photosynthesis to keep the community stable.[67] By comparison, the generalist approach is utilized differently by the white rot fungi that have generalist enzymes recruited for their growth and degradation of their surroundings. These generalist enzymes are capable of degrading a wide range of organic pollutants that can often include MPs of concern.[68] These white rot fungi are able to bridge the gap that both the UASB and CAS face when it comes to requiring specificity to degrade MPs of choice. While it is true that microbes are able to degrade specific MPs very effectively, treating wastewater by exposure to white rot fungi could result in a possible first step towards tackling the least common MPs. This generalist approach is also the white rot fungi's downfall, as

they utilize MPs and waste materials as their primary growth material, and considering that MPs are present in low concentrations, it is unlikely for a species of fungi to be able to subsist off of this chemical alone. This is where the idea of specific cultivation of microbes to address these issues comes in. Because many of the MPs released into the waste stream are pharmaceuticals or other trace contaminants, it is often possible to treat MPs when they are at a higher concentration before being diluted in a population's water system.[26,69]

Ideally, if a MP is known to be widely used in a specific location, such as a hospital or industrial site, cultivation of microbes brought up specifically to grow and consume these MPs at the source can be implemented to reduce MP load on local WWTPs.[26,69] This technique involves the culturing and growth of these specific microbes in a reactor parallel to the treatment system. By occasionally injecting the system with these microbial specialists, a majority of the MP of interest can be degraded before it enters a community's water system. The downfall to this however is that because these microbes are so specialized, they are incapable of surviving in natural environments outside of a highly MP saturated system, and as such will often die off, requiring more frequent injections to consistently remove MPs, resulting in high operational involvement. A helpful middle ground between too specific and too general is the use of membrane bioreactors.

While the UASB utilizes a suspended membrane type system, providing surfaces for microbes to grow on and maintaining these membranes has also been studied.[18,70] This system is comparable to the UASB. Utilizing fixed growth locations to allow for a larger diversity of microbes to exist, and not necessarily requiring the introduction of oxygen to operate, this system could be considered a possible addition or alternative to the UASB given the correct scenario. While the UASB keeps the option available for some planktonic microbes to survive and interact with MPs, membrane bioreactors have large surface area available for adherent microbes to attach

to and grow on. Additional steps can be taken to improve the ability of an anaerobic reactor to provide the desired treatment of water, including the addition of a filter at the top of an anaerobic column to reduce the loss of microbes and provide one final stopgap for MPs to be caught, the issue of course being the necessary recycling of the filter to ensure continued efficacy.

### 1.5 Research Objectives

As discussed in the preceding, there is much we already know about the two systems presented, the CAS and UASB. However, our understanding of MP removal in the UASB is vastly inferior to our understanding of the capabilities and limitations of the CAS interacting with a wide range of MPs. This knowledge gap stems from our lack of understanding of biological processes present in each reactor. To bridge this gap, our research focused on three points of interest. First, we investigated the present effectiveness of both the UASB and CAS systems at removing MPs. We set out to quantify as many MPs present as possible across both systems utilizing advanced analytical techniques, and define criteria that indicated effective MP removal in a reactor. Once we had a set of criteria that defined what it meant to have an MP effectively removed, we were curious about the pathway by which the MP was being removed, sorption or biotransformation. This spurred our second question: how can we compare the biotransformation rates of notable MPs across both reactors? To attempt to answer this question we utilized bench-scale batch test bioreactors spiked with MPs of interest to monitor the disappearance rate. By utilizing several different bioreactors we determined which MPs were disappearing due to biotransformation and which were removed by sorption to biological material. The question remained: into what did the MPs of interest biotransform? This was our third research question. While ignoring the MPs removed by sorption, we utilized predictive software to generate possible transformation pathways that produced a wide range of possible products. By first observing each full-scale system *in-situ*

to determine which MPs were present, followed by batch tests to determine how MPs were being removed, capped off with an analysis of biotransformation products, we believe that we have effectively addressed some of the knowledge gap surrounding the effectiveness of both a conventional and emerging system handling MPs.

# CHAPTER 2 - Differential Biotransformation of Micropollutants in Conventional Activated Sludge and Up-flow Anaerobic Sludge Blanket Processes

**Abstract**

Biological wastewater treatment processes exhibit variable extents of organic micropollutant (MP) biotransformation. We hypothesize that the unique wastewater microbial communities in conventional activated sludge (CAS) and up-flow anaerobic sludge blanket (UASB) processes will perform different types of MP biotransformations at different rates. To test this hypothesis, we collected influent and effluent samples from a full-scale CAS and a pilot-scale UASB. We measured each sample by means of high-resolution mass spectrometry and measured 39 MPs in both systems. We identify 17 MPs that exhibited better removal in the CAS and 16 MPs that exhibited better removal in the UASB. We also conducted batch experiments in bioreactors seeded with wastewater microbial communities harvested from the CAS and UASB into which we spiked 24 MPs of interest. Analysis of biotransformation products revealed that those formed exclusively in the batch CAS experiments were the result of oxidations and those formed in both batch CAS and UASB experiments were the result of redox-independent hydrolyses. Androsterone yielded different biotransformation products in the CAS and UASB experiments that align with the disparate redox conditions. Together, our data provide novel insights on the relative functioning of two wastewater microbial communities. Our study demonstrates the potential of the UASB to biotransform MPs and the relative biotransformation potential of CAS and UASB processes. We also present structures of nine biotransformation products, five of which have not been previously reported in the literature.

## 2.1 Introduction

Micropollutants (MPs) are defined as man-made organic chemicals that can have a negative impact on the environment at concentrations in the ng $L^{-1}$ to µg $L^{-1}$ range.[1,71] For example, pharmaceuticals and personal care products are down-the-drain chemicals that are conveyed to wastewater treatment plants (WWTPs) where they may only be partially removed before discharge to receiving surface waters.[16,71,72] Surface water systems that receive wastewater effluent often contain complex mixtures of organic MPs that are known to have adverse effects on aquatic ecosystems.[20,73] A variety of studies have reported higher incidences of antibiotic resistance, endocrine effects, and reduced ecosystem diversity and functioning in effluent-dominated surface water systems.[72,74] These observations motivate studies into enhanced removal of MPs during wastewater treatment.[46]

Conventional wastewater treatment relies on biological processes (*e.g.*, conventional activated sludge (CAS) processes) to remove bulk carbon and nutrients from wastewater.[75,76] Decades of studies of CAS processes have likewise demonstrated that some MPs can be removed during conventional wastewater treatment.[26,53,77–79] There are two main mechanisms of MP removal: sorption to sludge[48–50] and biotransformation.[51–54] Sorption to sludge is relevant for more hydrophobic MPs that have $logK_{ow}$ values greater than 4 or $logK_{oc}$ values that, when multiplied by the fraction of organic carbon ($f_{oc} \approx 0.35$ for wastewater sludge), result in a $logK_d$ with a value greater than 1.[48] Data has shown that adsorption can be a primary mechanism of MP removal for triclosan, perfluoroalkyl substances (PFASs), and fragrances used in personal care products.[40,80] Most MPs are polar or semi-polar and are not removed to a significant extent by means of adsorption.[81] Previous research has demonstrated that some MPs are readily biotransformed by the wastewater microbial communities in a CAS processes around the world whereas other MPs are

never biotransformed in CAS processes.[53,77,79,82,83] The vast majority of MPs exhibit variable extents of biotransformation in CAS processes around the world reflecting some relationship to operational and environmental conditions that shape the structure and functioning of the wastewater microbial community.[14,17,78,84,85] However, very little is known about the operational and environmental conditions that might enhance the biotransformation of certain MPs and therefore it is not yet possible to optimize the performance of WWTPs for the removal of MPs.

Although most WWTPs operate CAS processes, there is an increasing interest in alternative biological processes that might be better suited for smaller population densities or operation in resource-limited regions of the world.[36,86] One such emerging biological process is the up-flow anaerobic sludge blanket (UASB) reactor.[29,84,86,87] UASBs are attractive because they are comparatively inexpensive to install and maintain and can generate methane, thus promoting a clean energy solution.[57] However, UASBs are not likely to become globally adopted because of their slower reaction kinetics due to energy constraints, and the specificity they provide is not conducive to a more universal set of pollutants. We expect UASBs to see the most use in decentralized treatment plants that would benefit from the energy saving factor of the reactor. Nevertheless, UASBs harbor microbial communities that have diverse niches thanks to granular colony formation such that the morphology and diversity of the wastewater microbial community is clearly distinct from that in a CAS process.[15,37,88,89] Whereas the UASB has the ability to perform at a competitive level to the CAS when accounting for the treatment of bulk carbon substrates, its potential to biotransform MPs has not been extensively studied.[86] We argue that the UASB is an interesting system to study because of the unique morphology, structure, and functioning of the wastewater microbial community.[70,86]

The objectives of this research were to: (1) characterize MP removal (or biotransformation) at a full-scale CAS process and in a pilot-scale UASB reactor operating at the same WWTP; (2) identify MPs that exhibit faster biotransformation in each of the biological processes; and (3) identify specific biotransformations for MPs that exhibit differential biotransformation. To meet these objectives, we first conducted a field sampling campaign in which we collected 1 L grab samples from the influent and effluent of both biological processes. Triplicate samples were collected at 1 hour intervals and effluent samples were offset from influent samples by the hydraulic retention time of each biological process. We also conducted batch experiments with wastewater microbial communities harvested from each of the biological processes and spiked with 24 MPs that exhibited biotransformation in at least one of the biological processes. We found that several MPs exhibited differential biotransformation rates that could be explained by the performance of different biotransformations. This is the first study to reveal specific biotransformations that determine biotransformation rates in biological processes operated in parallel.

## 2.2 Materials and Methods

### *2.2.1 Micropollutant standards and reagents*

We selected 175 MPs for target quantification in this study. The selected MPs are commonly observed in WWTPs and consist of pharmaceuticals, industrial chemicals, pesticides, human metabolites, and food additives. These MPs also encompass a broad range of MP chemical structures. Stock solutions of all 175 MPs were prepared at 1 g $L^{-1}$ in either LC-MS-grade methanol (OmniSolv, VWR), nanopure water (EMD Millipore), LC-MS-grade acetonitrile (Fisher Chemical), ethanol (Decon Labs), or dimethyl sulfoxide (Macron Fine Chemicals) and stored at -20°C. A standard mixture of all 175 MPs was created in nanopure water at 5 mg $L^{-1}$ and stored

at -20°C. A list of the 175 MPs, along with their CAS numbers, chemical formulas, and analytical parameters are provided in **Table A1** of the **Appendix**. Similarly, a mixture of 51 isotope-labeled internal standards (ILIS) was created in nanopure water at 5 mg $L^{-1}$ and stored at -20°C. A list of the 51 ILISs is provided in **Table A2**. Finally, an experimental mixture containing a subset of 24 of the MPs at a concentration of 20 mg $L^{-1}$ was prepared in nanopure water and stored at -20°C until use in batch experiments. A list of the 24 MPs used in batch experiments is provided in **Table A3**.

*2.2.2 Field sampling campaign*

We designed a field sampling campaign to characterize MP removal (or biotransformation) at a full-scale CAS WWTP and in a pilot-scale UASB reactor operating at the same WWTP. The sampling campaign took place on September 20, 2022. Grab samples were collected in 4 L amber glass bottles that had been cleaned in an acid bath and pre-rinsed with methanol to remove any impurities. Triplicate samples were collected at 1 hour intervals and effluent samples were offset from influent samples by the hydraulic retention time of the CAS (8 hours) and the UASB (9.5 hours). The influent sample for the CAS process (noted as *CAS_inf* in this thesis) was collected after primary settling and before mixing with the return activated sludge. The effluent sample for the CAS process (*CAS_eff*) was collected at the downstream end of the aeration basin. The UASB process is operated as a pilot-scale system at the same WWTP and receives raw, unfiltered wastewater from the WWTP. The influent sample for the UASB process (*UASB_inf*) was collected from the outlet of the peristaltic pump that feeds untreated wastewater to the UASB. The effluent sample from the UASB process (*UASB_eff*) was collected at the downstream end of the UASB. Schematics of CAS and UASB processes along with sample locations are provided in **Figures A1**

and **A2** of the **Appendix**. Bulk performance of the CAS and UASB processes is summarized in **Table A4** of the **Appendix**.

*2.2.3 Preparation of wastewater samples*

All samples were filtered through Bunn coffee filters at the WWTP immediately after sampling to remove any unwanted solids and residue that would interfere with subsequent sample preparation. Triplicate influent samples (*CAS_inf*, *UASB_inf*) were collected in the morning, stored on ice at the WWTP until all triplicate samples were collected, and then immediately transported to the laboratory for sample preparation. Upon arrival in the laboratory, subsamples were collected to measure total suspended solids (TSS) and to archive biomass according to standard protocols as previously described.[90,91] The wastewater samples were then filtered with glass-fiber filters (grade GF/F, diameter 4.7 cm, pore size 0.7 µm, VWR) to remove suspended solids and subsequently with cellulose acetate filters (diameter 4.7 cm, pore size 0.45 µm, VWR) to generate sample filtrate for solid-phase extraction SPE. One liter of each triplicate influent sample (*CAS_inf*, *UASB_inf*) was titrated to a pH of 6.5 using dilute formic acid and spiked with 20 µL of the ILIS mixture such that each sample had an ILIS concentration of 100 ng $L^{-1}$ before loading onto mixed-bed SPE cartridges to concentrate the samples by a factor of 1000 as previously described.[92,93] Briefly, the self-packed mixed bed cartridges contained three layers separated by polyethylene frits. The bottom layer consisted of 200 mg Envi-CARB (Supelclean) media. The middle layer was 350 mg of a 1:1:1.5 (by mass) mixture of Strata X-AW (Phenomenex), Strata X-CW (Phenomenex), and Isolute ENV+ (Biotage), respectively. The final layer was 200 mg of Oasis HLB (Waters) media. The cartridges were conditioned before sample loading by passing a mixture of 5 mL methanol and 10 mL nanopure water through each cartridge. The sample loading rate was controlled at approximately 1-3 drops per second. The cartridges were eluted using 6 mL of 50:50

v/v ethyl acetate/methanol with 0.5% ammonia, 3 mL of 50:50 v/v ethyl acetate/methanol with 1.7% formic acid, and 2 mL of pure methanol. The extracts were evaporated under a stream of high-purity nitrogen to 100 µL and reconstituted to 1 mL using nanopure water. The extracted samples were filtered (regenerated cellulose, 0.45 µm, Thermo Scientific) and stored at -20 ˚C and in the dark until analysis. We also prepared a 9-point calibration curve by spiking the mixture of the 175 MPs into 1 L of nanopure water to generate standards at concentrations of 0, 1, 5, 25, 50, 100, 250, 500, and 750 ng $L^{-1}$. The calibration standards were likewise spiked with 20 µL of the ILIS mixture and loaded onto the mixed-bed SPE cartridges and eluted as described in the preceding. Triplicate effluent samples (*CAS_eff*, *UASB_eff*) were collected in the afternoon, stored on ice at the WWTP until all triplicate samples were collected, and then immediately transported to the laboratory for sample preparation and SPE as described in the preceding.

*`2.2.4 Batch experiments with wastewater microbial communities from the UASB*

Batch experiments with wastewater microbial communities harvested from the UASB were conducted in 100 mL clear glass serum bottles and under anaerobic conditions. Wastewater microbial communities were harvested from the base of the UASB in 2 L amber glass bottles with no headspace. Harvested wastewater microbial communities were then immediately transported to the laboratory and placed in an anaerobic chamber with a controlled atmosphere containing pure $N_2$ and $H_2$. The wastewater microbial communities were aseptically transferred into a 1L clear glass bottle and diluted with deoxygenated wastewater effluent to a final TSS concentration of approximately 1 g $L^{-1}$. We then transferred 30 mL of the diluted wastewater microbial communities into triplicate serum bottles covered in aluminum and crimp sealed them with an airtight rubber stopper. The reactors were then spiked with the mixture of 24 MPs using a hypodermic needle to achieve a starting concentration of 100 µg $L^{-1}$ for each MP and placed on a rotary shaker at 20 °C;

all incubation experiments were spiked within 5 h of sample collection. We collected 0.5 mL samples from each reactor after 5 min, 2 h, 6 h, 18 h, 30 h, 54 h, 69 h, 95 h, 118 h, 140 h, 165 h, and 190 h, transferred the samples to a 1.5 mL centrifuge tube (Eppendorf), and centrifuged the samples at 13000 rpm for 5 min at 4 °C. Then, 400 µL of the supernatant was transferred to a 2 mL amber glass vial (VWR), capped, and stored at -20 °C until analysis. An adsorption control experiment was conducted with the same procedure, except adsorption control reactors were autoclaved twice (120 °C, 1.3 bar, 20 min, 4 h apart) and spiked 24 h after the start of the active incubation experiment and sampled at an interval of 5 min, 2 h, 6 h, 18 h, 30 h, 45 h, 71 h, 94 h, 116 h, 140 h, and 167 h. A final control reactor was included to assess background concentrations of the 24 MPs and microbial activity in the absence of spiked MPs; this reactor contained the wastewater microbial community but was not spiked with MPs.

*2.2.5 Batch experiments with wastewater microbial communities from the CAS*

Batch experiments with wastewater microbial communities harvested from the CAS were conducted in 100 mL amber glass reactors (Corning) covered in aluminum and in triplicate as previously described.[90,91] Wastewater microbial communities were harvested from the middle of the CAS in 2 L amber glass bottles with headspace to allow them to remain aerobic. We did not dilute the wastewater microbial communities harvested from the CAS because the TSS was already approximately 1 g $L^{-1}$. We then transferred 30 mL of the diluted wastewater microbial communities into the amber glass reactors. The reactors were then spiked with the MP mixture solution to achieve a starting concentration of 100 µg $L^{-1}$ for each MP and placed on a rotary shaker at 20 °C. We collected 0.5 mL samples from each reactor after 5 min, 2 h, 6 h, 18 h, 24 h, 26 h, 30 h, 42 h, 54 h, 74 h, 94 h, 118 h, 143 h, 165 h, and 190 h, transferred the samples to a 1.5 mL centrifuge tube (Eppendorf), and centrifuged the samples at 13000 rpm for 5 min at 4 °C. Then, 400 µL of

the supernatant was transferred to a 2 mL amber glass vial (VWR), capped, and stored at -20 °C until analysis. An adsorption control experiment was conducted with the same procedure, except adsorption control reactors were autoclaved twice (120 °C, 1.3 bar, 20 min, 4 h apart) and spiked 24 h after the start of the active incubation experiment and sampled at an interval of 5 min, 2 h, 6 h, 18 h, 30 h, 50 h, 70 h, 94 h, 119 h, 142 h, and 166 h. A final control reactor was included to assess background concentrations of the 24 MPs and microbial activity in the absence of spiked MPs; this reactor contained the wastewater microbial community but was not spiked with MPs.

*2.2.6 Sample analysis*

We adopted a previously described analytical method for MP quantification and transformation product (TP) identification in samples collected in the field campaign and in the batch experiments.[90,91,94–96] Briefly, samples were measured using reversed-phase liquid chromatography (Ultimate 3000, Thermo Scientific) coupled to high-resolution quadrupole-orbitrap mass spectrometry (QExactive, Thermo Scientific) with 30 µL injections of samples stored at 4 °C during the analysis. Samples were separated using a mobile phase gradient of LC-MS grade water (OmniSolv, 58201, solvent A) and methanol (OmniSolv, 58215, solvent B) – both containing 0.1% (v/v) formic acid – over an XBridge C18 column (Waters, 186003021, particle size: 3.5 µm, flow rate: 0.2 mL/min, gradient properties: 0 – 5 min: 5% B, 5 – 21 min: 5% B – 95% B (linear increase), 21 – 25 min: 95% B, 25 – 30 min: 5% B). We performed full-scan MS acquisitions (100-1000 m/z, resolution 140,000) in electrospray ionization positive-negative switch mode. Data dependent MS2 spectra were acquired at the exact masses and retention times of all target MPs and ILISs with additional MS2 spectra collected for the TopN MS features if the inclusion list was not triggered. For absolute quantification of target analytes, we used ILIS normalized peak areas obtained with Xcalibur Quanbrowser (Thermo Scientific, Version

4.0.27.19) and a calibration series (concentration range: 0-750 µg/L after passing through SPE) with 1/x least-squares regression. Analytical parameters for each target MP and its assigned ILIS are provided in **Table A1** and **Table A2**.

*2.2.7 Biotransformation product analysis*

We used the Eawag-Pathway Prediction System (Eawag-PPS)[97] to generate a list of predicted TPs with masses greater than 100 Da for select MPs that were biotransformed in the batch experiments. We generated SMILES for each of the predicted TPs and used ChemDraw Professional (2018 version 18.2.0.48) to calculate the exact mass of the $[M+H]^+$ and $[M-H]^-$ ions for each predicted TP. We then used Xcalibur Qualbrowser (Thermo Scientific, Version 4.0.27.19) to visually screen HRMS acquisitions for evidence of TP formation in the samples from the batch experiment or the *CAS_eff* and *UASB_eff* samples. Evidence of biotransformation product formation includes: (i) peak areas greater than E5; (ii) reasonable peak shape; (iii) presence of a peak in the active reactors and absence of a peak (or a peak area less than E4) in control reactors; and (iv) increasing or increasing and then decreasing peak area over time.

*2.2.8 Quality Control*

Because the wastewater samples from the field sampling campaign had a relatively complex matrix, some of the 51 ILISs that we included in this study did not ionize efficiently. Therefore, we had to eliminate some ILISs from our processing method and reassign some of the target MPs to ILISs that could be measured across all samples. The results of this exercise are summarized in the last column in **Table A2**. Next, we used an in-house R script to match MS2 fragments to candidate target MP peaks in the wastewater samples from the sampling campaign (R script available for download at github.com/cmc493). Confirmed MP detection in any sample required at least one diagnostic fragment in one of the triplicate sample measurements. We only

report concentrations of MPs where linear calibration curves consisted of at least three points and had an $R^2$ greater than 0.85. Data quality parameters such as $R^2$ and LOQ for target MPs are provided in **Table A5**.

*2.2.9 Data Analysis*

We used *CAS_eff*, *UASB_eff*, MP removal (*%R_CAS* or *%R_UASB*) and pseudo first-order biotransformation rate constants ($k_{bio,CAS}$ *or* $k_{bio,UASB}$) as metrics to describe the performance of the two biological processes. The %R was calculated as:

$$\%R = 100 * \frac{C_{inf} - C_{eff}}{C_{inf}} \quad (1)$$

where $C_{inf}$ is the concentration of an MP in the influent sample for the CAS or UASB (*CAS_inf* or *UASB_inf*) and $C_{eff}$ is the concentration of an MP in the effluent sample for the CAS or UASB (*CAS_eff* or *UASB_efff*).

The $k_{bio}$ was calculated as:

$$k_{bio} = \ln\left(\frac{C_{eff}}{C_{inf}}\right)\left(-\frac{1}{HRT}\right) \quad (2)$$

where $C_{inf}$ is the concentration of an MP in the influent sample for the CAS or UASB (*CAS_inf* or *UASB_inf*), $C_{eff}$ is the concentration of an MP in the effluent sample for the CAS or UASB (*CAS_eff* or *UASB_efff*), and HRT is the hydraulic retention time of the CAS or the UASB process. We estimated a $k_{bio}$ for each MP by performing linear regression on the sum squared differences between our three separate samples. When accounting for sorption's effect on the calculation for $k_{bio}$, the autoclaved controls demonstrated that sorption is nearly instantaneous as previously reported in other works.[98] Since sorption is considered to be instantaneous the only influence it holds on $k_{bio}$ is determining what is considered to be $C_{inf}$. In this study we determined that for each MP its $C_{inf}$ would be the calculated average of the first data point across the three reactors, given

that this average was not below the observed concentration present in the sorption batch reactor, in which case the new $C_{inf}$ would be the first concentration observed in the autoclaved reactor.

