MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES

THE FATE OF ORGANIC MICROPOLLUTANTS IN HIGH-RATE CONTACT STABILIZATION PROCESS INTEGRATED WITH ANAEROBIC DIGESTION

DİLARA SANCAR

Ph.D. THESIS

Department of Environmental Engineering

Thesis Supervisor
Prof. Dr. Neslihan SEMERCİ

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Prof. Dr. Zehra Semra CAN

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Dilara Sancar, a Doctor of Philosophy student of Marmara University Institute for Graduate Studies in Pure and Applied Sciences, defended her thesis entitled “The Fate of Organic Micropollutants in High-Rate Contact Stabilization Process Integrated with Anaerobic Digestion”, June 11, 2026 and has been found to be