## 2.3 Results and Discussion

### *2.3.1 Summary data from sampling of full-scale CAS and UASB*

We collected triplicate grab samples from the influent and effluent of a full-scale CAS and pilot scale UASB process at the same WWTP. The average TSS of the UASB was 21.8 g/L and the TSS of the CAS was 1.2 g/L. Other operational parameters are provided in **Table A4**. We prepared the samples and measured them for up to 175 MPs. After implementing quality control measures, we quantified 94 MPs in at least one sample. We quantified 65 MPs in the influent to the CAS process and 58 in the influent to the UASB process. Of the 65 MPs quantified in the influent of the CAS process, 61 were also quantified in the effluent. Of the 58 MPs quantified in the influent of the UASB process, 50 were also quantified in the effluent. When comparing the two systems, we identified 39 MPs that were present in the influent samples from both the CAS and UASB processes. Average concentrations of the 39 MPs measured in each sample are provided in **Table A6**.

### *2.3.2 Comparing MP removal performance in both systems*

We first aimed to evaluate the performance of the CAS and UASB for removing (or biotransforming) the 39 MPs that were measured in the influent samples from both processes. To do this we defined three metrics. First, we used a direct comparison of the average *CAS_eff* and *UASB_eff* data for each of the 39 MPs. Because we collected samples at three time points and measured each of the samples in triplicate, these data represent the average of nine measurements for each of the 39 MPs. We reasoned that the process that achieved a lower average *C_eff* for a given MP was performing better for that MP because they are biological processes operating in

parallel at the same WWTP. Second, we calculated MP removal (*%R_CAS* or *%R_UASB*) as defined in **Equation 1**. MP removal was again calculated using the average of nine measurements

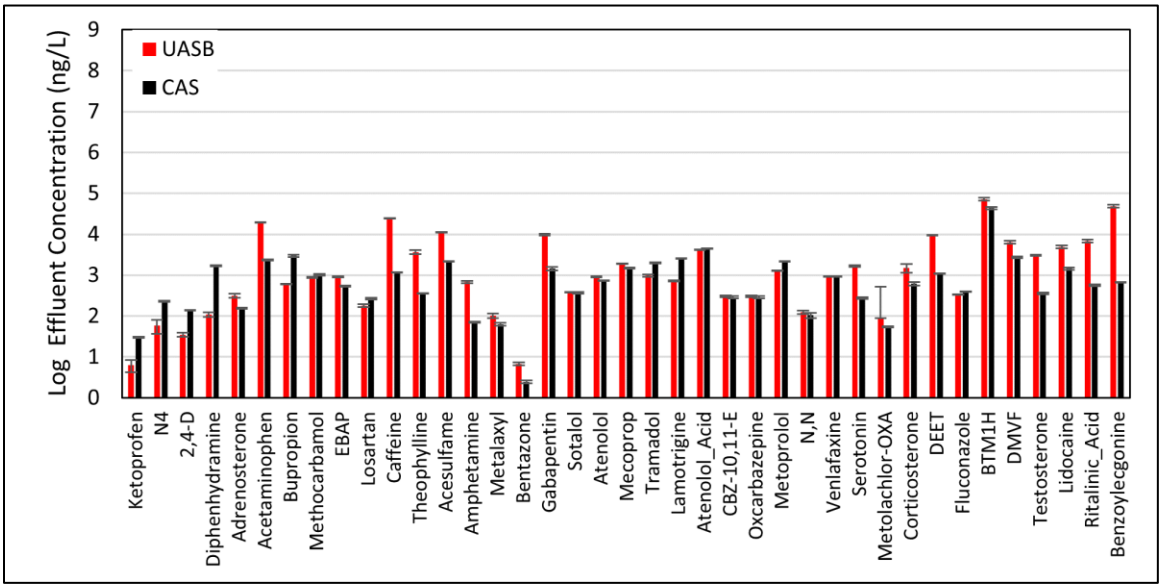


**Figure 2.1**: A summary of the effluent concentrations (*CAS-eff* and *UASB_eff*) of the 39 MPs that were measured in both the CAS and UASB samples. Note: due to size limitations the following MPs have been abbreviated: N4 = N4-acetylsulfamethoxazole, EBAP = Ethyl_butylacetylaminopropionate, CBZ-10,11-E = Carbamazepine-10,11-epoxide, N,N = N,N-didesmethylvenlafaxine, BTM1H = Benzotriazole_methyl-1H, DMVF = Desmethylvenlafaxine

for each MP. This metric allows us to compare the performance of each of the wastewater microbial communities and accounts for the different *C_inf* concentrations for each of the processes. Third, we calculated pseudo first-order biotransformation rate constants ($k_{bio,CAS}$ *or* $k_{bio,UASB}$) as defined in **Equation 2**. Biotransformation rate constants were calculated with the average of nine measurements and incorporates the hydraulic retention time (HRT) of the respective process. This metric allows us to compare the relative activity of the wastewater microbial communities by normalizing the removal to the amount of time available for biotransformation.

A direct comparison of the average *CAS_eff* and *UASB_eff* data for each of the 39 MPs is provided in **Figure 2.1**. These data demonstrate that eleven of the 39 MPs have significantly lower effluent concentrations in the UASB versus the CAS. These include ketoprofen, N4-

acetylsulfamethosazole, 2,4-D, diphenhydramine, bupropion, methocarbamol, losartan, tramadol, lamotrigine, metoprolol, and fluconazole (two tailed t-test, $p < 0.05$). It is difficult to assess whether the effluent concentrations are the result of adsorption to the sludge solids or biotransformation. One could expect more adsorption in the UASB because the TSS was 18.2x higher in the UASB than the CAS. We compared the $logK_{ow}$ and $logK_{oc}$ values (**Table A3**) of the

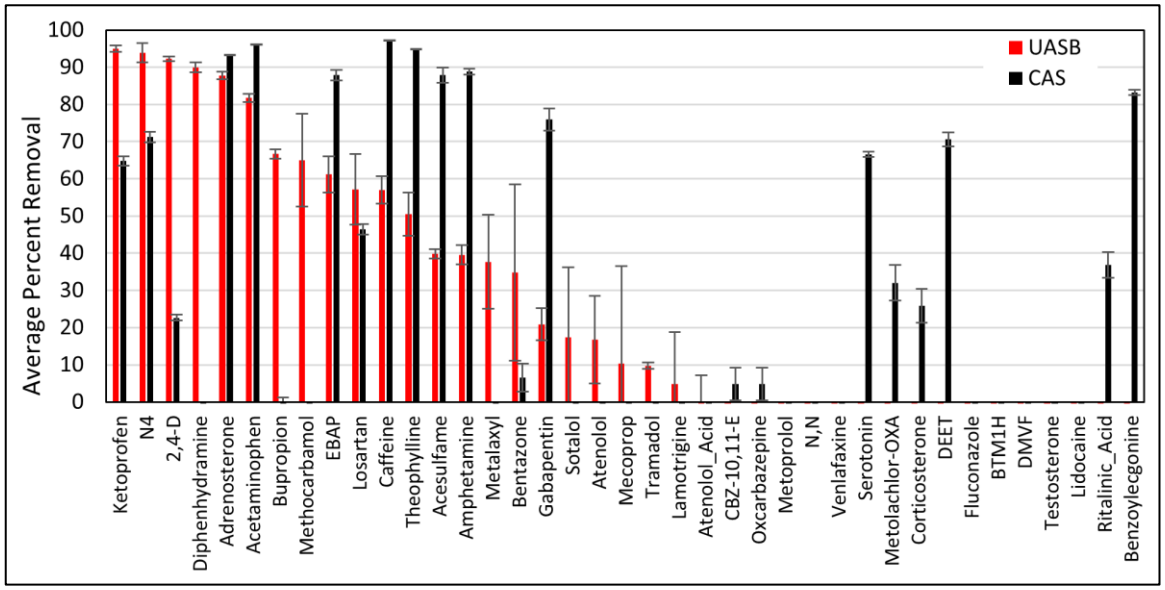


**Figure 2.2**: A summary of the MP Removal (*%R_CAS* and *%R_UASB*) of the 39 MPs that were measured in both the CAS and UASB samples. Note: due to size limitations the following MPs have been abbreviated: N4=N4-acetylsulfamethoxazole, EBAP=Ethyl_butylacetylaminopropionate, CBZ-10,11-E=Carbamazepine-10,11-epoxide, N,N=N,N-didesmethylvenlafaxine, BTM1H=Benzotriazole_methyl-1H, DMVF=Desmethylvenlafaxine.

eleven MPs that had lower effluent concentrations in the UASB to the other 28 MPs and found no statistically significant difference (two tailed t-test, p-value > 0.05). This finding suggests that biotransformation in the UASB is likely important for at least for some of these eleven MPs. Six other MPs did not exhibit statistically significant effluent concentrations between the UASB and the CAS (t-test, $p > 0.05$). These include atenolol acid, carbamazepine-10,11-epoxide, oxcarbemazepine, sotalol, corticosterone, and venlafaxine. These are primarily MPs that are known to be persistent during biological wastewater treatment.[99,100] The remaining 22 MPs exhibited significantly lower effluent concentration in the CAS and are presumed to be better removed in the CAS process based on this metric.

A direct comparison of the average %R_*CAS* and %R_*UASB* data for each of the 39 MPs is provided in **Figure 2.2**. These data demonstrate that twelve of the 39 MPs have significantly higher *%R_UASB* than *%R_CAS* (t-test, p-value < 0.05) These include ketoprofen, N4-acetylsulfamethosazole, 2,4-D, diphenhydramine, bupropion, methocarbamol, metalaxyl, sotolol, atenolol, mecoprop, tramadol, and lamotrigine. Eight of these also had lower *UASB_eff* than *CAS_eff* highlighting the better performance of the UASB for these MPs using two independent metrics. The other four MPs either had nearly identical effluent concentrations in the UASB and the CAS or lower *CAS_eff* than *UASB_eff* (metalaxyl, sotolol, atenolol, and mecoprop). In these cases, accounting for the higher *UASB_inf* concentration (relative to *CAS_inf*) demonstrates better removal of these MPs. We believe that the lower concentration of these MPs in the *CAS_inf* sample is due to some removal during primary sedimentation. Interestingly, losartan, metoprolol, and fluconazole were found to have lower effluent concentrations in the UASB than the CAS, but the percent removal was either higher in the CAS (losartan) or the same in the UASB and CAS (metoprolol and fluconazole). Indeed, there were nine MPs that exhibited no removal or negative removal in both systems including atenolol acid, metoprolol, N,N-didesmethylvenlafaxine, venlafaxine, fluconazole, benzotriazole-methyl-1H, desmethylvenlafaxine, testosterone, and lidocaine. These MPs have exhibited varying degrees of removal in biological treatment processes

in other studies.[99,100] The remaining 18 MPs exhibited significantly higher removal in the CAS and are presumed to be better removed in the CAS based on this metric (t-test, $p < 0.05$).

A direct comparison of the pseudo first-order biotransformation rate constants ($k_{bio,CAS}$ *or* $k_{bio,UASB}$) for each of the 39 MPs is provided in **Figure 2.3**. These data demonstrate that seven of the 39 MPs have significantly higher $k_{bio,UASB}$ than $k_{bio,CAS}$ (t-test, $p < 0.05$). These include ketoprofen, diphenhydramine, bupropion, metalaxyl, sotolol, tramadol, and lamotrigine. Five of these MPs also had lower *UASB_eff* than *CAS_eff* and higher *%R_UASB* than *%R_CAS* highlighting the better performance of the UASB for these five MPs using all three of our defined metrics. In fact, ten of the 15 MPs that were identified as being better removed in the UASB by at least one of our metrics were also identified by at least one other metric; the exceptions are losartan,

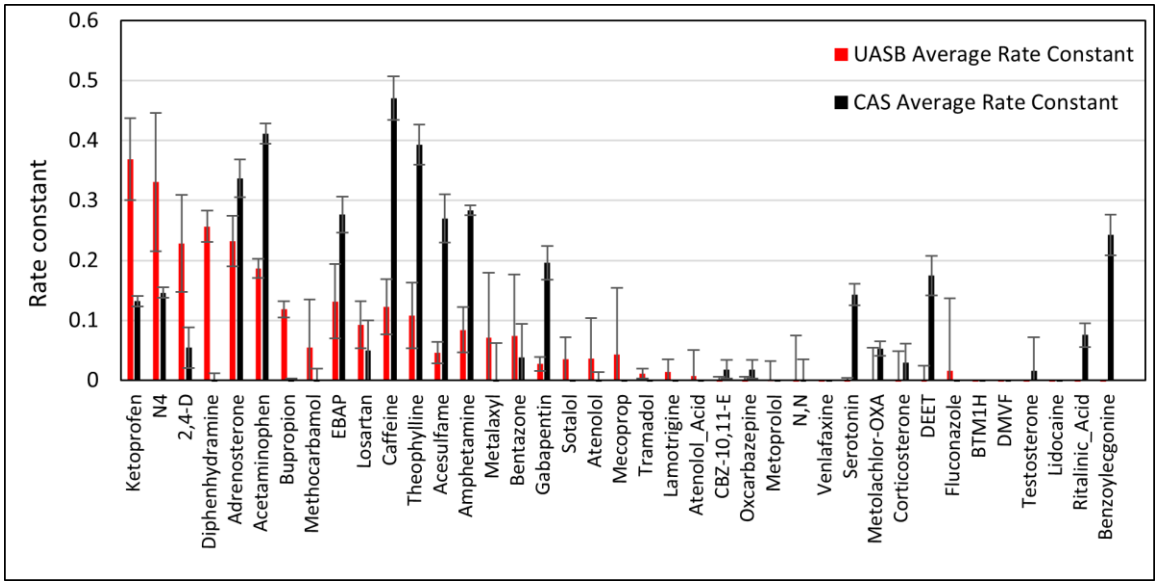


**Figure 2.3**: A summary of the pseudo first-order biotransformation rate constants ($k_{bio,CAS}$ *or* $k_{bio,UASB}$) of the 39 MPs that were measured in both the CAS and UASB samples. Note: due to size limitations the following MPs have been abbreviated: N4=N4-acetylsulfamethoxazole, EBAP=Ethyl_butylacetylaminopropionate, CBZ-10,11-E=Carbamazepine-10,11-epoxide, N,N=N,N-didesmethylvenlafaxine, BTM1H=Benzotriazole_methyl-1H, DMVF=Desmethylvenlafaxine

metoprolol, fluconazole, atenolol, and mecoprop which were only identified by one metric. We also report on six MPs that exhibited pseudo first-order biotransformation rate constants equal to zero (or a negative value suggesting formation, which is a phenomenon previously reported in the

literature).[21,101,102] These include metoprolol, N,N-didesmethylvenlafaxine, venlafaxine, benzotriazole-methyl-1H, desmethylvenlafaxine, and lidocaine. We also report on thirteen MPs that did not exhibit significantly different rate constants between the UASB and the CAS. These include N4-acetylsulfamethosazole, 2,4-D, adrenosterone, methocarbamol, losartan, bentazone, atenolol, mecoprop, atenolol acid, metolachlor-oxa, corticosterone, testosterone, and fluconazole (t-test, $p > 0.05$). The remaining 13 MPs exhibited significantly higher $k_{bio,CAS}$ than $k_{bio,UASB}$. These include acetaminophen, ethyl-butylacetylaminopropionic acid, caffeine, theophylline, acesulfame, amphetamine, gabapentin, carbamazepine-10,11-epoxide, oxcarbazepine, serotonin, DEET, ritalinic acid, and benzoylecgonine. All of these were identified by at least one of our other metrics as MPs that are better removed in the CAS system.

In summary, our analysis identified 16 MPs that are better removed in the UASB (ketoprofen, N4-acetylsulfamethosazole, 2,4-D, diphenhydramine, bupropion, methocarbamol, losartan, metalaxyl, bentazone, sotolol, atenolol, metoprolol, mecoprop, tramadol, lamotrigine, and fluconazole) and 17 MPs that are better removed in the CAS (adrenosterone, acetaminophen, ethyl-butylacetylaminopropionic acid, caffeine, theophylline, acesulfame, amphetamine, gabapentin, carbamazepine-10,11-epoxide, oxcarbazepine, serotonin, metolachlor-oxa, corticosterone, DEET, testosterone, ritalinic acid, and benzoylecgonine). Six of the MPs exhibited similar removal in both systems. It is interesting to note that there was nearly an even split of MPs that were better removed by the two processes. This was also somewhat unexpected because previous studies show that MPs are readily biotransformed in aerobic biological processes whereas anaerobic biological processes often perform MP biotransformations more slowly or not at all.[28,91,103] To investigate these findings further, we performed batch experiments to assess biotransformation rates and explore the formation of biotransformation products.

*2.3.3 Batch experiments – biotransformation kinetics*

We performed batch experiments in bioreactors seeded with microbial communities harvested from the UASB and CAS processes. The microbial communities from the UASB were sampled and handled in an anaerobic environment and were diluted by a factor of 33.3x to a concentration of 1 g $L^{-1}$ with deoxygenated WWTP effluent prior to experimentation. This was done to replicate the biomass concentration in the CAS. We selected 12 MPs that were found to be better removed in the pilot-scale UASB (ketoprofen, N4-acetylsulfamethosazole, 2,4-D, diphenhydramine, bupropion, methocarbamol, losartan, bentazone, sotolol, mecoprop, tramadol, and lamotrigine) and 12 MPs that were found to be better removed in the full-scale CAS (adrenosterone, acetaminophen, ethyl-butylacetylaminopropionic acid, caffeine, theophylline, acesulfame, amphetamine, gabapentin, serotonin, DEET, ritalinic acid, and benzoylecgonine). A mixture of the 24 MPs was spiked into each of the triplicate prepared bioreactors to reach a starting concertation of each MP of 100 µg $L^{-1}$. Samples were collected over a period of eight days and the resulting data were used to estimate pseudo first-order biotransformation rate constants. More details on how the data were processed to generate pseudo first-order rate constants (including evaluation of data from control experiments) are provided in text in the **Appendix** and the results of the analysis are presented in **Figure 2.4**.

The data in **Figure 2.4** demonstrate that eighteen of the 24 MPs were biotransformed in the UASB batch experiments and all 24 of the MPs were biotransformed in the CAS batch experiments. The six MPs that exhibited no biotransformation in the UASB experiments include three that were removed to greater extents in the pilot-scale UASB (mecoprop, tramadol, and lamotrigine) and three that were removed to greater extents in the CAS (amphetamine, gabapentin, and ritalinic acid). Notably, only 2,4-D exhibited faster biotransformation kinetics in the UASB batch experiments versus the CAS batch experiments. Together, these observations suggest that the batch systems are not replicating the MP biotransformation rates that were observed in the pilot-scale UASB and the full-scale CAS. There are several likely reasons for this discrepancy. First, we diluted the UASB sludge by a factor of 33.3x to ensure that we have equivalent biomass densities in the respective batch experiments to allow us to compare the relative activities of the two microbial communities. However, dilution can lower the diversity of the microbial community through the bottleneck effect and can essentially eliminate low abundance members of the

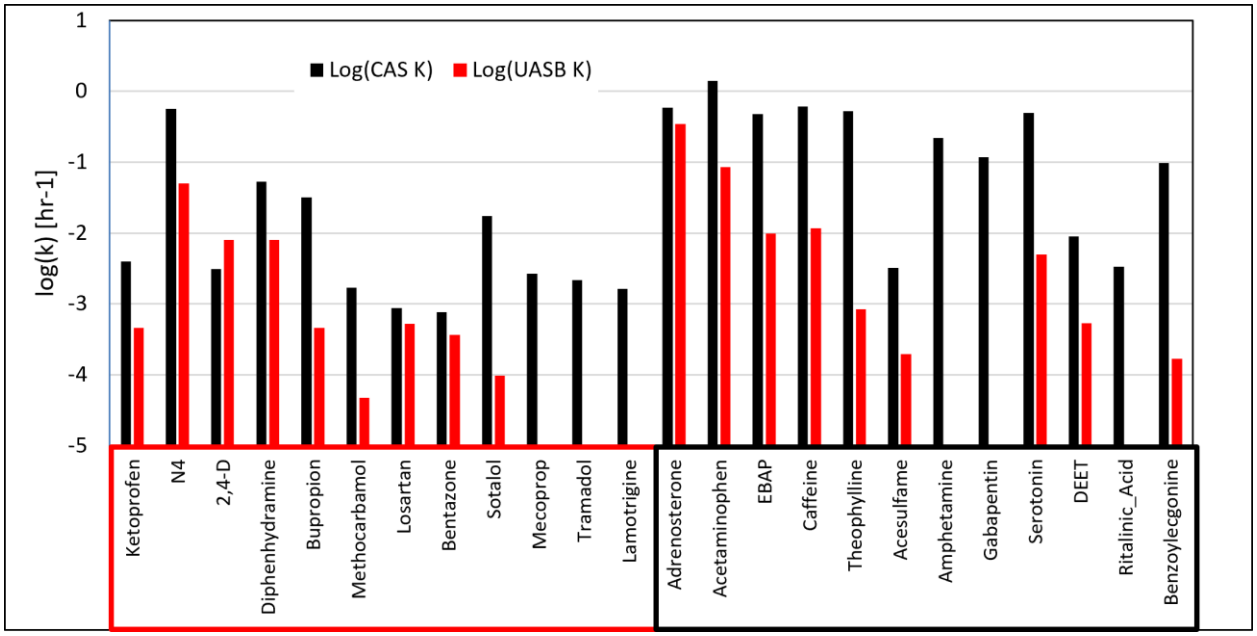


**Figure 2.4**: A summary of the pseudo first-order biotransformation rate constants ($k_{bio,CAS}$ *or* $k_{bio,UASB}$) of the 24 MPs that were measured in the batch experiments. The MPs in the red box are those that were better removed in the pilot-scale UASB and those in the black box are those that were better removed in the CAS. Note: N4 = N4-acetylsulfamethoxazole, EBAP = Ethyl butylacetylaminopropionate

microbial community that may participate in MP biotransformations.[104] This could explain why we observed no biotransformation of mecoprop, tramadol, and lamotrigine in the batch experiments with the UASB sludge. It is also possible that some of the removal observed in the pilot-scale system was driven by adsorption to sludge and that removal by means of adsorption significantly decreased when we diluted the sludge. Although this is a plausible explanation for some MPs, an analysis of $logK_{ow}$ and $logK_{oc}$ values (**Table A3**) along with literature reported sludge-water distribution coefficients[48,80,105] suggests that sorption is not a major removal mechanisms for these MPs. Finally, we note that harvesting wastewater microbial communities from full-scale or pilot-scale systems that are operating at steady-state and placing them in batch reactor in the lab is likely to cause some changes in microbial community structure and function. This has been discussed in the literature to some extent when evaluating MP biotransformations in bioreactors seeded with sludge from CAS processes,[106,107] and is likewise relevant here for the experiments with the UASB sludge. In the latter case, the bioreactors were established under anaerobic conditions in an atmosphere consisting of $N_2$ and $H_2$. These conditions might not align with those in the pilot-scale system which further complicates this comparison.

Nevertheless, it is important to emphasize that we did see biotransformation of 16 MPs in the batch experiments seeded with sludge from the UASB. This is an important observation in its own right because the performance of wastewater microbial communities from UASB systems to biotransform MPs has not been studied to a significant extent. Also, we note that the difference in the magnitudes of the rate constants between the UASB and CAS batch experiments are smaller for the MPs that were observed to be better removed in the pilot-scale UASB (red box in **Figure 2.4**). To explore this latter observation more deeply, we corrected the rate constants for the MPs in the UASB experiment to account for the 33.3x dilution factor used to equalize the biomass

concentrations. The results of this analysis are presented in **Figure 2.5**. Here we can see that six of the 9 MPs that were biotransformed in our batch experiments and were better removed in the pilot-scale UASB have greater rate constants in the UASB experiments than in the CAS experiments. The other three MPs have nearly identical rate constants in both sets of batch experiments. In contrast, only four of 9 MPs that were biotransformed in our batch experiments and were better removed in the full-scale CAS have greater rate constants in the UASB experiments than in the CAS experiments. The differences here are relatively small and should be tested for statistical significance. Nevertheless, this correction demonstrates that biotransformation rates measured in batch can represent trends observed at pilot- and full-scale when one corrects for biomass dilution.

*2.3.4 Batch experiments – biotransformation products*

Our final objective was to screen the high-resolution mass spectral acquisitions from the

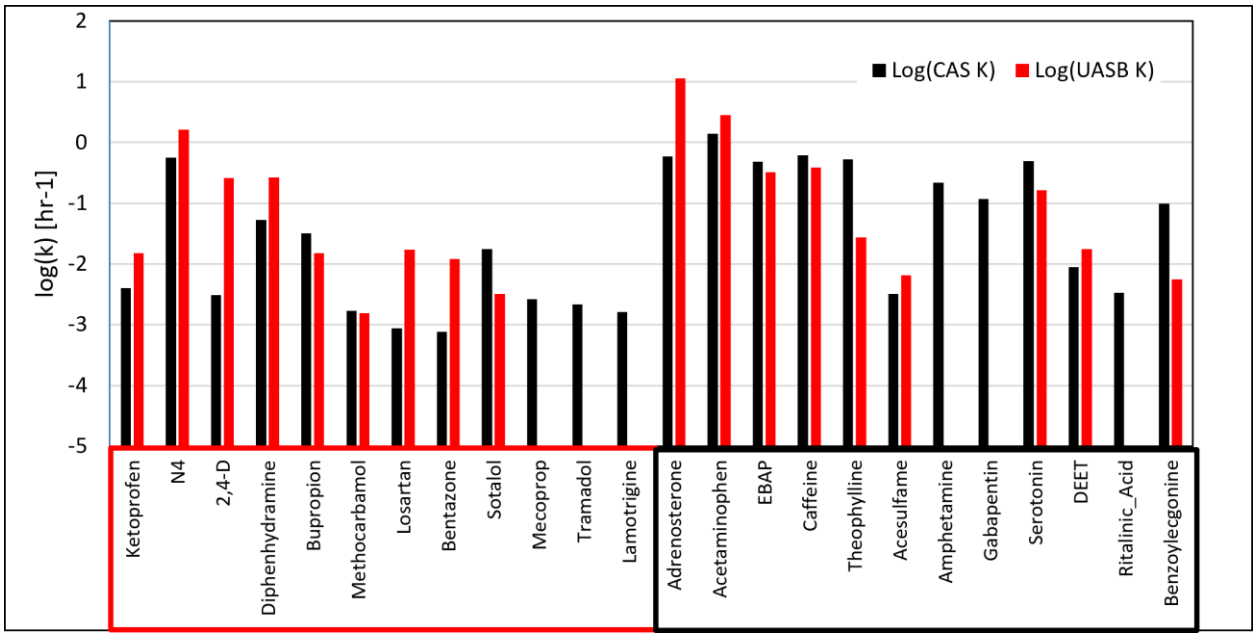


**Figure 2.5**: A summary of the pseudo first-order biotransformation rate constants ($k_{bio,CAS}$ *or* $k_{bio,UASB}$) of the 24 MPs that were measured in the batch experiments correcting for dilution of UASB sludge. The MPs in the red box are those that were better removed in the pilot-scale UASB and those in the black box are those that were better removed in the CAS. Note: N4 = N4-acetylsulfamethoxazole, EBAP = Ethyl butylacetylaminopropionate

batch experiments for evidence of biotransformation product formation. This type of evidence will

allow us to infer whether there were differential metabolic activities between the two types of wastewater microbial communities. To do this, used the Eawag-PPS to predict biotransformation products for 14 of the total 24 parent MPs. The selected 14 MPs were parent MPs that were observed to have a log(k) rate constant higher than 0.01 $hr^{-1}$ in the CAS batch test, and a log(k) rate constant higher than 0.005 $hr^{-1}$ in the UASB batch test. We reasoned that only those MPs with a measurable rate constant would generate TPs that we could actually measure. We performed this prediction with relative reasoning turned off and allowed the Eawag-PPS to predict both aerobic and anaerobic biotransformations. This resulted in a list of 104 predicted first-generation biotransformation products for the 14 parent MPs. We used the exact masses of the $[M+H]^+$ and $[M-H]^-$ parent ions for each of the predicted TPs to screen our high-resolution mass spectral acquisitions. More details on our approach to biotransformation product screening is provided in the **Appendix** along with a summary of our findings in **Table A7**.

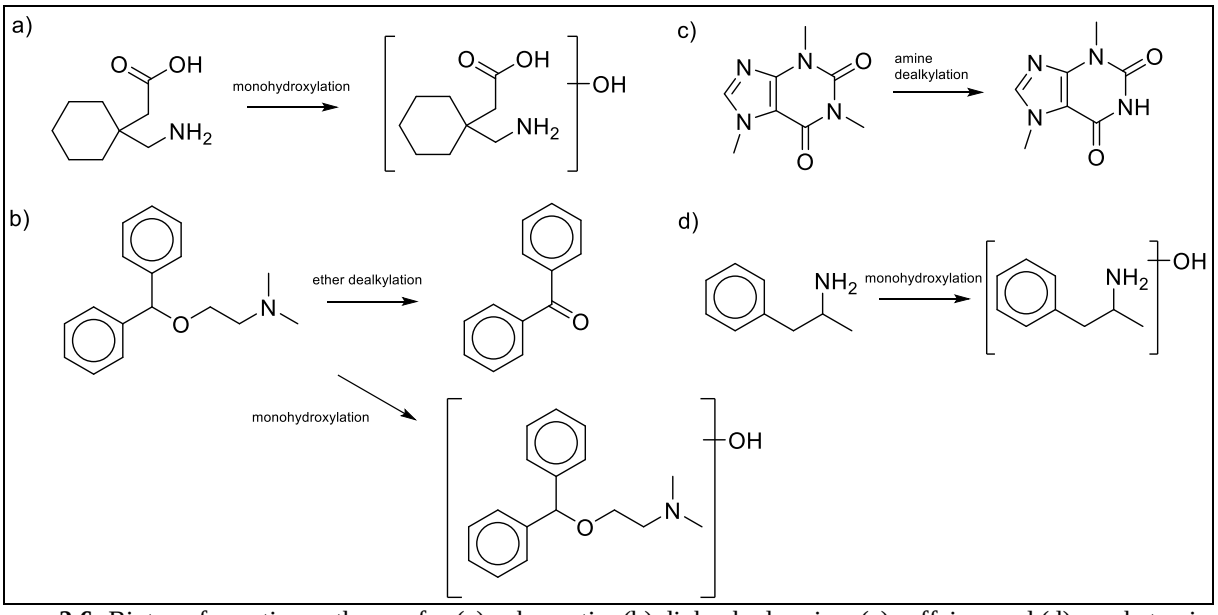


**Figure 2.6:** Biotransformation pathways for (a) gabapentin, (b) diphenhydramine, (c) caffeine, and (d) amphetamine.

Using the conservative criteria for TP identification established in the Methods section, we screened for TPs in batch experiments in which the observed biotransformation rate constant was

greater than 0.005 $hr^{-1}$ (for UASB) or greater than 0.01 $hr^{-1}$ (for CAS). There were 14 MPs that met our biotransformation rate constant threshold for at least one of the batch systems. We identified nine TPs for seven of the 14 parent MPs in at least one of the batch experiments. A summary of those transformation products is provided in **Figures 2.6** through **2.8**. It must be noted that no evidence of predicted biotransformation products was observed for eight of the 14 parent MPs. These included 2,4-D (rate constant threshold met in both UASB and CAS experiments), acetaminophen (both), serotonin (both), caffeine (UASB), diphenhydramine (UASB), benzoylecgonine (CAS), bupropion (CAS), theophylline (CAS). We contend that biotransformation products for these TPs were either not accurately predicted or subsequently biotransformed into other products before they could be measured.[108] **Figure 2.6** shows biotransformation pathways for gabapentin, diphenhydramine, caffeine, and amphetamine. TPs for these MPs were measured exclusively in the experiments seeded with wastewater microbial communities harvested from the CAS system. The observed biotransformations include monohydroxylations (gabapentin, diphenhydramine, and amphetamine), ether dealkylation (diphenhydramine), and amine dealkylation (caffeine). These are all oxidations that have previously been reported in aerobic environments.[109] However, to the best of our knowledge, this is the first report of transformation products for gabapentin (monohydroxylation) and diphenhydramine (monohydroxylation and ether dealkylation) in a CAS system. **Figure 2.7** shows biotransformation pathways for N-acetylsulfamethoxazole and ethyl-butylacetylaminopropionate.

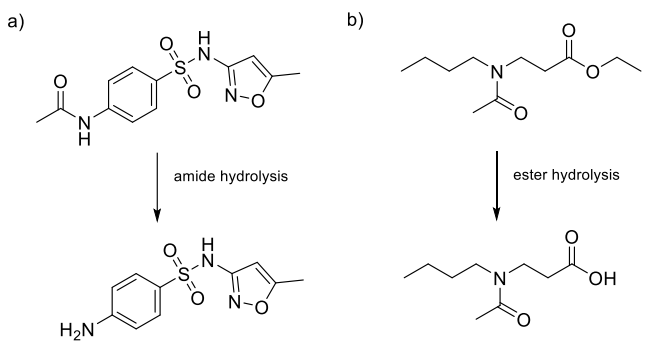


**Figure 2.7**: Biotransformation pathways for (a) N-acetylsulfamethoxazole and (b) ethyl-butylacetylamino-propionate.

TPs for these MPs were measured in the experiments seeded with both wastewater microbial communities harvested from the CAS or UASB system. The observed biotransformations are ester hydrolysis (ethyl-butylacetylamino-propionate) and amide hydrolyses (acetylsulfamethoxazole). Interestingly, these biotransformations are not expected to be redox dependent which can explain why we see these types of biotransformations in experiments seeded with wastewater microbial communities harvested from CAS and UASB systems. Both of these biotransformations have been previously reported in other types of biological wastewater processes, further highlighting the ubiquity at which these biotransformation may be performed.[110] Finally, **Figure 2.8** shows the biotransformation pathways for adrenosterone which exhibited different TPs in CAS and UASB experiments. In the CAS experiment we observe the formation of a monohydroxylation product which is an oxidation that one would expect in an aerobic environment, but to the best of our

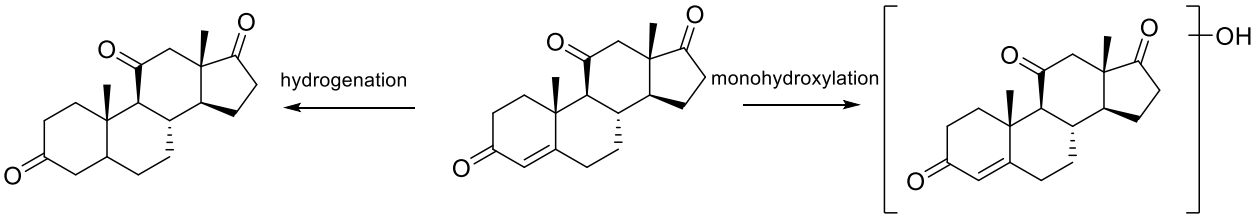


**Figure 2.8**: Biotransformation pathways for adrenosterone.

knowledge has not been previously reported.[111] In the UASB we observe a hydrogenation reaction which has not been previously reported but could be expected in an anaerobic environment.[112] This latter observation is of particular interest because it demonstrates rapid yet differential biotransformation of a MP in CAS and UASB processes. Together, data from our batch experiments demonstrate that MPs can be biotransformed with relevant rates in batch experiments and the observed biotransformation products align with the expected metabolisms of the respective wastewater microbial communities.

## 2.4 Summary and Conclusions

The objectives of this research were to: (1) characterize MP removal (or biotransformation) at a full-scale CAS and in a pilot-scale UASB reactor operating at the same WWTP; (2) identify MPs that exhibit faster biotransformation in each of the biological processes; and (3) identify specific biotransformations for MPs that exhibit differential biotransformation. We integrated the results of a field sampling campaign with batch experiments to explore MP removal (or biotransformation) in both reactor types in real and controlled environments. Our data and analyses lead to several key conclusions:

- When using metrics of effluent concentration, removal percentage, and estimated biotransformation rate constants to evaluate the performance of the CAS and UASB for 39 MPs, we observe 16 MPs that are better removed in the pilot-scale UASB and 17 MPs that are better removed in the full-scale CAS. This rather even distribution of MPs is interesting, if not unexpected, and suggests that UASB reactors have the potential to remove MPs during wastewater treatment.
- Batch experiments seeded with wastewater microbial communities from the pilot-scale UASB and the full-scale CAS and spiked with 24 MPs can be used to simulate the pilot- and full-scale systems. Observed biotransformation rate constants do not necessarily match field data, which we attribute to dilution of the sludge and transfer of the microbial communities into different environments.
- Our analysis of biotransformation products was conservative and somewhat limited, but did reveal the formation of nine biotransformation products from seven MPs. Five of the biotransformation products are reported here for the first time. TPs identified exclusively in CAS experiments were found to be the result of oxidations and TPs identified in CAS and UASB experiments were found to be the result of redox-independent hydrolyses.

Interestingly, adrenosterone was rapidly biotransformed in the UASB and CAS and we observed the formation of different biotransformation products that align with our expectations considering the different redox conditions.

Overall, our data provide novel insights on the relative functioning of two wastewater microbial communities operated under different redox conditions. Our study demonstrates the potential of the UASB reactor and reports on several TPs that have not been previously reported in the literature. Future work will explore the formation of the TPs in the pilot- and full-scale systems.

# CHAPTER 3 – Summary and Future Work

The research presented in this thesis describes the comparison of two different biological systems for their potential to remove organic micropollutants (MPs) from water, the conventional activated sludge system (CAS) and the up-flow anaerobic sludge blanket bioreactor (UASB). This was accomplished by first analyzing the properties of the reactors present at the IAWWTP. To quantify the differences between the two systems, we developed three separate criteria to define MP removal: concentration in effluent, percent removal in system, and pseudo-first order rate constants of removal. By collecting and analyzing the influent and effluent of both systems, we determined that a number of MPs exhibited variable removals across both systems. After compiling the MPs that had the most pronounced differences, while also having a sufficiently high rate constant, we proceeded to perform batch tests on 24 MPs to determine how much of their removal was due to sorption to sludge or biotransformation from the microbes present in the system. By spiking the MPs into active and inactivated reactors, utilizing one blank reactor to account for initial MP presence, we observed the transformation of the 24 MPs across eight days. By analyzing the concentration of each MP across the eight days, we were able to confirm that while several chemicals had the possibility of being removed due to sorption to sludge, there were many that exhibited degradation via biotransformation. To determine if biotransformation had occurred, we utilized the EAWAG Pathway Prediction System (EAWAG-PPS) to generate possible chemical transformation pathways, given each system's environment (pH, dissolved oxygen levels, etc.). We then screened our samples for the possible transformation products predicted from EAWAG-PPS. By comparing the presence of each transformation product to the original removal of the parent MP, we determined which of the 24 MPs were biotransformed in each system. Five of the observed biotransformations are first documented in this experiment. Of

the 24 MPs, adrenosterone was the only MP that exhibited different removal pathways in each system. Together, our data provide novel insights on the relative functioning of two wastewater microbial communities. Our study demonstrates the potential of the UASB to biotransform MPs and the relative biotransformation potential of CAS and UASB processes.

While it is exciting to witness different biotransformations across separate reactors and redox conditions, there are still several factors that are yet to be studied. There are three large knowledge gaps that are relevant in this study. The first is understanding "who" is present in each reactor: what microbial taxa are responsible for the observed biotransformations? The second is a deeper understanding of the full transformation pathways available for the MPs analyzed as well as other MPs still present in our waste streams. Finally, the third gap in our knowledge is the lack of toxicology studies on the transformation products produced. In order for these questions to be answered the following steps could be taken for each knowledge gap.

To understand who is present in microbial reactors, a meta-omics analysis should be employed. As there are several different types of metaomics, it is important to consider which approach is most appropriate to answer our question. We predict that the use of metagenomics, metatranscriptomics, and metaproteomics in conjunction to one another can help to identify what microbes are present in each system. Metagenomics has the potential to illuminate the DNA present in a given environment, and if sequenced this DNA could tell us what microbes are present at a given timepoint. Metatranscriptomics provides the information required to determine which genes are being expressed in a system, and what is being transcribed by the cells. By analyzing the transcription products resulting from gene expression, we can hopefully determine which mechanisms are being employed to transform each of the TPs. Finally metaproteomics should be done in conjunction with sampling. This is because metaproteomics is able to determine what

proteins are activated in the system, thus giving clarity to any findings found in transcriptomics. With a collection of a microbial community's DNA, protein transcription records, and protein activation records, we could potentially map out what the exact pathway of each MP of interest takes to become transformed via biotransformation. Other studies have demonstrated the power of analyzing a wastewater microbial community with different metaomics approaches, resulting in a better characterization of unculturable microbes than ever before. Given time this characterization could aide in determining the pathways these MPs while biotransforming.[113,114]

We successfully observed the biotransformation of several MPs, however all transformations observed were the product of a prediction software that had only predicted the possible first step of transformation for each of the MPs. Prediction software is a useful first step in anticipating how MPs can interact with their environment.[115] This is to say that while we did observe biotransformations of MPs, we did not necessarily observe the final product of these MPs. The ability to characterize any chemical's transformation pathway to mineralization (complete biotransformation into $H_2O$ and $CO_2$) is an incredibly difficult task that, while not impossible, would require a researcher to have the ability to characterize all intermediates, no matter how low their concentration, at a pace frequent enough to observe all permutations of transformation. In order to characterize the mineralization potential of an MP in a biological treatment system, you would ideally know what microbes are present in the system by utilizing meta-omics for microbe characterization. Once you are aware of the possible pathways a MP could take to reach any transformation product, you would then need to undergo batch testing and collect samples continuously across several timepoints to observe concentrations of known MPs and transformation products. This would need to be repeated several times, each time narrowing the possible pathways based on observed, and not observed transformation products. Finally, at some

point your observed transformation pathway should coincide with a model's predicted transformation product. This can be done a bit more expeditiously by completing a nontarget screening where you are unaware of the possible TPs forming and you simply observe and try to quantify the TPs present. Utilizing either method you would have the confirmed transformation present in a batch reactor, which can then be screened for in the full scale reactor. An alternative to repetition of batch reactors is utilizing higher power prediction software that can be calibrated to each specific system of interest. With enough data surrounding how MPs biodegrade, all possible paths of degradation should hypothetically be predicted, and one biotransformation test should be sufficient to determine which path is correct in that system. Some of these pathways may not lead to mineralization into $CO_2$ and $H_2O$, and may instead reach a recalcitrant transformation state that is unlikely to transform in a reasonable timeframe.[116,117]

While it is not necessary for a MP to reach mineralization to be considered removed, it is often the preferred method of removal as $CO_2$ and $H_2O$ have negligible environmental impacts. This is where our final knowledge gap could be addressed. We are still ignorant of the effects caused by the transformation products observed in this study on a natural environment they may encounter. As discussed, the removal of the MPs we observed in our batch tests were monohydroxylation, hydrogenation, ether dealkylation, amine dealkylation, amide hydrolysis and ester hydrolysis reactions. All of these reactions are excellent first steps in causing confirmational change of a MP, however they do not guarantee that the new product is safe for discharge. By understanding the full transformation pathway that an MP can undertake, we would have a better understanding of what molecules we are discharging to surface waters. Once we know what molecules are present, we are able to perform toxicology analyses to determine not only their effect on living organisms, but we can also determine and predict their environmental fate based on their

final chemical structure.[116,118] With a greater understanding of what MPs of concern are becoming, along with their ultimate environmental fate and effect, we can better implement changes in our society. These changes could include making MP parent chemicals more degradable, implementing new political policy on what is deemed acceptable for release to the environment, and future research into more effective waste management and treatment.

Ultimately this research has begun to explore the MP removal effectiveness of a potential alternative to CAS reactors. While this research was not undertaken to determine the sociological efficacy of installing UASB reactors as opposed to CAS reactors, our hope is that by demonstrating that although the UASB may still be in pilot scale, it has properties comparable to a full-scale CAS at a treatment plant. By having a small system display its effectiveness, we hope that if there are underserved communities who lack the funds to construct a large scale CAS aeration basin and WWTP, that the UASB can be a comparably inexpensive option to alleviate some of their MP contamination concerns in addition to the processing of their bulk carbon, nitrogen, and phosphorus. Additionally, our research demonstrates that by having a wider range of biodiversity present across systems driven by differences in redox conditions, different transformation pathways can occur. While adrenosterone was the only MP found to have a different pathway in each system, it is unlikely that it is the only compound in existence that displays this property. The differential redox conditions across both reactors cultivated different microbial communities and our studies have shown that this difference and biodiversity are responsible for the unique biotransformation pathways observed.

Lastly, we hypothesize a few immediate next steps beyond closing the knowledge gaps discussed above. Looking to the future works of this project we recommend that researchers not only conduct a metaomics analysis of the communities present in each reactor as they are sampled,

but also to increase the sampling frequency of both reactors, to be a continuous sample to allow for longer temporal analysis. A recent publication has revealed that MP removal is dependent on microbial ecology that varies across time. By sampling continuously across a longer timescale, hopefully a future researcher would be able to note the differences in MP removal and determine what state results in the best removal of MPs.[45] While we initially took grab samples taken across each system's respective HRT, in order to get a more comprehensive understanding of a system across any given day, taking continuous composite samples can allow the researcher to create a profile of a system on a given day. By taking composite samples across a longer time period, such as a week or a month, the future researcher will likely begin to see different MP patterns emerge in either or both systems. By comparing these MP concentration trends to the meta-omics performed on the community at a given time point, the researcher could then hopefully determine the composition of the microbial community as it responds to the MP concentration present in each reactor. This analysis would aid in exploring and comparing the benefits of either reactor. By determining the MP and chemical degradation potential of the system and the microbes, point source biodegradation reactors could be implemented to dramatically reduce the amount of MPs entering a water system. This should always be the ultimate goal of the project, to explore and create pathways of biological chemical remediation that are able to improve water quality in the natural environment.

# REFERENCES


(1) Schwarzenbach, R. P.; Escher, B. I.; Fenner, K.; Hofstetter, T. B.; Johnson, C. A.; von Gunten, U.; Wehrli, B. The Challenge of Micropollutants in Aquatic Systems. *Science (1979)* **2006**, *313* (5790), 1072–1077. https://doi.org/10.1126/science.1127291.

(2) Helbling, D. E.; Johnson, D. R.; Honti, M.; Fenner, K. Micropollutant Biotransformation Kinetics Associate with WWTP Process and Microbial Community Characteristics. *Environ Sci Technol* **2012**, *46* (19), 10579–10588. https://doi.org/10.1021/es3019012.

(3) Persson, L.; Carney Almroth, B. M.; Collins, C. D.; Cornell, S.; de Wit, C. A.; Diamond, M. L.; Fantke, P.; Hassellöv, M.; MacLeod, M.; Ryberg, M. W.; Søgaard Jørgensen, P.; Villarrubia-Gómez, P.; Wang, Z.; Hauschild, M. Z. Outside the Safe Operating Space of the Planetary Boundary for Novel Entities. *Environ Sci Technol* **2022**, acs.est.1c04158. https://doi.org/10.1021/acs.est.1c04158.

(4) Steffen, W.; Richardson, K.; Rockström, J.; Cornell, S. E.; Fetzer, I.; Bennett, E. M.; Biggs, R.; Carpenter, S. R.; De Vries, W.; De Wit, C. A.; Folke, C.; Gerten, D.; Heinke, J.; Mace, G. M.; Persson, L. M.; Ramanathan, V.; Reyers, B.; Sörlin, S. Planetary Boundaries: Guiding Human Development on a Changing Planet. *Science (1979)* **2015**, *347* (6223). https://doi.org/10.1126/science.1259855.

(5) Woermann, M.; Kontchou, J. A.; Sures, B. Micropollutant-Loaded Powdered Activated Carbon Released from Waste Water Treatment Plants: A Risk for Sediment-Dwelling Organisms? *Environ Sci Eur* **2021**, *33* (1). https://doi.org/10.1186/s12302-021-00531-w.

(6) Peschke, K.; Geburzi, J.; Kor̈hler, H. R.; Wurm, K.; Triebskorn, R. Invertebrates as Indicators for Chemical Stress in Sewage-Influenced Stream Systems: Toxic and Endocrine Effects in Gammarids and Reactions at the Community Level in Two Tributaries of Lake Constance, Schussen and Argen. *Ecotoxicol Environ Saf* **2014**, *106*, 115–125. https://doi.org/10.1016/j.ecoenv.2014.04.011.

(7) Stamm, C.; Räsänen, K.; Burdon, F. J.; Altermatt, F.; Jokela, J.; Joss, A.; Ackermann, M.; Eggen, R. I. L. Unravelling the Impacts of Micropollutants in Aquatic Ecosystems: Interdisciplinary Studies at the Interface of Large-Scale Ecology. In *Advances in Ecological Research*; Academic Press Inc., 2016; Vol. 55, pp 183–223. https://doi.org/10.1016/bs.aecr.2016.07.002.

(8) Tlili, A.; Montuelle, B.; Bérard, A.; Bouchez, A. Impact of Chronic and Acute Pesticide Exposures on Periphyton Communities. *Science of the Total Environment* **2011**, *409* (11), 2102–2113. https://doi.org/10.1016/j.scitotenv.2011.01.056.

(9) Groh, K.; vom Berg, C.; Schirmer, K.; Tlili, A. Anthropogenic Chemicals As Underestimated Drivers of Biodiversity Loss: Scientific and Societal Implications.

*Environmental Science and Technology*. American Chemical Society January 18, 2022, pp 707–710. https://doi.org/10.1021/acs.est.1c08399.

(10) Czekalski, N.; Berthold, T.; Caucci, S.; Egli, A.; Bürgmann, H. Increased Levels of Multiresistant Bacteria and Resistance Genes after Wastewater Treatment and Their Dissemination into Lake Geneva, Switzerland. *Front Microbiol* **2012**, *3* (MAR). https://doi.org/10.3389/fmicb.2012.00106.

(11) Tilson, H. A. *Developmental Neurotoxicology of Endocrine Disruptors and Pesticides: Identification of Information Gaps and Research Needs*.

(12) Berg, V.; Lyche, J. L.; Gutleb, A. C.; Lie, E.; Skaare, J. U.; Aleksandersen, M.; Ropstad, E. Distribution of PCB 118 and PCB 153 and Hydroxylated PCB Metabolites (OH-CBs) in Maternal, Fetal and Lamb Tissues of Sheep Exposed during Gestation and Lactation. *Chemosphere* **2010**, *80* (10), 1144–1150. https://doi.org/10.1016/j.chemosphere.2010.06.022.

(13) Grandclément, C.; Seyssiecq, I.; Piram, A.; Wong-Wah-Chung, P.; Vanot, G.; Tiliacos, N.; Roche, N.; Doumenq, P. From the Conventional Biological Wastewater Treatment to Hybrid Processes, the Evaluation of Organic Micropollutant Removal: A Review. *Water Research*. Elsevier Ltd 2017, pp 297–317. https://doi.org/10.1016/j.watres.2017.01.005.

(14) Onesios, K. M.; Yu, J. T.; Bouwer, E. J. Biodegradation and Removal of Pharmaceuticals and Personal Care Products in Treatment Systems: A Review. *Biodegradation*. July 2009, pp 441–466. https://doi.org/10.1007/s10532-008-9237-8.

(15) Margot, J.; Lochmatter, S.; Barry, D. A.; Holliger, C. Role of Ammonia-Oxidizing Bacteria in Micropollutant Removal from Wastewater with Aerobic Granular Sludge. *Water Science and Technology* **2016**, *73* (3), 564–575. https://doi.org/10.2166/wst.2015.514.

(16) Oberoi, A. S.; Jia, Y.; Zhang, H.; Khanal, S. K.; Lu, H. Insights into the Fate and Removal of Antibiotics in Engineered Treatment Systems: A Critical Review. *Environ Sci Technol* **2019**, *53* (13), 7234–7264. https://doi.org/10.1021/acs.est.9b01131.

(17) Stadlmair, L. F.; Letzel, T.; Drewes, J. E.; Grassmann, J. Enzymes in Removal of Pharmaceuticals from Wastewater: A Critical review of Challenges, Applications and Screening Methods for Their Selection. *Chemosphere* **2018**, *205*, 649–661. https://doi.org/10.1016/j.chemosphere.2018.04.142.

(18) Besha, A. T.; Gebreyohannes, A. Y.; Tufa, R. A.; Bekele, D. N.; Curcio, E.; Giorno, L. Removal of Emerging Micropollutants by Activated Sludge Process and Membrane Bioreactors and the Effects of Micropollutants on Membrane Fouling: A Review. *Journal of Environmental Chemical Engineering*. Elsevier Ltd June 1, 2017, pp 2395–2414. https://doi.org/10.1016/j.jece.2017.04.027.

(19) Eggen, R. I. L.; Hollender, J.; Joss, A.; Schärer, M.; Stamm, C. Reducing the Discharge of Micropollutants in the Aquatic Environment: The Benefits of Upgrading Wastewater Treatment Plants. *Environ Sci Technol* **2014**, *48* (14), 7683–7689. https://doi.org/10.1021/es500907n.

(20) Stadler, L. B.; Ernstoff, A. S.; Aga, D. S.; Love, N. G. Micropollutant Fate in Wastewater Treatment: Redefining ``Removal''. *Environ Sci Technol* **2012**, *46* (19), 10485–10486. https://doi.org/10.1021/es303478w.

(21) Jelic, A.; Gros, M.; Ginebreda, A.; Cespedes-Sánchez, R.; Ventura, F.; Petrovic, M.; Barcelo, D. Occurrence, Partition and Removal of Pharmaceuticals in Sewage Water and Sludge during Wastewater Treatment. *Water Res* **2011**, *45* (3), 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010.

(22) Zumstein, M. T.; Helbling, D. E. Biotransformation of Antibiotics: Exploring the Activity of Extracellular and Intracellular Enzymes Derived from Wastewater Microbial Communities. *Water Res* **2019**, *155*, 115–123. https://doi.org/10.1016/j.watres.2019.02.024.

(23) Fenner, K.; Scheringer, M. The Need for Chemical Simplification As a Logical Consequence of Ever-Increasing Chemical Pollution. *Environmental Science and Technology*. American Chemical Society November 2, 2021, pp 14470–14472. https://doi.org/10.1021/acs.est.1c04903.

(24) Shoener, B. D.; Bradley, I. M.; Cusick, R. D.; Guest, J. S. Energy Positive Domestic Wastewater Treatment: The Roles of Anaerobic and Phototrophic Technologies. *16* (6), 1204–1222. https://doi.org/10.1039/c3em00711a.

(25) Mungray, A. K.; Murthy, Z. V. P.; Tirpude, A. J. Post Treatment of Up-Flow Anaerobic Sludge Blanket Based Sewage Treatment Plant Effluents: A Review. *22* (1), 220–237. https://doi.org/10.5004/dwt.2010.1788.

(26) Zhou, N. A.; Lutovsky, A. C.; Andaker, G. L.; Gough, H. L.; Ferguson, J. F. Cultivation and Characterization of Bacterial Isolates Capable of degrading Pharmaceutical and Personal Care Products for Improved removal in Activated Sludge Wastewater Treatment. *Biodegradation* **2013**, *24* (6), 813–827. https://doi.org/10.1007/s10532-013-9630-9.

(27) Kassab, G.; Halalsheh, M.; Klapwijk, A.; Fayyad, M.; van Lier, J. B. Sequential Anaerobic-Aerobic Treatment for Domestic Wastewater - A Review. *101* (10), 3299–3310. https://doi.org/10.1016/j.biortech.2009.12.039.

(28) Gonzalez-Gil, L.; Krah, D.; Ghattas, A. K.; Carballa, M.; Wick, A.; Helmholz, L.; Lema, J. M.; Ternes, T. A. Biotransformation of Organic Micropollutants by Anaerobic Sludge Enzymes. *Water Res* **2019**, *152*, 202–214. https://doi.org/10.1016/j.watres.2018.12.064.

(29) Lim, S. J.; Kim, T.-H. Applicability and Trends of Anaerobic Granular Sludge Treatment Processes. *60*, 189–202. https://doi.org/10.1016/j.biombioe.2013.11.011.

(30) Abdelgadir, A.; Chen, X.; Liu, J.; Xie, X.; Zhang, J.; Zhang, K.; Wang, H.; Liu, N. Characteristics, Process Parameters, and Inner Components of Anaerobic Bioreactors. https://doi.org/10.1155/2014/841573.

(31) Amani, T.; Nosrati, M.; Mousavi, S. M. Response Surface Methodology Analysis of Anaerobic Syntrophic Degradation of Volatile Fatty Acids in an Upflow Anaerobic Sludge Bed Reactor Inoculated with Enriched Cultures. *17* (1), 133–144. https://doi.org/10.1007/s12257-011-0248-7.

(32) Chernicharo, C. A. L.; van Lier, J. B.; Noyola, A.; Bressani Ribeiro, T. Anaerobic Sewage Treatment: State of the Art, Constraints and Challenges. *14* (4), 649–679. https://doi.org/10.1007/s11157-015-9377-3.

(33) Liotta, F.; Chatellier, P.; Esposito, G.; Fabbricino, M.; Van Hullebusch, E. D.; Lens, P. N. L.; Pirozzi, F. Current Views on Hydrodynamic Models of Nonideal Flow Anaerobic Reactors. *45* (20), 2175–2207. https://doi.org/10.1080/10643389.2015.1010426.

(34) Donoso-Bravo, A.; Mailier, J.; Martin, C.; Rodriguez, J.; Aceves-Lara, C. A.; Vande Wouwer, A. Model Selection, Identification and Validation in Anaerobic Digestion: A Review. *45* (17), 5347–5364. https://doi.org/10.1016/j.watres.2011.08.059.

(35) Ghattas, A. K.; Fischer, F.; Wick, A.; Ternes, T. A. Anaerobic Biodegradation of (Emerging) Organic Contaminants in the Aquatic Environment. *Water Research*. Elsevier Ltd 2017, pp 268–295. https://doi.org/10.1016/j.watres.2017.02.001.

(36) Li, W.-W.; Yu, H.-Q. Anaerobic Granule Technologies for Hydrogen Recovery from Wastes: The Way Forward. *43* (12), 1246–1280. https://doi.org/10.1080/10643389.2011.644218.

(37) Subramanyam, R. Physicochemical and Morphological Characteristics of Granular Sludge in Upflow Anaerobic Sludge Blanket Reactors. *30* (5), 201–212. https://doi.org/10.1089/ees.2012.0347.

(38) Latif, M. A.; Ghufran, R.; Wahid, Z. A.; Ahmad, A. Integrated Application of Upflow Anaerobic Sludge Blanket Reactor for the Treatment of Wastewaters. *45* (16), 4683–4699. https://doi.org/10.1016/j.watres.2011.05.049.

(39) Perez, J.; Aldana, G.; Cardenas, C. Upflow Anaerobic Sludge Blanket Reactor (UASB) Performance through Sludge Age Load and Kinetic Coefficients. *35* (1), 98–108.

(40) Alvarino, T.; Suarez, S.; Lema, J.; Omil, F. Understanding the Sorption and Biotransformation of Organic in Innovative Biological Wastewater Treatment. *SCIENCE*

*OF THE TOTAL ENVIRONMENT* **2018**, *615*, 297–306. https://doi.org/10.1016/j.scitotenv.2017.09.278.

(41) Iranpour, R.; Magallanes, A.; Zermen Ä O, M.; Varsh, V.; Abrishamchi, A.; Stenstrom, M. *ASSESSMENT OF AERATION BASIN PERFORMANCE EFFICIENCY: SAMPLING METHODS AND TANK COVERAGE*. www.elsevier.com/locate/watres.

(42) Steele, P.; Warner, R.; Rosso, D. Oxygen Transfer Comparison of Jets and Coarse Bubble Aeration in Concentrated Sludge. *Water Environ Res* **2023**, *95* (5), e10869. https://doi.org/10.1002/wer.10869.

(43) Nadarajah, N.; Allen, D. G.; Fulthorpe, R. R. Composition of Activated Sludge Settling and Planktonic Bacterial Communities Treating Industrial Effluent and Their Correlation to Settling Problems. *Appl Microbiol Biotechnol* **2010**, *88* (5), 1205–1214. https://doi.org/10.1007/s00253-010-2829-3.

(44) McCarty, P. L.; Bae, J.; Kim, J. Domestic Wastewater Treatment as a Net Energy Producer-Can This Be Achieved? *Environ Sci Technol* **2011**, *45* (17), 7100–7106. https://doi.org/10.1021/es2014264.

(45) Rich, S. L.; Helbling, D. E. Daily Monitoring at a Full-Scale Wastewater Treatment Plant Reveals Temporally Variable Micropollutant Biotransformations. *Environ Sci Technol* **2023**. https://doi.org/10.1021/acs.est.3c02408.

(46) Zhou, N. A.; Gough, H. L. Enhanced Biological Trace Organic Contaminant Removal: A Lab-Scale with Bisphenol A-Degrading Bacteria Sphingobium Sp BiD32. *Environ Sci Technol* **2016**, *50* (15), 8057–8066. https://doi.org/10.1021/acs.est.6b00727.

(47) Pomiès, M.; Choubert, J. M.; Wisniewski, C.; Coquery, M. Modelling of Micropollutant Removal in Biological Wastewater Treatments: A Review. *Science of the Total Environment*. January 5, 2013, pp 733–748. https://doi.org/10.1016/j.scitotenv.2012.11.037.

(48) Ying, Z.; Droste, R. L. Sorption of Microconstituents onto Primary Sludge. *Water Science and Technology* **2015**, *72* (5), 779–784. https://doi.org/10.2166/wst.2015.270.

(49) Stevens-Garmon, J.; Drewes, J. E.; Khan, S. J.; McDonald, J. A.; Dickenson, E. R. V. Sorption of Emerging Trace Organic Compounds onto Wastewater Sludge Solids. *Water Res* **2011**, *45* (11), 3417–3426. https://doi.org/10.1016/j.watres.2011.03.056.

(50) Hörsing, M.; Ledin, A.; Grabic, R.; Fick, J.; Tysklind, M.; Jansen, J. la C.; Andersen, H. R. Determination of Sorption of Seventy-Five Pharmaceuticals in Sewage Sludge. *Water Res* **2011**, *45* (15), 4470–4482. https://doi.org/10.1016/j.watres.2011.05.033.

(51) Kennes-Veiga, D. M.; Gonzalez-Gil, L.; Carballa, M.; Lema, J. M. Enzymatic Cometabolic Biotransformation of Organic Micropollutants in wastewater Treatment Plants: A Review. *Bioresour Technol* **2022**, *344* (B). https://doi.org/10.1016/j.biortech.2021.126291.

(52) Tran, N. H.; Urase, T.; Ngo, H. H.; Hu, J.; Ong, S. L. Insight into Metabolic and Cometabolic Activities of Autotrophic and heterotrophic Microorganisms in the Biodegradation of Emerging Trace Contaminants. *Bioresour Technol* **2013**, *146*, 721–731. https://doi.org/10.1016/j.biortech.2013.07.083.

(53) Fischer, K.; Majewsky, M. Cometabolic Degradation of Organic Wastewater Micropollutants by Activated Sludge and Sludge-Inherent Microorganisms. *Applied Microbiology and Biotechnology*. Springer Verlag 2014, pp 6583–6597. https://doi.org/10.1007/s00253-014-5826-0.

(54) Kumwimba, M. N.; Meng, F. Roles of Ammonia-Oxidizing Bacteria in Improving Metabolism and cometabolism of Trace Organic Chemicals in Biological Wastewater Processes: A Review. *SCIENCE OF THE TOTAL ENVIRONMENT* **2019**, *659*, 419–441. https://doi.org/10.1016/j.scitotenv.2018.12.236.

(55) Chong, S.; Sen, T. K.; Kayaalp, A.; Ang, H. M. The Performance Enhancements of Upflow Anaerobic Sludge Blanket (UASB) Reactors for Domestic Sludge Treatment - A State-of-the-Art Review. *46* (11), 3434–3470. https://doi.org/10.1016/j.watres.2012.03.066.

(56) Buzzini, A. P.; Pires, E. C. Evaluation of a Upflow Anaerobic Sludge Blanket Reactor with Partial Recirculation of Effluent Used to Treat Wastewaters from Pulp and Paper Plants. *98* (9), 1838–1848. https://doi.org/10.1016/j.biortech.2006.06.030.

(57) Abbasi, T.; Abbasi, S. A. Formation and Impact of Granules in Fostering Clean Energy Production and Wastewater Treatment in Upflow Anaerobic Sludge Blanket (UASB) Reactors. *Renewable and Sustainable Energy Reviews*. April 2012, pp 1696–1708. https://doi.org/10.1016/j.rser.2011.11.017.

(58) Wang, J.; Wang, X.; Zhao, Z.; Li, J. Organics and Nitrogen Removal and Sludge Stability in Aerobic Granular Sludge Membrane Bioreactor. *Appl Microbiol Biotechnol* **2008**, *79* (4), 679–685. https://doi.org/10.1007/s00253-008-1466-6.

(59) Li, N.; Quan, X.; Zhuo, M.; Zhang, X.; Quan, Y.; Liang, P. Enhancing Methanogenesis of Anaerobic Granular Sludge by Incorporating Fe/Fe Oxides Nanoparticles Aided with Biofilm Disassembly Agents and Mediating Redox Activity of Extracellular Polymer Substances. *Water Res* **2022**, *216*. https://doi.org/10.1016/j.watres.2022.118293.

(60) Butkovskyi, A.; Sevenou, L.; Meulepas, R. J. W.; Hernandez Leal, L.; Zeeman, G.; Rijnaarts, H. H. M. Micropollutant Removal from Black Water and Grey Water Sludge in a UASB-GAC Reactor. *Water Science and Technology* **2018**, *77* (4), 1137–1148. https://doi.org/10.2166/wst.2017.640.

(61) Wolff, D.; Helmholz, L.; Castronovo, S.; Ghattas, A. K.; Ternes, T. A.; Wick, A. Micropollutant Transformation and Taxonomic Composition in Hybrid MBBR – A Comparison of Carrier-Attached Biofilm and Suspended Sludge. *Water Res* **2021**, *202*. https://doi.org/10.1016/j.watres.2021.117441.

(62) Luo, J.; Chen, H.; Han, X.; Sun, Y.; Yuan, Z.; Guo, J. Microbial Community Structure and Biodiversity of Size-Fractionated Granules in a Partial Nitritation-Anammox Process. *FEMS Microbiol Ecol* **2017**, *93* (6). https://doi.org/10.1093/femsec/fix021.

(63) Xu, S.; Zhang, L.; Huang, S.; Zeeman, G.; Rijnaarts, H.; Liu, Y. Improving the Energy Efficiency of a Pilot-Scale UASB-Digester for Low Temperature Domestic Wastewater Treatment. *Biochem Eng J* **2018**, *135*, 71–78. https://doi.org/10.1016/j.bej.2018.04.003.

(64) Moon, H. C.; Song, I. S. Enzymatic Hydrolysis of FoodWaste and Methane Production Using UASB Bioreactor. *Int J Green Energy* **2011**, *8* (3), 361–371. https://doi.org/10.1080/15435075.2011.557845.

(65) Gomec, C. Y. High-Rate Anaerobic Treatment of Domestic Wastewater at Ambient Operating Temperatures: A Review on Benefits and Drawbacks. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*. August 2010, pp 1169–1184. https://doi.org/10.1080/10934529.2010.493774.

(66) Arias, A.; Alvarino, T.; Allegue, T.; Suárez, S.; Garrido, J. M.; Omil, F. An Innovative Wastewater Treatment Technology Based on UASB and IFAS for Cost-Efficient Macro and Micropollutant Removal. *J Hazard Mater* **2018**, *359*, 113–120. https://doi.org/10.1016/j.jhazmat.2018.07.042.

(67) de Wilt, A.; Butkovskyi, A.; Tuantet, K.; Leal, L. H.; Fernandes, T. V.; Langenhoff, A.; Zeeman, G. Micropollutant Removal in an Algal Treatment System Fed with Source Separated Wastewater Streams. *J Hazard Mater* **2016**, *304*, 84–92. https://doi.org/10.1016/j.jhazmat.2015.10.033.

(68) Mir-Tutusaus, J. A.; Baccar, R.; Caminal, G.; Sarrà, M. Can White-Rot Fungi Be a Real Wastewater Treatment Alternative for Organic Micropollutants Removal? A Review. *Water Research*. Elsevier Ltd July 1, 2018, pp 137–151. https://doi.org/10.1016/j.watres.2018.02.056.

(69) Verlicchi, P.; Galletti, A.; Petrovic, M.; BarcelÓ, D. Hospital Effluents as a Source of Emerging Pollutants: An Overview of Micropollutants and Sustainable Treatment Options. *Journal of Hydrology*. August 2010, pp 416–428. https://doi.org/10.1016/j.jhydrol.2010.06.005.

(70) Faria, C. V.; Ricci, B. C.; Silva, A. F. R.; Amaral, M. C. S.; Fonseca, F. V. Removal of Micropollutants in Domestic Wastewater by Expanded Granular Sludge Bed Membrane Bioreactor. *Process Safety and Environmental Protection* **2020**, *136*, 223–233. https://doi.org/10.1016/j.psep.2020.01.033.

(71) Richardson, S. D.; Ternes, T. A. Water Analysis: Emerging Contaminants and Current Issues. *Analytical Chemistry*. June 15, 2011, pp 4616–4648. https://doi.org/10.1021/ac200915r.

(72) Gago-Ferrero, P.; Bletsou, A. A.; Damalas, D. E.; Aalizadeh, R.; Alygizakis, N. A.; Singer, H. P.; Hollender, J.; Thomaidis, N. S. Wide-Scope Target Screening of >2000 Emerging Contaminants in Wastewater Samples with UPLC-Q-ToF-HRMS/MS and Smart Evaluation of Its Performance through the Validation of 195 Selected Representative Analytes. *J Hazard Mater* **2020**, *387*. https://doi.org/10.1016/j.jhazmat.2019.121712.

(73) Sherman, A.; Mejia, W. A. G.; Padilla, N. R.; Carpenter, C. M. G.; Rich, S. L.; Moncada, E. T.; Helbling, D. E. Target and Suspect Screening for Biocides in Drinking Water Resources of Honduras. *H2Open Journal* **2022**, *5* (1), 84–97. https://doi.org/10.2166/h2oj.2022.140.

(74) Fan, W. Q.; Yanase, T.; Morinaga, H.; Gondo, S.; Okabe, T.; Nomura, M.; Komatsu, T.; Morohashi, K. I.; Hayes, T. B.; Takayanagi, R.; Nawata, H. Atrazine-Induced Aromatase Expression Is SF-1 Dependent: Implications for Endocrine Disruption in Wildlife and Reproductive Cancers in Humans. *Environ Health Perspect* **2007**, *115* (5), 720–727. https://doi.org/10.1289/ehp.9758.

(75) Vuono, D. C.; Benecke, J.; Henkel, J.; Navidi, W. C.; Cath, T. Y.; Munakata-Marr, J.; Spear, J. R.; Drewes, J. E. Disturbance and Temporal Partitioning of the Activated Sludge. *ISME JOURNAL* **2015**, *9* (2), 425–435. https://doi.org/10.1038/ismej.2014.139.

(76) Oosterhuis, M.; Ringoot, D.; Hendriks, A.; Roeleveld, P. Thermal Hydrolysis of Waste Activated Sludge at Hengelo Wastewater Treatment Plant, the Netherlands. *Water Science and Technology* **2014**, *70* (1), 1–7. https://doi.org/10.2166/wst.2014.107.

(77) Krah, D.; Ghattas, A. K.; Wick, A.; Bröder, K.; Ternes, T. A. Micropollutant Degradation via Extracted Native Enzymes from Activated Sludge. *Water Res* **2016**, *95*, 348–360. https://doi.org/10.1016/j.watres.2016.03.037.

(78) Winkler, M. K. H.; Kleerebezem, R.; De Bruin, L. M. M.; Verheijen, P. J. T.; Abbas, B.; Habermacher, J.; Van Loosdrecht, M. C. M. Microbial Diversity Differences within Aerobic Granular Sludge and Activated Sludge Flocs. *Appl Microbiol Biotechnol* **2013**, *97* (16), 7447–7458. https://doi.org/10.1007/s00253-012-4472-7.

(79) Men, Y.; Han, P.; Archermann, S.; Yu, Y.; Helbling, D.; Johnson, D.; Wagner, M.; Fenner, K. Roles Played by Ammonia Oxidizers of a Nitrifying Activated Sludge in Micropollutant Biotransformation. *ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY* **2017**, *253*.

(80) Ternes, T. A.; Herrmann, N.; Bonerz, M.; Knacker, T.; Siegrist, H.; Joss, A. A Rapid Method to Measure the Solid-Water Distribution Coefficient (K d) for Pharmaceuticals and

Musk Fragrances in Sewage Sludge. *Water Res* **2004**, *38* (19), 4075–4084. https://doi.org/10.1016/j.watres.2004.07.015.

(81) Nam, S. W.; Choi, D. J.; Kim, S. K.; Her, N.; Zoh, K. D. Adsorption Characteristics of Selected Hydrophilic and Hydrophobic Micropollutants in Water Using Activated Carbon. *J Hazard Mater* **2014**, *270*, 144–152. https://doi.org/10.1016/j.jhazmat.2014.01.037.

(82) Gulde, R.; Meier, U.; Schymanski, E. L.; Kohler, H. P. E.; Helbling, D. E.; Derrer, S.; Rentsch, D.; Fenner, K. Systematic Exploration of Biotransformation Reactions of Amine-Containing Micropollutants in Activated Sludge. *Environ Sci Technol* **2016**, *50* (6), 2908–2920. https://doi.org/10.1021/acs.est.5b05186.

(83) Men, Y.; Achermann, S.; Helbling, D. E.; Johnson, D. R.; Fenner, K. Relative Contribution of Ammonia Oxidizing Bacteria and Other Members of nitrifying Activated Sludge Communities to Micropollutant. *Water Res* **2017**, *109*, 217–226. https://doi.org/10.1016/j.watres.2016.11.048.

(84) Winkler, M. K. H.; Meunier, C.; Henriet, O.; Mahillon, J.; Suárez-Ojeda, M. E.; Del Moro, G.; De Sanctis, M.; Di Iaconi, C.; Weissbrodt, D. G. An Integrative Review of Granular Sludge for the Biological Removal of Nutrients and Recalcitrant Organic Matter from Wastewater. *Chemical Engineering Journal*. Elsevier B.V. March 15, 2018, pp 489–502. https://doi.org/10.1016/j.cej.2017.12.026.

(85) Wu, L.; Ning, D.; Zhang, B.; Li, Y.; Zhang, P.; Shan, X.; Zhang, Q.; Brown, M.; Li, Z.; Van Nostrand, J. D.; Ling, F.; Xiao, N.; Zhang, Y.; Vierheilig, J.; Wells, G. F.; Yang, Y.; Deng, Y.; Tu, Q.; Wang, A.; Zhang, T.; He, Z.; Keller, J.; Nielsen, P. H.; Alvarez, P. J. J.; Criddle, C. S.; Wagner, M.; Tiedje, J. M.; He, Q.; Curtis, T. P.; Stahl, D. A.; Alvarez-Cohen, L.; Rittmann, B. E.; Wen, X.; Zhou, J.; Acevedo, D.; Agullo-Barcelo, M.; Andersen, G. L.; de Araujo, J. C.; Boehnke, K.; Bond, P.; Bott, C. B.; Bovio, P.; Brewster, R. K.; Bux, F.; Cabezas, A.; Cabrol, L.; Chen, S.; Etchebehere, C.; Ford, A.; Frigon, D.; GÃ$^{3}$mez, J. S.; Griffin, J. S.; Gu, A. Z.; Habagil, M.; Hale, L.; Hardeman, S. D.; Harmon, M.; Horn, H.; Hu, Z.; Jauffur, S.; Johnson, D. R.; Keucken, A.; Kumari, S.; Leal, C. D.; Lebrun, L. A.; Lee, J.; Lee, M.; Lee, Z. M. P.; Li, M.; Li, X.; Liu, Y.; Luthy, R. G.; MendonÃ§a-Hagler, L. C.; de Menezes, F. G. R.; Meyers, A. J.; Mohebbi, A.; Oehmen, A.; Palmer, A.; Parameswaran, P.; Park, J.; Patsch, D.; Reginatto, V.; de los Reyes, F. L.; Noyola, A.; Rossetti, S.; Sidhu, J.; Sloan, W. T.; Smith, K.; de Sousa, O. V.; Stephens, K.; Tian, R.; Tooker, N. B.; De los Cobos Vasconcelos, D.; Wakelin, S.; Wang, B.; Weaver, J. E.; West, S.; Wilmes, P.; Woo, S. G.; Wu, J. H.; Wu, L.; Xi, C.; Xu, M.; Yan, T.; Yang, M.; Young, M.; Yue, H.; Zhang, Q.; Zhang, W.; Zhang, Y.; Zhou, H. Global Diversity and Biogeography of Bacterial Communities in Wastewater Treatment Plants. *Nat Microbiol* **2019**, *4* (7), 1183–1195. https://doi.org/10.1038/s41564-019-0426-5.

(86) Pronk, M.; Giesen, A.; Thompson, A.; Robertson, S.; Van Loosdrecht, M. Aerobic Granular Biomass Technology: Advancements in Design, Applications and Further Developments. *Water Pract Technol* **2017**, *12* (4), 987–996. https://doi.org/10.2166/wpt.2017.101.

(87) Burzio, C.; Ekholm, J.; Modin, O.; Svahn, O.; Persson, F.; Van Erp, T.; Gustavsson, D. J. I.; Wilén, B.-M. *Removal of Organic Micropollutants from Municipal Wastewater by Aerobic Granular*. https://ssrn.com/abstract=4068000.

(88) Lim, S. J.; Fox, P. Evaluation of a Static Granular Bed Reactor Using a Chemical Oxygen Demand Balance and Mathematical Modeling. *102* (11), 6399–6404. https://doi.org/10.1016/j.biortech.2011.03.031.

(89) Zheng, M. X.; Wang, K. J.; Zuo, J. E.; Yan, Z.; Fang, H.; Yu, J. W. Flow Pattern Analysis of a Full-Scale Expanded Granular Sludge Bed-Type Reactor under Different Organic Loading Rates. *107*, 33–40. https://doi.org/10.1016/j.biortech.2011.11.102.

(90) Zumstein, M. T. M. T. T.; Helbling, D. E. D. E. Biotransformation of Antibiotics: Exploring the Activity of Extracellular and Intracellular Enzymes Derived from Wastewater Microbial Communities. *Water Res* **2019**, *155* (155), 115–123. https://doi.org/10.1016/j.watres.2019.02.024.

(91) Rich, S. L.; Zumstein, M. T.; Helbling, D. E. Identifying Functional Groups That Determine Rates of Micropollutant Biotransformations Performed by Wastewater Microbial Communities. *Environ Sci Technol* **2021**, acs.est.1c06429. https://doi.org/10.1021/acs.est.1c06429.

(92) Pochodylo, A. L. A. L.; Helbling, D. E. D. E. Emerging Investigators Series: Prioritization of Suspect Hits in a Sensitive Suspect Screening Workflow for Comprehensive Micropollutant Characterization in Environmental Samples. *Environ Sci (Camb)* **2017**, *3* (1), 54–65.

(93) Carpenter, C. M. G. G.; Helbling, D. E. Widespread Micropollutant Monitoring in the Hudson River Estuary Reveals Spatiotemporal Micropollutant Clusters and Their Sources. *Environ Sci Technol* **2018**, *52* (11), 6187–6196. https://doi.org/10.1021/acs.est.8b00945.

(94) Helbling, D. E.; Hollender, J.; Kohler, H.-P. E. P. E.; Singer, H.; Fenner, K. High-Throughput Identification of Microbial Transformation Products of Organic Micropollutants. *Environ Sci Technol* **2010**, *44* (17), 6621–6627. https://doi.org/10.1021/es100970m.

(95) Helbling, D. E.; Hollender, J.; Kohler, H.-P. E.; Fenner, K. Structure-Based Interpretation of Biotransformation Pathways of Amide-Containing Compounds in Sludge-Seeded Bioreactors. *Environ Sci Technol* **2010**, *44* (17), 6628–6635. https://doi.org/10.1021/es101035b.

(96) Helbling, D. E.; Johnson, D. R.; Honti, M.; Fenner, K. Micropollutant Biotransformation Kinetics Associate with WWTP Process Parameters and Microbial Community Characteristics. *Environ Sci Technol* **2012**, *46* (19), 10579–10588.

(97) Gao, J.; Ellis, L. B. M.; Wackett, L. P. The University of Minnesota Biocatalysis/Biodegradation Database: Improving Public Access. *Nucleic Acids Res* **2010**, *38*, D488–D491. https://doi.org/10.1093/nar/gkp771.

(98) Helbling, D. E.; Hollender, J.; Kohler, H.-P. E.; Fenner, K. *SUPPORTING INFORMATION FOR: A Structure-Based Interpretation of Biotransformation Pathways of Amide-Containing Compounds in Sludge-Seeded Bioreactors*.

(99) Aukema, K. G.; Escalante, D. E.; Maltby, M. M.; Bera, A. K.; Aksan, A.; Wackett, L. P. In Silico Identification of Bioremediation Potential: Carbamazepine and Other Recalcitrant Personal Care Products. *Environ Sci Technol* **2017**, *51* (2), 880–888. https://doi.org/10.1021/acs.est.6b04345.

(100) Kaiser, E.; Prasse, C.; Wagner, M.; Bröder, K.; Ternes, T. A. Transformation of Oxcarbazepine and Human Metabolites of Carbamazepine and Oxcarbazepine in Wastewater Treatment and Sand Filters. *Environ Sci Technol* **2014**, *48* (17), 10208–10216. https://doi.org/10.1021/es5024493.

(101) Paíga, P.; Correia, M.; Fernandes, M. J.; Silva, A.; Carvalho, M.; Vieira, J.; Jorge, S.; Silva, J. G.; Freire, C.; Delerue-Matos, C. Assessment of 83 Pharmaceuticals in WWTP Influent and Effluent Samples by UHPLC-MS/MS: Hourly Variation. *Science of the Total Environment* **2019**, *648*, 582–600. https://doi.org/10.1016/j.scitotenv.2018.08.129.

(102) Polesel, F.; Andersen, H. R.; Trapp, S.; Plósz, B. G. Removal of Antibiotics in Biological Wastewater Treatment Systems - A Critical Assessment Using the Activated Sludge Modeling Framework for Xenobiotics (ASM-X). *Environ Sci Technol* **2016**, *50* (19), 10316–10334. https://doi.org/10.1021/acs.est.6b01899.

(103) Helbling, D. E.; Johnson, D. R.; Lee, T. K.; Scheidegger, A.; Fenner, K. A Framework for Establishing Predictive Relationships between Specific 16S RRNA Sequence Abundances and Biotransformation Rates. *Water Res* **2015**, *70*, 471–484. https://doi.org/10.1016/j.watres.2014.12.013.

(104) LeClair, J. S.; Wahl, L. M. The Impact of Population Bottlenecks on Microbial Adaptation. *Journal of Statistical Physics*. Springer New York LLC July 1, 2018, pp 114–125. https://doi.org/10.1007/s10955-017-1924-6.

(105) Lopez, I.; Borzacconi, L. UASB Reactor Hydrodynamics: Residence Time Distribution and Proposed Modelling Tools. *31* (6), 591–600. https://doi.org/10.1080/09593331003646638.

(106) Johnson, D. R.; Lee, T. K.; Park, J.; Fenner, K.; Helbling, D. E. The Functional and Taxonomic Richness of Wastewater Treatment Plant Microbial Communities Are Associated with Each Other and with Ambient Nitrogen and Carbon Availability. *Environ Microbiol* **2015**, *17* (12), 4851–4860. https://doi.org/10.1111/1462-2920.12429.

(107) Johnson, D. R.; Helbling, D. E.; Lee, T. K.; Park, J.; Fenner, K.; Kohler, H. P. E.; Ackermann, M. Association of Biodiversity with the Rates of Micropollutant Biotransformations among Full-Scale Wastewater Treatment Plant Communities. *Appl Environ Microbiol* **2015**, *81* (2), 666–675. https://doi.org/10.1128/AEM.03286-14.

(108) Cui, H.; Chang, H.; Zheng, H.; Wan, Y. Determination and Occurrence of Sulfonamide Transformation Products in Surface Waters. *Science of the Total Environment* **2021**, *779*. https://doi.org/10.1016/j.scitotenv.2021.146562.

(109) Ma, X.; Chen, H.; Chen, R.; Hu, X. Direct and Activated Chlorine Dioxide Oxidation for Micropollutant Abatement: A Review on Kinetics, Reactive Sites, and Degradation Pathway. *Water (Switzerland)*. MDPI July 1, 2022. https://doi.org/10.3390/w14132028.

(110) Achermann, S.; Falås, P.; Joss, A.; Mansfeldt, C. B.; Men, Y.; Vogler, B.; Fenner, K. Trends in Micropollutant Biotransformation along a Solids Retention Time Gradient. *Environ Sci Technol* **2018**, *52* (20), 11601–11611. https://doi.org/10.1021/acs.est.8b02763.

(111) Kim, Y. H.; Cha, C. J.; Engesser, K. H.; Kim, S. J. Degradation of Various Alkyl Ethers by Alkyl Ether-Degrading Actinobacteria Isolated from Activated Sludge of a Mixed Wastewater Treatment. *Chemosphere* **2008**, *73* (9), 1442–1447. https://doi.org/10.1016/j.chemosphere.2008.07.074.

(112) Wilson, R. M.; Tfaily, M. M.; Rich, V. I.; Keller, J. K.; Bridgham, S. D.; Zalman, C. M.; Meredith, L.; Hanson, P. J.; Hines, M.; Pfeifer-Meister, L.; Saleska, S. R.; Crill, P.; Cooper, W. T.; Chanton, J. P.; Kostka, J. E. Hydrogenation of Organic Matter as a Terminal Electron Sink Sustains High CO2:CH4 Production Ratios during Anaerobic Decomposition. *Org Geochem* **2017**, *112*, 22–32. https://doi.org/10.1016/j.orggeochem.2017.06.011.

(113) McDaniel, E. A.; Wahl, S. A.; Ishii, S.; Pinto, A.; Ziels, R.; Nielsen, P. H.; McMahon, K. D.; Williams, R. B. H. Prospects for Multi-Omics in the Microbial Ecology of Water Engineering. *Water Research*. Elsevier Ltd October 15, 2021. https://doi.org/10.1016/j.watres.2021.117608.

(114) Herold, M.; Martínez Arbas, S.; Narayanasamy, S.; Sheik, A. R.; Kleine-Borgmann, L. A. K.; Lebrun, L. A.; Kunath, B. J.; Roume, H.; Bessarab, I.; Williams, R. B. H.; Gillece, J. D.; Schupp, J. M.; Keim, P. S.; Jäger, C.; Hoopmann, M. R.; Moritz, R. L.; Ye, Y.; Li, S.; Tang, H.; Heintz-Buschart, A.; May, P.; Muller, E. E. L.; Laczny, C. C.; Wilmes, P. Integration of Time-Series Meta-Omics Data Reveals How Microbial Ecosystems Respond to Disturbance. *Nat Commun* **2020**, *11* (1). https://doi.org/10.1038/s41467-020-19006-2.

(115) Huang, Y.; Qiang, Z.; Sun, Z.; Li, M. Micropollutant Degradation by UV/H2O2 in Drinking Water: Facilitated Prediction through Combination of Model Simulation and Portable Measurement. *Water Res* **2022**, *221*. https://doi.org/10.1016/j.watres.2022.118794.

(116) Bhatt, P.; Bhandari, G.; Bilal, M. Occurrence, Toxicity Impacts and Mitigation of Emerging Micropollutants in the Aquatic Environments: Recent Tendencies and Perspectives. *J Environ Chem Eng* **2022**, *10* (3). https://doi.org/10.1016/j.jece.2022.107598.

(117) Kudlek, E.; Dudziak, M. Toxicity and Degradation Pathways of Selected Micropollutants in Water Solutions during the O3 and O3/H2O2 Process. *Desalination Water Treat* **2018**, *117*, 88–100. https://doi.org/10.5004/dwt.2018.22096.

(118) Escher Beate I. and Baumgartner, R. and L. J. and F. K. Predicting the Ecotoxicological Effects of Transformation Products. In *Transformation Products of Synthetic Chemicals in the Environment*; Boxall, A. B. A., Ed.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2009; pp 205–244. https://doi.org/10.1007/698_2_015.

# APPENDIX

# LIST OF FIGURES IN APPENDIX

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## Materials and Methods

*Micropollutant standards and reagents*

**Table A1:** List of 175 MPs with CAS numbers, chemical formulas, adducts, exact masses, retention times, and diagnostic fragments.

| Micropollutant | CAS Number | Chemical Formula | Adduct | Exact Mass (m/z) | RT (min) | Diagnostic Fragment (m/z) |
|---|---|---|---|---|---|---|
| 10,11-Dihydrocarbamazepine | 3564-73-6 | C15H14N2O | $[M+H]^+$ | 239.1179 | 6.7 | 194.10 |
| 2-(Methylthio)benzothiazole | 615-22-5 | C8H7NS2 | $[M+H]^+$ | 182.0092 | 8.5 | 135.01 |
| 2,2,4-Trimethyl-1,2-dihydroquinoline | 147-47-7 | C12H15N | $[M+H]^+$ | 174.1277 | 6.9 | 107.07 |
| 2,2'-Dithiobis(benzothiazole) | 120-78-5 | C14H8N2S4 | $[M+H]^+$ | 332.9642 | 13.9 | 109.01 |
| 2,4-D | 94-75-7 | C8H6Cl2O3 | $[M-H]^-$ | 218.9621 | 7.7 | 160.96 |
| 2,6-Dimethoxyphenol | 91-10-1 | C8H10O3 | $[M+H]^+$ | 155.0708 | 4.7 | 95.05 |
| 2-Aminobenzothiazole | 136-95-8 | C7H6N2S | $[M+H]^+$ | 151.0324 | 2.7 | 109.01 |
| 2-Ethyl-2-phenylmalonamide | 7206-76-0 | C11H14N2O2 | $[M+H]^+$ | 207.1134 | 3.1 | 119.09 |
| 2-Mercaptobenzothiazole | 149-30-4 | C7H5NS2 | $[M+H]^+$ | 167.9936 | 5.6 | 135.01 |
| Abacavir | 136470-78-5 | C14H18N6O | $[M+H]^+$ | 287.1620 | 3.1 | 191.10 |
| Acebutolol | 37517-30-9 | C18H28N2O4 | $[M+H]^+$ | 337.2122 | 4.0 | 319.20 |
| Acesulfame | 33665-90-6 | C4H5NO4S | $[M-H]^-$ | 161.9856 | 1.5 | 82.03 |
| Acetaminophen | 103-90-2 | C8H9NO2 | $[M+H]^+$ | 152.0706 | 2.1 | 110.06 |
| Acetazolamide | 59-66-5 | C4H6N4O3S2 | $[M+H]^+$ | 222.9954 | 2.2 | 181.97 |
| Adenosine | 58-61-7 | C10H13N5O4 | $[M+H]^+$ | 268.1040 | 1.3 | 136.06 |
| Adrenalone | 99-45-6 | C9H11NO3 | $[M+H]^+$ | 182.0812 | 1.1 | 146.06 |
| Adrenosterone | 382-45-6 | C19H24O3 | $[M+H]^+$ | 301.1803 | 6.2 | 257.15 |
| Alachlor | 15972-60-8 | C14H20ClNO2 | $[M+H]^+$ | 270.1255 | 10.1 | 162.13 |
| Alachlor OA | 171262-17-2 | C14H19NO4 | $[M-H]^-$ | 264.1241 | 6.1 | 160.11 |
| Albuterol | 18559-94-9 | C13H21NO3 | $[M+H]^+$ | 240.1594 | 2.0 | 148.08 |
| Allopurinol | 315-30-0 | C5H4N4O | $[M+H]^+$ | 137.0463 | 1.3 | 90.95 |
| Amisulpride | 71675-85-9 | C17H27N3O4S | $[M+H]^+$ | 370.1795 | 3.0 | 242.05 |
| Amitriptyline | 50-48-6 | C20H23N | $[M+H]^+$ | 278.1903 | 7.3 | 91.05 |
| Amphetamine | 300-62-9 | C9H13N | $[M+H]^+$ | 131.1121 | 3.0 | 91.05 |
| Ampicillin | 69-53-4 | C16H19N3O4S | $[M+H]^+$ | 350.1169 | 4.6 | 192.05 |
| Arecoline | 63-75-2 | C8H13NO2 | $[M+H]^+$ | 156.1019 | 1.1 | 113.06 |
| Atenolol | 29122-68-7 | C14H22N2O3 | $[M+H]^+$ | 267.1703 | 2.0 | 190.09 |
| Atenolol Acid | 56392-14-4 | C14H21NO4 | $[M+H]^+$ | 268.1543 | 3.0 | 191.07 |
| Atomoxetine | 83015-26-3 | C17H21NO | $[M+H]^+$ | 256.1696 | 6.6 | 224.08 |
| Atorvastatin | 134523-00-5 | C33H35FN2O5 | $[M+H]^+$ | 559.2603 | 10.7 | 440.22 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Atrazine | 1912-24-9 | C8H14ClN5 | $[M+H]^+$ | 216.1011 | 6.9 | 174.05 |
| Atrazine-2-hydroxy | 2163-68-0 | C8H15N5O | $[M+H]^+$ | 198.1349 | 3.5 | 156.09 |
| Atrazine-desethyl | 6190-65-4 | C6H10ClN5 | $[M+H]^+$ | 188.0697 | 4.6 | 146.02 |
| Atropine | 55-48-1 | C17H23NO3 | $[M+H]^+$ | 290.1751 | 3.5 | 260.16 |
| Baclofen | 1134-47-0 | C10H12ClNO2 | $[M+H]^+$ | 214.0635 | 3.3 | 151.03 |
| Bentazone | 25057-89-0 | C10H12N2O3S | $[M-H]^-$ | 239.0496 | 6.3 | 175.09 |
| Benzophenone | 119-61-9 | C13H10O | $[M+H]^+$ | 183.0810 | 8.7 | 105.03 |
| Benzothiazole | 95-16-9 | C7H5NS | $[M+H]^+$ | 136.0216 | 5.4 | 109.01 |
| Benzotriazole | 95-14-7 | C6H5N3 | $[M+H]^+$ | 120.0556 | 3.6 | 92.05 |
| Benzoylecgonine | 519-09-5 | C16H19NO4 | $[M+H]^+$ | 290.1387 | 3.7 | 168.10 |
| Bupropion | 34911-55-2 | C13H18ClNO | $[M+H]^+$ | 240.1156 | 4.8 | 166.04 |
| Butalbital | 77-26-9 | C11H16N2O3 | $[M-H]^-$ | 223.1083 | 5.8 | 136.37 |
| Caffeine | 58-08-2 | C8H10N4O2 | $[M+H]^+$ | 195.0877 | 3.2 | 138.07 |
| Candesartan | 139481-59-7 | C24H20N6O3 | $[M+H]^+$ | 441.1670 | 7.8 | 263.13 |
| Carbamazepine | 298-46-4 | C15H12N2O | $[M+H]^+$ | 237.1022 | 6.4 | 194.10 |
| Carbamazepine-10,11-epoxide | 36507-30-9 | C15H12N2O2 | $[M+H]^+$ | 253.0972 | 5.1 | 180.08 |
| Carbendazim | 10605-21-7 | C9H9N3O2 | $[M+H]^+$ | 192.0768 | 2.8 | 160.05 |
| Carisoprodol | 78-44-4 | C12H24N2O4 | $[M+H]^+$ | 261.1816 | 7.3 | 158.12 |
| Celecoxib | 169590-42-5 | C17H14F3N3O2S | $[M+H]^+$ | 382.0837 | 10.5 | 362.08 |
| Cetirizine | 83881-51-0 | C21H25ClN2O3 | $[M+H]^+$ | 389.1627 | 7.8 | 201.05 |
| Chlorpheniramine | 132-22-9 | C16H19ClN2 | $[M+H]^+$ | 275.1309 | 4.8 | 230.07 |
| Cimetidine | 51481-61-9 | C10H16N6S | $[M+H]^+$ | 253.1236 | 2.1 | 159.07 |
| Ciprofloxacin | 85721-33-1 | C17H18FN3O3 | $[M+H]^+$ | 332.1405 | 3.5 | 288.15 |
| Citalopram | 59729-33-8 | C20H21FN2O | $[M+H]^+$ | 325.1711 | 5.5 | 262.10 |
| Citric acid | 77-92-9 | C6H8O7 | $[M-H]^-$ | 191.0186 | 1.2 | 87.92 |
| Clarithromycin | 81103-11-9 | C38H69NO13 | $[M+H]^+$ | 748.4842 | 8.3 | 590.39 |
| Climbazole | 38083-17-9 | C15H17ClN2O2 | $[M+H]^+$ | 293.1057 | 6.6 | 197.07 |
| Clindamycin | 18323-44-9 | C18H33ClN2O5S | $[M+H]^+$ | 425.1872 | 5.6 | 126.13 |
| Codeine | 76-57-3 | C18H21NO3 | $[M+H]^+$ | 300.1594 | 2.2 | 243.10 |
| Corticosterone | 50-22-6 | C21H30O4 | $[M+H]^+$ | 347.2217 | 7.6 | 121.06 |
| Cotinine | 486-56-6 | C10H12N2O | $[M+H]^+$ | 177.1022 | 1.2 | 146.06 |
| Coumarin | 91-64-5 | C9H6O2 | $[M+H]^+$ | 147.0441 | 4.7 | 91.05 |
| DEET | 134-62-3 | C12H17NO | $[M+H]^+$ | 192.1383 | 7.1 | 119.05 |
| Desmethylvenlafaxine | 93413-62-8 | C16H25NO2 | $[M+H]^+$ | 264.1958 | 3.9 | 201.13 |
| Dexamethasone | 50-02-2 | C22H29FO5 | $[M+H]^+$ | 393.2072 | 7.6 | 237.13 |
| Dextromethorphan | 125-71-3 | C18H25NO | $[M+H]^+$ | 272.2009 | 5.5 | 215.14 |
| Diclofenac | 15307-86-5 | C14H11Cl2NO2 | $[M+H]^+$ | 296.0240 | 11.1 | 215.05 |
| Diethyl phthalate | 84-66-2 | C12H14O4 | $[M+H]^+$ | 223.0965 | 7.4 | 149.02 |
| Dimethyl phthalate | 131-11-3 | C10H10O4 | $[M+H]^+$ | 195.0652 | 5.3 | 149.02 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Diphenhydramine | 58-73-1 | C17H21NO | $[M+H]^+$ | 256.1701 | 5.6 | 167.09 |
| Efavirenz | 154598-52-4 | C14H9ClF3NO2 | $[M+H]^+$ | 316.0352 | 11.2 | 244.01 |
| Emtricitabine | 143491-57-0 | C8H10FN3O3S | $[M+H]^+$ | 248.0500 | 2.0 | 130.04 |
| Estrone | 53-16-7 | C18H22O2 | $[M+H]^+$ | 271.1698 | 8.7 | 157.06 |
| Ethyl Butylacetylaminopropionate | 52304-36-6 | C11H21NO3 | $[M+H]^+$ | 216.1594 | 6.3 | 188.10 |
| Famciclovir | 104227-87-4 | C14H19N5O4 | $[M+H]^+$ | 322.1510 | 3.3 | 202.11 |
| Famotidine | 76824-35-6 | C8H15N7O2S3 | $[M+H]^+$ | 338.0527 | 2.1 | 189.03 |
| Fenofibric Acid | 42017-89-0 | C17H15ClO4 | $[M+H]^+$ | 319.0732 | 10.9 | 233.04 |
| Fexofenadine | 83799-24-0 | C32H39NO4 | $[M+H]^+$ | 502.2958 | 6.9 | 466.27 |
| Fluconazole | 86386-73-4 | C13H12F2N6O | $[M+H]^+$ | 307.1113 | 4.3 | 220.07 |
| Flucytosine | 2022-85-7 | C4H4FN3O | $[M+H]^+$ | 130.0411 | 2.0 | 71.06 |
| Fluoxetine | 54910-89-3 | C17H18F3NO | $[M+H]^+$ | 310.1413 | 7.9 | 265.16 |
| Fluridone | 59756-60-4 | C19H14F3NO | $[M+H]^+$ | 330.1100 | 8.0 | 310.10 |
| Furosemide | 54-31-9 | C12H11ClN2O5S | $[M-H]^-$ | 328.9999 | 5.5 | 204.98 |
| Gabapentin | 60142-96-3 | C9H17NO2 | $[M+H]^+$ | 172.1332 | 2.9 | 137.10 |
| Gabapentin-lactam | 64744-50-9 | C9H15NO | $[M+H]^+$ | 154.1227 | 3.0 | 137.10 |
| Gemfibrozil | 25812-30-0 | C15H22O3 | $[M+H]^+$ | 251.1641 | 13.0 | 83.09 |
| Hexamethylphosphoramide | 680-31-9 | C6H18N3OP | $[M+H]^+$ | 180.1260 | 4.3 | 135.07 |
| Hydrochlorothiazide | 58-93-5 | C7H8ClN3O4S2 | $[M-H]^-$ | 295.9572 | 2.2 | 204.98 |
| Hydrocodone | 125-29-1 | C18H21NO3 | $[M+H]^+$ | 300.1599 | 2.3 | 199.08 |
| Hydrocortisone | 50-23-7 | C21H30O5 | $[M+H]^+$ | 363.2171 | 6.6 | 121.06 |
| Ibuprofen | 15687-27-1 | C13H18O2 | $[M+Na]^+$ | 229.1199 | 11.5 | 151.04 |
| Iodocarb | 55406-53-6 | C8H12INO2 | $[M+H]^+$ | 281.9958 | 7.0 | 164.92 |
| Iohexol | 66108-95-0 | C19H26I3N3O9 | $[M+H]^+$ | 821.8876 | 1.6 | 803.88 |
| Iopromide | 73334-07-3 | C18H24I3N3O8 | $[M+H]^+$ | 791.8770 | 2.3 | 572.90 |
| Irbesartan | 138402-11-6 | C25H28N6O | $[M+H]^+$ | 429.2397 | 7.5 | 386.22 |
| Irgarol | 28159-98-0 | C11H19N5S | $[M+H]^+$ | 254.1434 | 7.4 | 198.08 |
| Isophorone Diisocyanate | 4098-71-9 | C12H18N2O2 | $[M+K]^+$ | 261.0995 | 6.7 | 204.96 |
| Ketamine | 6740-88-1 | C13H16ClNO | $[M+H]^+$ | 238.0998 | 3.6 | 179.06 |
| Ketoprofen | 22071-15-4 | C16H14O3 | $[M+H]^+$ | 255.1016 | 8.1 | 209.10 |
| Lamotrigine | 84057-84-1 | C9H7Cl2N5 | $[M+H]^+$ | 256.0151 | 4.4 | 210.98 |
| Levamisole | 14769-73-4 | C11H12N2S | $[M+H]^+$ | 205.0794 | 2.5 | 178.07 |
| Levetiracetam | 102767-28-2 | C8H14N2O2 | $[M+H]^+$ | 171.1123 | 2.6 | 126.09 |
| Levofloxacin | 100986-85-4 | C18H20FN3O4 | $[M+H]^+$ | 362.1511 | 3.6 | 261.10 |
| Lidocaine | 137-58-6 | C14H22N2O | $[M+H]^+$ | 235.1805 | 3.4 | 86.10 |
| Losartan | 114798-26-4 | C22H23ClN6O | $[M+H]^+$ | 423.1695 | 7.5 | 235.10 |
| Mabuterol | 56341-08-3 | C13H18ClF3N2O | $[M+H]^+$ | 311.1138 | 4.4 | 237.04 |
| Mecoprop | 93-65-2 | C10H11ClO3 | $[M-H]^-$ | 213.0324 | 9.3 | 141.01 |
| Melamine | 108-78-1 | C3H6N6 | $[M+H]^+$ | 127.0727 | 1.1 | 85.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Meprobamate | 57-53-4 | C9H18N2O4 | $[M+H]^+$ | 219.1345 | 5.2 | 203.14 |
| Metalaxyl | 57837-19-1 | C15H21NO4 | $[M+H]^+$ | 280.1543 | 7.2 | 160.11 |
| Metaxalone | 1665-48-1 | C12H15NO3 | $[M+H]^+$ | 222.1125 | 6.7 | 133.10 |
| Metformin | 657-24-9 | C4H11N5 | $[M+H]^+$ | 130.1087 | 1.1 | 71.06 |
| Methadone | 76-99-3 | C21H27NO | $[M+H]^+$ | 310.2165 | 7.0 | 265.16 |
| Methocarbamol | 532-03-6 | C11H15NO5 | $[M+H]^+$ | 242.1028 | 4.5 | 163.08 |
| Metolachlor | 51218-45-2 | C15H22ClNO2 | $[M+H]^+$ | 284.1412 | 10.1 | 111.04 |
| Metolachlor ESA | 171118-09-5 | C15H23NO5S | $[M+H]^+$ | 330.1370 | 6.5 | 298.11 |
| Metolachlor OA | 152019-73-3 | C15H21NO4 | $[M-H]^-$ | 278.1398 | 7.6 | 158.10 |
| Metoprolol | 51384-51-1 | C15H25NO3 | $[M+H]^+$ | 268.1907 | 4.0 | 159.08 |
| Metronidazole | 443-48-1 | C6H9N3O3 | $[M+H]^+$ | 172.0717 | 2.0 | 128.05 |
| Morphine | 57-27-2 | C17H19NO3 | $[M+H]^+$ | 286.1438 | 1.4 | 201.09 |
| N,N-Didesmethylvenlafaxine | 93413-77-5 | C15H23NO2 | $[M+H]^+$ | 250.1802 | 5.2 | 147.08 |
| N4-Acetylsulfamethoxazole | 21312-10-7 | C12H13N3O4S | $[M+H]^+$ | 296.0700 | 4.5 | 198.02 |
| Nadolol | 42200-33-9 | C17H27NO4 | $[M+H]^+$ | 310.2013 | 3.3 | 254.14 |
| Naproxen | 22204-53-1 | C14H14O3 | $[M+H]^+$ | 231.1016 | 8.6 | 185.10 |
| Nicotine | 54-11-5 | C10H14N2 | $[M+H]^+$ | 163.1230 | 1.1 | 102.97 |
| Norfloxacin | 70458-96-7 | C16H18FN3O3 | $[M+H]^+$ | 320.1405 | 3.4 | 276.15 |
| Ofloxacin | 82419-36-1 | C18H20FN3O4 | $[M+H]^+$ | 362.1511 | 3.3 | 318.16 |
| Oxazepam | 604-75-1 | C15H11ClN2O2 | $[M+H]^+$ | 287.0558 | 7.5 | 241.05 |
| Oxcarbazepine | 28721-07-5 | C15H12N2O2 | $[M+H]^+$ | 253.0972 | 5.1 | 180.08 |
| Oxybenzone | 131-57-7 | C14H12O3 | $[M+H]^+$ | 229.0864 | 10.4 | 151.04 |
| Paraxanthine | 611-59-6 | C7H8N4O2 | $[M+H]^+$ | 181.0726 | 2.9 | 124.05 |
| Penciclovir | 39809-25-1 | C10H15N5O3 | $[M+H]^+$ | 254.1248 | 1.3 | 152.06 |
| Pentobarbital | 57-33-0 | C11H18N2O3 | $[M+H]^+$ | 227.1396 | 2.7 | 86.06 |
| Pentoxifylline | 6493-05-6 | C13H18N4O3 | $[M+H]^+$ | 279.1457 | 4.5 | 181.07 |
| Perfluorobutanoic Acid (PFBA) | 375-22-4 | C4HF7O2 | $[M-H]^-$ | 212.9792 | 4.3 | 168.99 |
| Perfluorooctanoic Acid (PFOA) | 335-67-1 | C8HF15O2 | $[M-H]^-$ | 412.9664 | 11.0 | 168.99 |
| Phenobarbital | 50-06-6 | C12H12N2O3 | $[M-H]^-$ | 231.0770 | 5.0 | 160.08 |
| Phenytoin | 57-41-0 | C15H12N2O2 | $[M+H]^+$ | 253.0977 | 6.2 | 182.10 |
| Primidone | 125-33-7 | C12H14N2O2 | $[M+H]^+$ | 219.1128 | 4.4 | 91.05 |
| Progesterone | 57-83-0 | C21H30O2 | $[M+H]^+$ | 315.2324 | 11.1 | 97.06 |
| Prometon | 1610-18-0 | C10H19N5O | $[M+H]^+$ | 226.1662 | 5.5 | 142.07 |
| Propazine | 139-40-2 | C9H16ClN5 | $[M+H]^+$ | 230.1167 | 8.3 | 146.02 |
| Propranolol | 525-66-6 | C16H21NO2 | $[M+H]^+$ | 260.1650 | 5.5 | 183.08 |
| Pseudoephedrine | 90-82-4 | C10H15NO | $[M+H]^+$ | 166.1229 | 2.5 | 148.11 |
| rac threo-Dihydrobupropion | 92264-82-9 | C13H20ClNO | $[M+H]^+$ | 242.1306 | 5.0 | 162.06 |
| Ranitidine | 66357-35-5 | C13H22N4O3S | $[M+H]^+$ | 315.1485 | 2.0 | 176.05 |
| Ritalinic Acid | 19395-41-6 | C13H17NO2 | $[M+H]^+$ | 220.1332 | 3.8 | 174.13 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Saccharin | 81-07-2 | C7H5NO3S | $[M-H]^-$ | 181.9917 | 2.3 | 61.97 |
| Serotonin | 50-67-9 | C10H12N2O | $[M+H]^+$ | 177.1022 | 1.1 | 146.06 |
| Siduron | 1982-49-6 | C14H20N2O | $[M+H]^+$ | 233.1654 | 8.5 | 137.07 |
| Simazine | 122-34-9 | C7H12ClN5 | $[M+H]^+$ | 202.0854 | 5.7 | 132.03 |
| Sitagliptin | 486460-32-6 | C16H15F6N5O | $[M+H]^+$ | 408.1254 | 4.3 | 235.08 |
| Sotalol | 3930-20-9 | C12H20N2O3S | $[M+H]^+$ | 273.1267 | 1.8 | 213.07 |
| Sucralose | 56038-13-2 | C12H19Cl3O8 | $[M+FA-H]^-$ | 441.0128 | 3.6 | 278.15 |
| Sulfadimethoxine | 122-11-2 | C12H14N4O4S | $[M+H]^+$ | 311.0809 | 4.7 | 156.08 |
| Sulfamethazine | 57-68-1 | C12H14N4O2S | $[M+H]^+$ | 279.0910 | 3.0 | 204.04 |
| Sulfamethoxazole | 723-46-6 | C10H11N3O3S | $[M+H]^+$ | 254.0594 | 3.8 | 156.01 |
| Sulfathiazole | 72-14-0 | C9H9N3O2S2 | $[M+H]^+$ | 256.0209 | 2.4 | 108.04 |
| Temazepam | 846-50-4 | C16H13ClN2O2 | $[M+H]^+$ | 301.0744 | 7.8 | 255.07 |
| Testosterone | 58-22-0 | C19H28O2 | $[M+H]^+$ | 289.2162 | 9.1 | 253.20 |
| Theophylline | 58-55-9 | C7H8N4O2 | $[M+H]^+$ | 181.0726 | 2.9 | 124.05 |
| Thiabendazole | 148-79-8 | C10H7N3S | $[M+H]^+$ | 202.0433 | 3.3 | 175.03 |
| Tramadol | 123154-38-1 | C16H25NO2 | $[M+H]^+$ | 264.1958 | 3.9 | 201.13 |
| Triamterene | 396-01-0 | C12H11N7 | $[M+H]^+$ | 254.1154 | 3.8 | 237.09 |
| Tributyl phosphate | 126-73-8 | C12H27O4P | $[M+H]^+$ | 267.1725 | 12.6 | 155.05 |
| Triclosan | 3380-34-5 | C12H7Cl3O2 | $[M-H]^-$ | 286.9439 | 13.1 | 165.89 |
| Triethyl phosphate | 78-40-0 | C6H15O4P | $[M+H]^+$ | 183.0781 | 5.1 | 98.98 |
| Trimethoprim | 738-70-5 | C14H18N4O3 | $[M+H]^+$ | 291.1452 | 3.0 | 245.10 |
| Triphenyl phosphate | 115-86-6 | C18H15O4P | $[M+H]^+$ | 327.0781 | 11.5 | 327.08 |
| Tris(1,3-dichloro-2-propyl)phosphate (TDCPP) | 13674-87-8 | C9H15Cl6O4P | $[M+H]^+$ | 428.8917 | 11.5 | 98.98 |
| Tris(2-chloroethyl) phosphate | 115-96-8 | C6H12Cl3O4P | $[M+H]^+$ | 284.9612 | 6.0 | 160.98 |
| Valsartan | 137862-53-4 | C24H29N5O3 | $[M+H]^+$ | 436.2343 | 9.4 | 235.10 |
| Venlafaxine | 93413-69-5 | C17H27NO2 | $[M+H]^+$ | 278.2115 | 5.1 | 58.07 |
| Verapamil | 52-53-9 | C27H38N2O4 | $[M+H]^+$ | 455.2910 | 6.0 | 303.21 |
| Warfarin | 81-81-2 | C19H16O4 | $[M+H]^+$ | 309.1121 | 9.0 | 251.07 |

**Table A2:** List of the 51 isotope labeled internal standards (ILISs).

| Internal Standard | CAS Number | Chemical Formula | RT (min) | Adduct | Extracted Mass (m/z) | Reference MP(s) |
|---|---|---|---|---|---|---|
| 17-beta-estradiol-d5 | 221093-45-4 | C18H19O2D5 | 1.0 | $[M+H]^+$ | 278.2162 | n/a |
| 2-methyl-3-isothiazolinone-d3 | n/a | C4D3H2NOS | 1.5 | $[M+H]^+$ | 119.0353 | Arecoline, Flucytosine, Melamine |
| Iodocarb-d9 | 1246815-08-6 | C8H3D9INO2 | 7.0 | $[M+H]^+$ | 291.0550 | Iodocarb, Candesartan |
| Acetaminophen-d4 | 64315-36-2 | C8D4H5NO2 | 2.1 | $[M+H]^+$ | 156.0963 | 2-Aminobenzothiazole, Acetaminophen, Cimetidine, Famotidine, Hydrochlorothiazide, Levamisole |
| Allopurinol-d2 | 916979-34-5 | C5H2D2N4O | 1.3 | $[M+H]^+$ | 139.0583 | Cotinine, Adrenalone, Allopurinol, Nicotine, Metformin, Penciclovir |
| Atenolol-d7 | 1202864-50-3 | C14D7H15N2O3 | 2.0 | $[M+H]^+$ | 274.2148 | Atenolol, Metronidazole, Pentobarbital |
| Atrazine-d5 | 163165-75-1 | C8H9D5Cl1N5 | 6.9 | $[M+H]^+$ | 221.1330 | 2,2,4-Trimethyl-1,2-dihydroquinoline, Atomoxetine, Atrazine-2-hydroxy, Atrazine-desethyl, Climbazole, Metaxalone, Methadone, Simazine |
| Azoxystrobin-d4 | 1346606-39-0 | C22H13D4N3O5 | 8.3 | $[M+H]^+$ | 408.1492 | Clarithromycin, Corticosterone, Oxazepam, Propazine, Testosterone |
| Bisphenol A-d16 | 96210-87-6 | C15D16O2 | 11.7 | $[M+H]^+$ | 245.2227 | n/a |
| Caffeine-13C3 | 78072-66-9 | [13]C3C5H10N4O2 | 3.2 | $[M+H]^+$ | 198.0977 | Caffeine, Levofloxacin, Paraxanthine |
| Carbamazepine-13C6 | n/a | [13]C6C9H12N2O | 6.3 | $[M+H]^+$ | 243.1224 | 10,11-Dihydrocarbamazepine, Carbamazepine, Carbamazepine-10,11-epoxide, DEET, Ethyl Butylacetylaminopropionate, Metolachlor-ESA, Oxcarbazepine |
| Carbaryl-d7 | 362049-56-7 | C12H4D7NO2 | 6.2 | $[M+H]^+$ | 209.1302 | Hydrocortisone, Prometon |
| Carbofuran-d3 | 1007459-98-4 | C12H12D3NO3 | 5.8 | $[M+H]^+$ | 225.1313 | 2-Mercaptobenzothiazole, Adrenosterone, Dexamethasone, Estrone, Ketoprofen, Pentoxifylline, Phenytoin |
| Celecoxib-d4 | 544686-20-6 | C17H10D4F3N3O2S | 10.5 | $[M+H]^+$ | 386.1083 | Celecoxib |
| Cimetidine-d3 | 1185237-29-9 | C10D3H13N6S | 2.1 | $[M+H]^+$ | 256.1418 | n/a |
| Ciprofloxacin-d8 | 1216659-54-9 | C17D8H10FN3O3 | 3.5 | $[M+H]^+$ | 340.1907 | Ciprofloxacin |
| Citalopram-d6 | 1246819-94-2 | C20D6H15FN2O | 5.5 | $[M+H]^+$ | 331.2087 | Benzothiazole, Bupropion, Citalopram, Coumarin, Diphenhydramine, |
| Dextromethorphan-d3 | 524713-56-2 | C18D3H22NO | 5.5 | $[M+H]^+$ | 275.2197 | Propranolol, Dextromethorphan, Triethyl phosphate |
| Diazinon-d10 | 100155-47-3 | C12D10H11N2O3PS | 7.8 | $[M+H]^+$ | 315.1711 | n/a |
| Diclofenac-13C6 | 15307-79-6 | [13]C6C8H11Cl2NO2 | 11.1 | $[M+H]^+$ | 302.0441 | Diclofenac, Fenofibric acid, Progesterone |
| Diltiazem-d4 | *112259-40-2* | C22D4H22N2O4S | 6.1 | $[M+H]^+$ | 419.1937 | Clindamycin, , Verapamil |

| | | | | | | |
|---|---|---|---|---|---|---|
| Dimethoate-d6 | 1219794-81-6 | C5H6D6NO3PS2 | 4.2 | $[M+H]^+$ | 236.0446 | 2,6-Dimethoxyphenol, Acebutolol, Fluconazole, Hexamethylphosphoramide, Lamotrigine, Mabuterol, Methocarbamol, Metoprolol, N4-Acetylsulfamethoxazole, Primidone, Sitagliptin |
| Erythromycin-13C,d3 | 114-07-8 | [13]CC36D3H64NO13 | 6.8 | $[M+H]^+$ | 738.4907 | n/a |
| Estrone-d2 | 350820-16-5 | C18H20D2O2 | 8.7 | $[M+H]^+$ | 273.1818 | Irgarol, Warfarin |
| Fexofenadine-d6 | 548783-71-7 | C32H33D6NO4 | 6.9 | $[M+H]^+$ | 508.3328 | , Fexofenadine |
| Fluoxetine-d5 | 1173020-43-3 | C17D5H13F3NO | 7.9 | $[M+H]^+$ | 315.1727 | Cetirizine, Fluoxetine, Fluridone |
| Gemfibrozil-d6 | 1184986-45-5 | C15H16D6O3 | 13.0 | $[M+H]^+$ | 257.2018 | Gemfibrozil |
| Ibuprofen-d3 | 121662-14-4 | C13D3H15O2 | 11.5 | $[M+H]^+$ | 232.1387 | Atorvastatin, Ibuprofen, Tributyl phosphate |
| Imidacloprid-d4 | 1015855-75-0 | C9D4H6Cl1N5O2 | 3.8 | $[M+H]^+$ | 260.0852 | NA |
| Iopromide-d3 | 1189947-73-6 | C18D3H21I3N3O8 | 2.3 | $[M+H]^+$ | 794.8959 | 2-(Methylthio)benzothiazole, 2-Ethyl-2-phenylmalonamide, Iopromide, Levetiracetam, Pseudoephedrine |
| Isoproturon-d6 | 217487-17-7 | C12H12D6N2O | 7.2 | $[M+H]^+$ | 213.1869 | Amitriptyline, Benzophenone, Carisoprodol, Diethyl phthalate, Dimethyl phthalate, Irbesartan, Isophorone Diisocyanate, Losartan, Metalaxyl, Temazepam, Valsartan |
| Mecoprop-d3 | 352431-15-3 | C10H8D3ClO3 | 9.3 | $[M-H]^-$ | 216.0501 | 2,4-D, Acesulfame, Bentazone, Butalbital, Mecoprop, , Metolachlor OA |
| Metformin-d6 | 1185166-01-1 | C4D6H5N5 | 1.1 | $[M+H]^+$ | 136.1464 | n/a |
| Metoprolol Acid-d5 | 1215404-47-9 | C14H16D5NO4 | 3.0 | $[M+H]^+$ | 273.1863 | Amphetamine, Atenolol acid, Acetazolamide, Albuterol, Amisulpride, Carbendazim, Codeine, , Gabapentin, Gabapentin-lactam, Norfloxacin, Ofloxacin |
| Morphine-d3 | 67293-88-3 | C17D3H16NO3 | 1.4 | $[M+H]^+$ | 289.1626 | Adenosine, Emtricitabine, Hydrocodone, Iohexol, Morphine, Serotonin, Sotalol |
| Naproxen-methoxy-d3 | 958293-79-3 | C14D3H11O3 | 8.6 | $[M+H]^+$ | 234.1204 | Metolachlor, Naproxen, Siduron |
| Oxybenzone-d3 | 131-57-7 | C14H9D3O3 | 10.4 | $[M+H]^+$ | 232.1048 | , Oxybenzone |
| Pirimicarb-d6 | 1015854-66-6 | C11D6H12N4O2 | 3.8 | $[M+H]^+$ | 245.1879 | Tramadol |
| rac-Efavirenz-d4 | 1246812-58-7 | C14H5D4ClF3NO2 | 11.1 | $[M+H]^+$ | 320.0597 | Efavirenz, Triphenyl phosphate |
| Ranitidine-d6 | 1185238-09-8 | C13H16D6N4O3S | 2.0 | $[M+H]^+$ | 321.1862 | Ranitidine |
| Sertraline-d3 | 1217741-83-7 | C17D3H14Cl2N | 8.3 | $[M+H]^+$ | 309.0999 | n/a |
| Sucralose-d6 | 1459161-55-7 | C12H13D6Cl3O8 | 3.6 | $[M-H]^-$ | 447.0499 | Alachlor OA, Citric acid, Furosemide, Phenobarbital, Saccharin, Sucralose |
| Sulfadimethoxine-d6 | 73068-02-7 | C12D6H8N4O4S | 4.7 | $[M+H]^+$ | 317.1185 | Ampicillin, Sulfadimethoxine, Sulfamethazine, Sulfamethoxazole, Sulfathiazole |
| Sulfamethoxazole-(phenyl-13C6) | 1196157-90-0 | [13]C6C4H11N3O3S | 3.8 | $[M+H]^+$ | 260.0795 | n/a |
| 13C8 – PFOA | n/a | [13]C8HF15O2 | 11.0 | $[M-H]^-$ | 420.9921 | PFBA, PFOA |

| | | | | | | |
|---|---|---|---|---|---|---|
| 13C8 – PFOS | n/a | [13]C8HF17O3S | 12.0 | $[M-H]^-$ | 506.9559 | n/a |
| Thiabendazole-d4 | 1190007-20-5 | C10H3D4N3S | 3.3 | $[M+H]^+$ | 206.0685 | Atropine, Baclofen, Benzotriazole, Benzoylecgonine, Famciclovir, Ketamine, Lidocaine, Nadolol, Ritalinic acid, Thiabendazole, Triamterene |
| Triclosan-d3 | 1020719-98-5 | C12H4D3Cl3O2 | 13.1 | $[M-H]^-$ | 289.9627 | Triclosan |
| Trimethoprim-d9 | 1189460-62-5 | C14D9H9N4O3 | 2.9 | $[M+H]^+$ | 300.2016 | Abacavir, Trimethoprim |
| Tris(2-chloroethyl)phosphate-d12 | 1276500-47-0 | C6D12Cl3O4P | 6.0 | $[M+H]^+$ | 297.0365 | , TDCPP, Tris(2-chloroethyl) phosphate |
| Venlafaxine-d6 | 1062606-12-5 | C17D6H21NO2 | 5.1 | $[M+H]^+$ | 284.2491 | Chlorpheniramine, Meprobamate, N,N-Didesmethylvenlafaxine, O-Desmethylvenlafaxine, rac threo-Dihydrobupropion, Venlafaxine |

n/a = information not available

**Table A3:** List of 24 MPs used in batch experiments with CAS numbers, chemical formulas, adducts, exact masses, retention times, and diagnostic fragments.

| Micropollutant | $pK_a$ | Species at neutral pH | logD | $logK_{oc}$ | $K_d$ |
|---|---|---|---|---|---|
| Acetaminophen | 9.46 | neutral | 0.75 | 1.32 | 7.31 |
| Caffeine | n/a | neutral | -0.7 | 0.98 | 0.07 |
| Adrenosterone | n/a | neutral | 2.81 | 2.1 | 44.06 |
| N4-acetylsulfamethoxazole | 5.88 | negative | -0.3 | 1.71 | 17.95 |
| Theophylline | 11.44 | neutral | -0.73 | 1 | 3.5 |
| Serotonin | 9.31 | positive | -1.01 | 1.19 | 5.42 |
| Ethyl_butylacetylaminopropionate | n/a | neutral | 0.94 | 1.65 | 15.63 |
| Amphetamine | 10.01 | positive | -0.55 | 1.88 | 26.55 |
| Gabapentin | 9.91 | neutral | 1.09 | -0.47 | 0.12 |
| Benzoylecgonine | 9.54 | neutral | -0.38 | -0.7 | 0.07 |
| Diphenhydramine | 8.87 | positive | 2.39 | 2.58 | 133.07 |
| Bupropion | 8.22 | positive | 2.89 | 3.21 | 567.63 |
| Sotalol | 9.43 | positive | -2.41 | 0.74 | 1.92 |
| DEET | n/a | neutral | 2.59 | 1.86 | 25.36 |
| Ketoprofen | 3.88 | negative | 0.69 | 2.08 | 42.08 |
| 2,4-D | 2.81 | negative | -0.87 | 1.77 | 20.61 |
| Mecoprop | 3.47 | negative | -0.23 | 1.98 | 33.42 |
| Tramadol | 9.23 | positive | 0.85 | 1.96 | 31.92 |
| Methocarbamol | 13.36 | neutral | 0.43 | 0.88 | 2.66 |
| Lamotrigine | 5.87 | neutral | 2.11 | 2.18 | 52.97 |
| Losartan | 8.30 | neutral | 5.27 | 3.2 | 554.71 |
| Bentazone | 2.03 | negative | 0.01 | 2.11 | 45.09 |
| Ritalinic_Acid | 10.08 | neutral | -0.11 | -0.48 | 0.12 |
| Acesulfame | 3.02 | negative | -1.43 | 0.35 | 0.78 |

*Field sampling campaign*

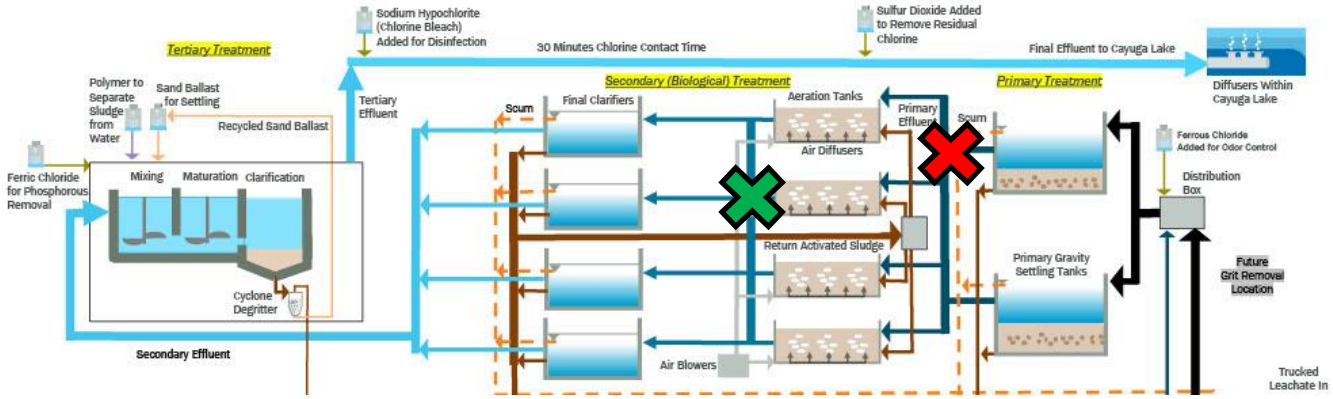


**Figure A10**: Process flow diagram of the studied WWTP along with sampling locations depicted with a red (CAS_inf) and green (CAS_eff) X symbol.

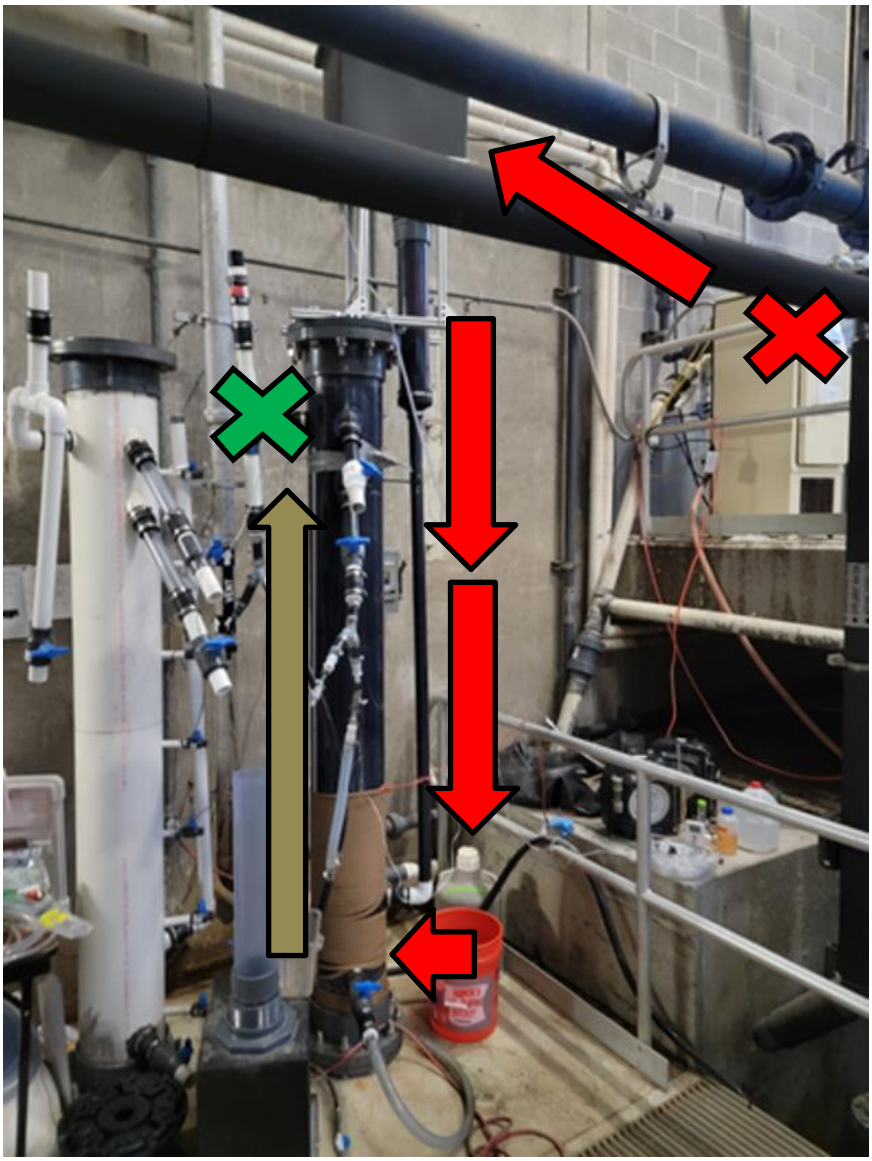

**Figure A11**: Process flow diagram of the studied UASB along with sampling locations depicted with a red (UASB_inf) and green (UASB_eff) X symbol.

**Table A4**: WWTP operational parameters measured during the sampling period.

| | UASB Inf | UASB Eff | CAS Inf | CAS Eff |
|---|---|---|---|---|
| **DO (mg/L)** | 1.8 | 2.69 | 1.92 | 0.86 |
| **pH** | 7.57 | 7.23 | 7.48 | 7.19 |
| **T (°C)** | 21.1 | 22.4 | 21.2 | 22 |
| **BOD (mg/L)** | 156.41 | 98.18 | 54.53 | 651.32 |
| **COD (mg/L)** | 568.0 | 232 | 269.2 | 1580 |
| **Ammonia NH3-N (mg/L)** | 59.8 | 42.9 | 35.3 | 31.6 |
| **TSS (mg/L)** | 145 | 24.0 | 24.0 | 1169 |
| **Fecal coliforms (CFU)** | >2419.6 | >2419.6 | >2419.6 | >2419.6 |
| **Total P (mg/L)** | 4.879 | 3.516 | 0.818 | 19.135 |
| | **UASB - top** | **UASB - bottom** | **CAS** | |
| **Sludge/Granule TSS (mg/mL)[1]** | 7.2 | 36.4 | 1.2 | |

[1]sludge was collected from the top and bottom of the UASB for TSS analysis.

Note: The data collected here is intended to demonstrate the potential and functional characteristics of both the UASB and the CAS systems. Much of these data fall under the expected norms of the respective systems. However, there are 2 observations that merit further comment:

-First, the UASB is an anaerobic system, but the DO level in both the influent and effluent samples are higher than one would expect. We attribute this to introduction of oxygen during sampling.

-Second, we expect to see biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), and total phosphorus (P) levels decrease across both systems. Instead we observed BOD/COD/TSS/P increase in the CAS effluent. We attribute this to the presence of bacterial flocs in the sample that were not filtered prior to analysis of BOD/COD/TSS/P.

**Table A5:** List of MPs with $R^2$, LOQ, and number of detections in *CAS_inf, CAS_eff, UASB_inf, UASB_eff* samples.

| Micropollutant | $R^2$ | LOQ (ng/L) | N UASB_inf | N UASB_eff | N CAS_inf | N CAS_eff |
|---|---|---|---|---|---|---|
| 10,11-dihydrocarbamazepine | 0.9978 | 1 | 0 | 0 | 0 | 0 |
| 2-Aminobenzothiazole | 0.9864 | 1 | 9 | 8 | 9 | 9 |
| 2-ethyl-2-phenyl_malonamide | 0.9819 | 25 | 9 | 0 | 8 | 0 |
| 2-Mercaptobenzothiazole | 0.0076 | 1 | 0 | 0 | 0 | 0 |
| 2-Methylthiobenzothiazole | 0.1013 | 1 | 0 | 0 | 0 | 0 |
| 2,2,4-Trimethyl-1,2-Dihydr | 1.0000 | 500 | 0 | 0 | 0 | 0 |
| 2,2_-Dithiobis(benzothiazole) | 0.0145 | 1 | 0 | 0 | 0 | 0 |
| 2,4-D | 0.9990 | 1 | 9 | 8 | 9 | 9 |
| 2,6-dimethoxyphenol | 0.0128 | 1 | 0 | 0 | 0 | 0 |
| Abacavir | 0.9934 | 25 | 4 | 0 | 9 | 0 |
| Acebutolol | 0.9786 | 50 | 0 | 0 | 0 | 2 |
| Acesulfame | 0.8882 | 1 | 9 | 9 | 9 | 9 |
| Acetaminophen | 0.9822 | 1 | 9 | 9 | 9 | 9 |
| Acetazolamide | 0.9986 | 25 | 0 | 9 | 0 | 3 |
| Acetochlor_Alachlor | 0.9759 | 5 | 3 | 0 | 0 | 0 |
| Adenosine | 0.0115 | 1 | 0 | 0 | 0 | 0 |
| Adrenalone | 0.0201 | 25 | 0 | 0 | 0 | 0 |
| Adrenosterone | 0.9916 | 1 | 9 | 9 | 9 | 9 |
| Alachlor-OXA | 0.9304 | 1 | 8 | 9 | 8 | 9 |
| Albuterol | 0.9943 | 1 | 9 | 9 | 9 | 9 |
| Allopurinol | 0.0190 | 50 | 0 | 0 | 0 | 0 |
| Amisulpride | 0.9815 | 25 | 0 | 0 | 0 | 0 |
| Amitriptyline | 0.9387 | 50 | 0 | 0 | 0 | 6 |
| Amphetamine | 0.9950 | 1 | 9 | 9 | 9 | 9 |
| Ampicillin | 1.0000 | 500 | 0 | 0 | 0 | 0 |
| Arecoline | 0.9027 | 5 | 9 | 8 | 5 | 1 |
| Atenolol | 0.9955 | 1 | 9 | 9 | 9 | 9 |
| Atenolol_Acid | 0.9969 | 1 | 9 | 9 | 9 | 9 |
| Atomoxetine | 0.8148 | 5 | 0 | 0 | 0 | 0 |
| Atorvastatin | 0.9571 | 25 | 0 | 0 | 6 | 0 |
| Atrazine | 0.9892 | 1 | 8 | 7 | 9 | 9 |
| Atrazine-2-hydroxy | 0.7951 | 5 | 0 | 0 | 0 | 0 |
| Atrazine-desethyl | 0.8799 | 250 | 0 | 9 | 0 | 0 |
| Atropine | 0.9506 | 50 | 4 | 9 | 0 | 9 |
| Baclofen | 0.9499 | 50 | 0 | 2 | 0 | 7 |
| Bentazone | 0.9749 | 1 | 9 | 9 | 9 | 9 |
| Benzophenone | 0.0015 | 1 | 0 | 0 | 0 | 0 |
| Benzothiazole | 0.0237 | 1 | 0 | 0 | 0 | 0 |
| Benzotriazole | 0.9556 | 100 | 9 | 9 | 9 | 9 |
| Benzotriazole_methyl-1H | 0.9903 | 1 | 9 | 9 | 9 | 9 |
| Benzoylecgonine | 0.9924 | 50 | 9 | 9 | 9 | 9 |
| Bupropion | 0.9954 | 25 | 9 | 9 | 9 | 9 |
| Butalbital | 0.9703 | 1 | 2 | 1 | 6 | 7 |
| Caffeine | 0.8979 | 50 | 9 | 9 | 9 | 9 |
| Candesartan | 0.9960 | 5 | 0 | 0 | 3 | 0 |
| Carbamazepine | 0.1958 | 1 | 0 | 0 | 0 | 0 |
| Carbamazepine-10,11-epoxide | 0.9950 | 1 | 9 | 9 | 9 | 9 |
| Carbendazim | 0.9980 | 25 | 1 | 9 | 0 | 9 |
| Carisoprodol | 0.9994 | 1 | 9 | 9 | 2 | 9 |
| Celecoxib | 0.9924 | 5 | 0 | 0 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cetirizine | 0.8999 | 1 | 9 | 0 | 9 | 9 |
| Chlorpheniramine | 0.9958 | 100 | 6 | 9 | 5 | 9 |
| Cimetidine | 0.8669 | 250 | 8 | 9 | 2 | 9 |
| Ciprofloxacin | 1.0000 | 250 | 0 | 0 | 0 | 0 |
| Citalopram | 0.9978 | 25 | 6 | 0 | 9 | 9 |
| Citric_Acid | 0.0007 | 50 | 0 | 0 | 0 | 0 |
| Clarithromycin | 0.9882 | 25 | 1 | 0 | 1 | 9 |
| Climbazole | 0.7393 | 1 | 0 | 0 | 0 | 0 |
| Clindamycin | 0.8677 | 25 | 4 | 7 | 8 | 9 |
| Codeine | 0.9914 | 25 | 2 | 9 | 0 | 9 |
| Corticosterone | 0.9988 | 1 | 9 | 9 | 9 | 9 |
| Cotinine | 0.9771 | 1 | 9 | 9 | 9 | 9 |
| Coumarin | 0.7260 | 1 | 0 | 0 | 0 | 0 |
| DEET | 0.9779 | 1 | 9 | 9 | 9 | 9 |
| Desmethylvenlafaxine | 0.9919 | 25 | 9 | 9 | 9 | 9 |
| Dexamethasone | 0.9392 | 1 | 7 | 4 | 9 | 6 |
| Dextromethorphan | 0.9989 | 25 | 0 | 0 | 1 | 9 |
| Diclofenac | 0.9972 | 5 | 6 | 0 | 9 | 8 |
| Diethyl_phthalate | 0.0218 | 1 | 0 | 0 | 0 | 0 |
| Dimethyl_phthalate | 0.4040 | 1 | 0 | 0 | 0 | 0 |
| Diphenhydramine | 0.9249 | 1 | 9 | 9 | 9 | 9 |
| Efavirenz | 0.9956 | 25 | 0 | 0 | 0 | 0 |
| Emtricitabine | 0.9501 | 50 | 9 | 9 | 9 | 9 |
| Estrone | 0.0315 | 1 | 0 | 0 | 0 | 0 |
| Ethyl_butylacetylaminoprop | 0.8874 | 1 | 9 | 9 | 9 | 9 |
| Famciclovir | 1.0000 | 500 | 0 | 0 | 0 | 0 |
| Famotidine | 1.0000 | 500 | 3 | 9 | 0 | 9 |
| Fenofibric_Acid | 0.9408 | 1 | 9 | 9 | 9 | 9 |
| Fexofenadine | 0.9944 | 1 | 8 | 0 | 9 | 2 |
| Fluconazole | 0.9884 | 25 | 7 | 9 | 9 | 9 |
| Flucytosine | 0.9731 | 25 | 9 | 9 | 9 | 9 |
| Fluoxetine | 0.6895 | 250 | 0 | 0 | 0 | 0 |
| Fluridone | 0.7890 | 1 | 0 | 0 | 0 | 0 |
| Furosemide | 0.9494 | 5 | 5 | 0 | 9 | 7 |
| Gabapentin | 0.9687 | 25 | 9 | 9 | 9 | 9 |
| Gabapentin_lactam | 0.9387 | 100 | 9 | 9 | 9 | 9 |
| Gemfibrozil | 0.4566 | 1 | 0 | 0 | 0 | 0 |
| Hexamethylphosphoramide | 0.9975 | 25 | 0 | 0 | 0 | 2 |
| Hydrochlorothiazide | 0.9903 | 5 | 9 | 9 | 9 | 9 |
| Hydrocodone | 0.9884 | 25 | 4 | 9 | 2 | 9 |
| Hydrocortisone | 0.9899 | 1 | 9 | 4 | 9 | 4 |
| Ibuprofen_Na | 0.9954 | 50 | 2 | 0 | 7 | 0 |
| Iodocarb | 0.0000 | 1 | 0 | 0 | 0 | 0 |
| Iohexol | 0.9468 | 250 | 9 | 9 | 9 | 9 |
| Iopromide | 0.9725 | 250 | 0 | 0 | 0 | 0 |
| Irbesartan | 0.9925 | 1 | 9 | 8 | 9 | 9 |
| Irgarol | 0.9893 | 1 | 0 | 0 | 0 | 0 |
| Isophorone_Diisocyanate_K | 0.9900 | 25 | 0 | 0 | 0 | 0 |
| Ketamine | 0.9558 | 50 | 0 | 7 | 0 | 9 |
| Ketoprofen | 0.9941 | 1 | 9 | 7 | 9 | 9 |
| Lamotrigine | 0.9930 | 50 | 9 | 9 | 9 | 9 |
| Levamisol | 0.9950 | 25 | 2 | 7 | 0 | 9 |
| Levetiracetam | 0.9777 | 5 | 9 | 0 | 9 | 0 |
| Levofloxacin | 0.9637 | 50 | 0 | 0 | 0 | 7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Lidocaine | 0.9231 | 50 | 9 | 9 | 9 | 9 |
| Losartan | 0.9893 | 5 | 9 | 9 | 9 | 9 |
| Mabuterol | 0.9929 | 50 | 0 | 0 | 0 | 0 |
| Mecoprop | 0.9989 | 1 | 9 | 9 | 9 | 9 |
| Melamine | 1.0000 | 750 | 1 | 1 | 1 | 7 |
| Meprobamate | 0.9757 | 25 | 0 | 6 | 0 | 1 |
| Metalaxyl | 0.9976 | 1 | 9 | 9 | 9 | 9 |
| Metaxalone | 0.5730 | 1 | 0 | 0 | 0 | 0 |
| Metformin | 0.5952 | 750 | 0 | 0 | 0 | 0 |
| Methadone | 0.8458 | 1 | 0 | 0 | 0 | 9 |
| Methocarbamol | 0.9853 | 50 | 8 | 9 | 9 | 9 |
| Metolachlor | 0.9659 | 1 | 7 | 9 | 9 | 9 |
| Metolachlor-ESA | 0.9878 | 5 | 6 | 5 | 9 | 9 |
| Metolachlor-OXA | 0.9973 | 1 | 9 | 9 | 9 | 9 |
| Metoprolol | 0.9782 | 25 | 9 | 9 | 9 | 9 |
| Metronidazole | 0.9883 | 5 | 3 | 0 | 8 | 9 |
| Morphine | 0.9986 | 5 | 8 | 9 | 9 | 2 |
| N,N-didesmethylvenlafaxine | 0.9860 | 5 | 7 | 9 | 6 | 9 |
| N4-acetylsulfamethoxazole | 0.9913 | 25 | 9 | 5 | 9 | 9 |
| Nadolol | 0.9706 | 25 | 3 | 9 | 1 | 9 |
| Naproxen | 0.1369 | 1 | 0 | 0 | 0 | 0 |
| Nicotine | 0.9276 | 1 | 9 | 9 | 9 | 9 |
| Norfloxacin | 0.9509 | 250 | 0 | 0 | 0 | 0 |
| Ofloxacin | 0.9485 | 50 | 0 | 0 | 0 | 6 |
| Oxazepam | 0.9694 | 5 | 2 | 0 | 3 | 8 |
| Oxcarbazepine | 0.9950 | 1 | 9 | 9 | 9 | 9 |
| Oxybenzone | 0.8724 | 1 | 6 | 0 | 9 | 3 |
| Theophylline | 0.9705 | 5 | 9 | 9 | 9 | 9 |
| Penciclovir | 0.8086 | 1 | 8 | 9 | 9 | 9 |
| Pentobarbital | 0.0433 | 1 | 0 | 0 | 0 | 0 |
| Pentoxyfylline | 0.9905 | 25 | 9 | 1 | 9 | 7 |
| Perfluorooctanoic_Acid | 0.9921 | 1 | 9 | 9 | 9 | 9 |
| PFBA | 0.9948 | 1 | 9 | 8 | 9 | 9 |
| Phenobarbital | 0.9438 | 5 | 3 | 0 | 7 | 7 |
| Phenytoin | 0.0050 | 1 | 0 | 0 | 0 | 0 |
| Primidone | 0.9937 | 50 | 3 | 9 | 0 | 9 |
| Progesterone | 0.0014 | 1 | 0 | 0 | 0 | 0 |
| Prometon | 0.9926 | 1 | 9 | 0 | 9 | 9 |
| Propazine | 0.8721 | 1 | 0 | 0 | 2 | 3 |
| Propranolol | 0.0093 | 1 | 0 | 0 | 0 | 0 |
| Pseudoephedrine | 0.9567 | 1 | 9 | 9 | 9 | 9 |
| rac-threo-Dihydrobupropion | 0.9964 | 5 | 9 | 9 | 9 | 9 |
| Ranitidine | 1.0000 | 500 | 0 | 0 | 0 | 0 |
| Ritalinic_Acid | 0.9803 | 50 | 9 | 9 | 9 | 9 |
| Saccharin | 0.9349 | 1 | 9 | 9 | 9 | 9 |
| Serotonin | 0.9925 | 1 | 9 | 9 | 9 | 9 |
| Siduron | 0.7962 | 1 | 0 | 0 | 0 | 0 |
| Simazine | 0.0356 | 1 | 0 | 0 | 0 | 0 |
| Sitagliptin | 0.9251 | 250 | 0 | 0 | 0 | 0 |
| Sotalol | 0.9968 | 5 | 9 | 9 | 9 | 9 |
| Sucralose_FA | 0.4073 | 1 | 0 | 0 | 0 | 0 |
| Sulfadimethoxine | 0.9774 | 100 | 0 | 0 | 0 | 0 |
| Sulfamethazine | 0.9100 | 250 | 0 | 0 | 0 | 0 |
| Sulfamethoxazole | 0.9754 | 250 | 6 | 2 | 7 | 6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sulfathiazole | 0.9672 | 50 | 0 | 0 | 0 | 0 |
| TDCPP | 0.8421 | 1 | 3 | 0 | 7 | 2 |
| Temazepam | 0.9909 | 1 | 6 | 8 | 9 | 9 |
| Testosterone | 0.9944 | 1 | 9 | 9 | 9 | 9 |
| Thiabendazole | 0.9735 | 25 | 0 | 2 | 0 | 9 |
| Tramadol | 0.9943 | 25 | 9 | 9 | 9 | 9 |
| Triamterene | 0.9766 | 50 | 0 | 0 | 0 | 7 |
| Tributyl_phosphate | 0.8598 | 1 | 9 | 9 | 9 | 9 |
| Triclosan | 0.0765 | 1 | 0 | 0 | 0 | 0 |
| Triethylphosphate | 0.6438 | 1 | 0 | 0 | 0 | 0 |
| Trimethoprim | 0.9952 | 1 | 4 | 0 | 9 | 1 |
| Triphenyl_phosphate | 0.5174 | 1 | 0 | 0 | 0 | 0 |
| Tris(2-chloroethyl)phosphate | 0.9429 | 1 | 5 | 8 | 9 | 9 |
| Valsartan | 0.5874 | 1 | 0 | 0 | 0 | 0 |
| Venlafaxine | 0.9909 | 1 | 9 | 9 | 9 | 9 |
| Verapamil | 0.9933 | 25 | 0 | 0 | 0 | 0 |
| Warfarin | 0.0234 | 1 | 0 | 0 | 0 | 0 |

## Results and Discussion

*Summary data from sampling of full-scale CAS and UASB.*

**Table A6:** List of 40 MPs measured in *CAS_inf* and *UASB_inf* and their average concentration in all samples.

| Micropollutant | $R^2$ | LOQ (ng/L) | Average UASB_inf [µg/L) | Average UASB_eff [µg/L) | Average CAS_inf [µg/L) | Average CAS_eff [µg/L) |
|---|---|---|---|---|---|---|
| Adrenosterone | 0.9916 | 1 | 294.2 | 331.6 | 229.5 | 159.0 |
| Bentazone | 0.9749 | 1 | 1.7 | 6.1 | 0.3 | 2.4 |
| Carbamazepine-10,11-epoxide | 0.9950 | 1 | 27.7 | 298.0 | 31.1 | 268.3 |
| Fluconazole | 0.9884 | 25 | 75.8 | 337.7 | 36.7 | 398.4 |
| Metalaxyl | 0.9976 | 1 | 22.6 | 90.5 | 5.0 | 68.7 |
| Metolachlor-OXA | 0.9973 | 1 | 7.9 | 76.6 | 8.1 | 53.0 |
| N,N-didesmethylvenlafaxine | 0.9860 | 5 | 14.3 | 115.1 | 6.5 | 80.4 |
| Oxcarbazepine | 0.9950 | 1 | 27.7 | 298.0 | 31.1 | 268.3 |
| Perfluorooctanoic_Acid | 0.9921 | 1 | 42.8 | 105.4 | 17.2 | 113.9 |
| Bupropion | 0.9954 | 25 | 194.3 | 626.6 | 286.1 | 3,086.6 |
| 2,4-D | 0.9990 | 1 | 30.8 | 30.3 | 21.1 | 134.2 |
| Sotalol | 0.9968 | 5 | 53.5 | 370.0 | 22.1 | 368.0 |
| Losartan | 0.9893 | 5 | 42.3 | 172.8 | 50.5 | 359.0 |
| Amphetamine | 0.9950 | 1 | 155.8 | 658.6 | 68.5 | 70.8 |
| Lamotrigine | 0.9930 | 50 | 81.7 | 712.2 | 114.5 | 2,833.4 |
| Methocarbamol | 0.9853 | 50 | 198.9 | 838.1 | 106.8 | 1,070.9 |
| Venlafaxine | 0.9909 | 1 | 71.1 | 858.2 | 70.4 | 930.8 |
| Benzoylecgonine | 0.9924 | 50 | 656.2 | 51,539.5 | 407.9 | 599.2 |
| Ethyl_butylacetylaminopropionate | 0.8874 | 1 | 354.4 | 890.0 | 377.0 | 429.9 |
| Ritalinic_Acid | 0.9803 | 50 | 124.6 | 6,721.5 | 91.1 | 501.9 |
| Serotonin | 0.9925 | 1 | 129.8 | 1,580.9 | 86.6 | 277.4 |
| Testosterone | 0.9944 | 1 | 114.2 | 2,862.8 | 31.2 | 282.9 |
| Caffeine | 0.8979 | 50 | 7,177.1 | 21,833.1 | 4,050.6 | 958.5 |
| Theophylline | 0.9705 | 5 | 871.3 | 3,116.5 | 660.0 | 289.1 |
| Atenolol | 0.9955 | 1 | 158.0 | 968.2 | 69.3 | 770.7 |
| Acesulfame | 0.8882 | 1 | 1,731.4 | 11,081.9 | 1,679.2 | 1,920.1 |
| Acetaminophen | 0.9822 | 1 | 11,104.3 | 18,705.1 | 6,069.7 | 2,267.0 |
| Atenolol_Acid | 0.9969 | 1 | 460.3 | 4,051.5 | 310.6 | 4,340.2 |
| Benzotriazole_methyl-1H | 0.9903 | 1 | 2,161.6 | 70,399.4 | 3,643.1 | 47,195.7 |
| DEET | 0.9779 | 1 | 720.6 | 9,657.7 | 396.7 | 984.9 |
| Desmethylvenlafaxine | 0.9919 | 25 | 208.0 | 6,338.7 | 175.8 | 2,662.3 |
| Gabapentin | 0.9687 | 25 | 1,215.8 | 9,413.4 | 612.9 | 1,289.4 |
| Lidocaine | 0.9231 | 50 | 175.2 | 5,229.8 | 88.6 | 1,316.8 |
| Mecoprop | 0.9989 | 1 | 349.4 | 1,732.0 | 79.5 | 1,463.4 |
| Metoprolol | 0.9782 | 25 | 127.1 | 1,247.5 | 105.4 | 2,096.7 |
| Tramadol | 0.9943 | 25 | 110.7 | 994.7 | 113.8 | 2,021.3 |
| Corticosterone | 0.9988 | 1 | 146.2 | 1,558.3 | 80.5 | 633.6 |
| Diphenhydramine | 0.9249 | 1 | 128.6 | 110.8 | 172.5 | 1,835.4 |
| N4-acetylsulfamethoxazole | 0.9913 | 25 | 92.5 | 83.5 | 82.3 | 256.7 |
| Ketoprofen | 0.9941 | 1 | 13.0 | 6.2 | 9.4 | 32.4 |

*Batch experiments – biotransformation kinetics.*

Our first batch test conducted was the analysis of biotransformation occurring in the UASB reactors. Because we had created triplicate batch reactors for each system, we observed three datapoints across each timepoint in our sample. In order to complete the analysis, we first averaged the concentration of each MP across the three reactors in any given time point and removed outliers. Outliers included any values that were below limit of quantification at an unexpected timepoint that disrupted the pattern of the concentration curve. Additionally, each MP was spiked into an autoclaved reactor to determine what amount of MP removal could be attributed to sorption, and finally by utilizing a bioreactor that did not have any MPs spiked in so that we could detect the possible "background noise" of MPs previously existing in the sample we collected. To establish that a MP was being removed, we graphed and compared the concentration of the MP across timepoints in the active bioreactors, with the concentration of MP found in the autoclaved bioreactors. If we found that the concentration of the MP in active bioreactors had dipped below the concentration of MP in autoclaved reactors, we knew that a biotransformation had to have occurred, possibly in conjunction with removal by sorption to biological materials. Additionally, we ensured that the MP disappearance curve was not present in the bioreactor that did not contain any MPs as that would indicate that we had observed a different chemical with the same mass as the target MP. Once we had determined that biological removal had occurred, we modeled both first and zero order biotransformation rate kinetics to determine which model better fit our data. After comparing the rate kinetics across all MPs, we determined that due to the curvilinear nature of MP degradation, first order rate kinetics would be most appropriate for this and future analyses.

For the CAS batch test reactor, due to the aerobic nature of the microbes in the system, we had several expectations of how MPs would transform. We expected MPs to have faster rate

kinetics in the CAS system due to oxygen being permitted as an electron acceptor. Additionally, because the microbial community is consistently being disturbed by O2 bubbling, and the biological density of the system is already 1g/L, we expected this batch test bioreactor to very closely mirror the system found at the IAWWTP. As described in the setup above we sampled the MPs of the batch test across an 8 day time period, while providing O2 for the microbes. Again, we had 3 separate active reactors, 1 reactor not containing MPs, and 1 autoclaved reactor to screen for initial MP presence. After analysis by HPLC-MS we were able to observe the concentration over time of the MPs in each reactor, and by the same method described in the UASB section, we took the average of MP concentrations across the three reactors and discarded any outliers found in our data. After graphically analyzing the concentration in active bioreactors, versus the concentration in autoclaved bioreactors, we were able to determine if MPs were exhibiting any removal patterns beyond sorption. Finally, we calculated both the first and zero order rate constants for each system, and because MP removal most accurately fits a non-linear curve, we elected to utilize first order reaction rate kinetics to compare the removal of MPs across both systems. Because we utilized first order rate kinetics to compare both systems we were able to compare the values of rate kinetics between both reactors without having to account for correction factors.

*Batch experiments – biotransformation products*

Once we had established a "removal" pattern for the parent MPs in each of our batch test reactors, we turned to searching for biotransformation products of the parent MPs to determine what reactions occurred in the bioreactors. We had already processed the data from the batch tests, specifically screening for the parent MPs, now we had to determine what would be an effective method of prediction to determine what transformation products (TPs) would be formed. To create our data pool of possible reactions to occur in both aerobic and anaerobic systems, we utilized a chemical pathway prediction system, EAWAG PPS. This system is a collection of 219 biotransformation rules documented and cataloged for use. While it does not contain every reaction possible, it is an excellent resource for predicting potential biological interactions with chemicals in aerobic and anaerobic conditions. We uploaded the smiles of our parent MPs, and turned relative reasoning off (this permits the system to report all possible TPs no matter how low their probability of existing is) as well as predicted reactions possible for both aerobic and anaerobic systems, and in return we got a total of 109 possible reaction products for our 24 MPs of interest. Each MP and TP has its own biotransformation rule, the reasoning behind the software reporting a possible TP. Once we had collected and processed the smiles of TPs, we began building a scanning method. This was done by adding or removing a proton from the TP chemical mass (depending on if the parent compound was better observed in positive or negative scan mode), we were able to create a new processing method for analyzing the data from our batch tests to determine what TPs may have been present.

After predicting all possible TPs given by the EAWAG PPS, we linked each TP to its respective parent compound and converted their smiles into an exact mass we could use for screening. We then went back through the data generated from the batch tests, and we screened

for the presence of peaks with an intensity of at least 1E5 to determine if a given transformation product was observed. This was repeated in both positive scan, where we added the weight of one electron, and in negative scan where we removed an additional electron in the weight. All parent compounds were found to be better observed in positive mode except for 2,4-D that exhibited clearer peaks when analyzed in negative mode for both systems. We then manually screened our data for TPs that displayed prominent peaks in both positive and negative scan mode. Once we had found the TPs that displayed peaks with the correct intensity in both systems, we were able to eliminate TPs that were predicted but not observed in our experiment. We then modified our processing method to search for the masses of the selected TPs across all timepoints and samples. After doing so we were able to generate a long report that allowed us to compare peak areas of TPs and their parent MPs. It is worth noting that because TPs are inherently transformations of the parent compounds they were derived from, we do not have authentic standards available for every possible chemical reaction that could have occurred, and as such measurements of peak area are not analogous to a defined concentration, but rather a comparison of intensity of presence when compared to other screened masses in the system. That being said, because we do have authentic standards for the parent compounds in the initial screening we are able to compare the parent peak area observed here to the original quantification.

Once we had the report of TP peak areas correlated with parent peak area, we were able to graphically analyze what reactions were observed in the batch test bioreactors, and predict possible transformation pathways for parent MPs. A collection of insight is provided in **Table A7** for each MP.

**Table A7:** Summary of observations from biotransformation product analysis.

| Chemical: | Observation in UASB | Observation in CAS |
|---|---|---|
| 2,4-D | Unlikely TP. Follows parent MP, after being initially present | Unlikely TP. Follows parent MP, after being initially present |
| Acetaminophen | No observed TP. No TPs were present above 1.7E7 threshold, but there was parent degredation | Immediate parent degradation with strong presence of TP3 in the autoclave, suggesting immediate transformation followed by sorbtion perhaps. If this is not the case then NO TP |
| Adrenosterone | Parent degrades and we see TP2 spike in response. I think we can confidently say that this is being transformed in the bioreactor. TP3 is close to the 1.7E7 threshold, but doesn't quite make the cut. | Parent immediately degrades into TP3 which in turn gets degraded. This is a completely different pathway and product from the UASB, and it can occur through a number of pathways. |
| Amphetamine | NO TP | Fast parent degradation and TP4 rise in accordance to the fall of parent MP indicates TP4 is likely the correct pathway. |
| Benzoylecgonine | NO TP, TP4 in autoclave was present at start and never wavered, and no MP degradation | Parent MP dropped quickly, and TP2 slowly rose in the autoclave reactor, however if no reactions are possible in autoclave reactor, NO TP |
| Bupropion | NO MP removal nor TP formation | Parent degradation, but no TP meeting criteria |
| Caffeine | Observed parent removal, constant presence of TP3, no rise in peak | Parent degrades immediately and becomes TP3 that spikes above the threshold and then goes back down, likely to be further degraded. This is likely the transformation pathway. |
| Diphenhydramine | Observed parent degradation, but no TP was present | Observed parent degradation followed by rise of TP4 in accordance. TP1 may have also been formed in some amount, although less than TP4. TP4 was also predicted in UASB, but was not found. I would argue that TP1 and TP4 are valid TPs. |
| Ethyl_butylacetylaminopropionate | Observed drop in Parent MP, with associated rise in TP5. Additionally there is some initial presence of TP 3, however this was also found in some degree in the nonchem, so it is likely that TP5 is the primary transformation in UASB, This is the same transformation pathway present in the CAS. | Observed parent Dropping down with TP4 rising above the threshold, indicating that TP4 is the primary transformation pathway. This is the same transformation pathway present in the UASB |
| Gabapentin | No fall in parent MP, however TP4 rose, indicating a false screening. | We observed a drop in parent MP followed by rise in TP4, that stayed constant once it reached its zenith, indicating that this is likely the terminal transformation pathway for the MP |
| N4-acetylsulfamethoxazole | Observed Parent fall in correspondence to TP4 rising to plateau at zenith. This is likely the terminal transformation pathway for this TP. Same | Observed parent MP fall with corresponding TP2 rise, indicating this is the primary pathway of transformation for this MP. This is the same as the UASB. This being said, |

| | | |
|---|---|---|
| | transformation as what is observed in CAS. | CAS had TP3 rise above the threshold at the tail of TP2, and given that TP3 is adding a hydroxyl group, I predict that this reaction is unique to the CAS. |
| Theophylline | NO TP | Parent MP disappearance, however all predicted TPs were either present in the beginning, and in the nonchem sample, or they did not meet the threshold. NO TP found |
| Serotonin | Observed Parent MP maybe disappearing, but only predicted TP did not rise to threshold. | Parent MP disappearance, and TP3 rise in response, in autoclave. If this is possible, then TP3 is the most likely pathway for the parent MP, if this is not possible, NO TP |
| Sotalol | TPs observed in both systems mirror the parent MP, indicating false screening in both reactors. | TPs observed in both systems mirror the parent MP, indicating false screening in both reactors. |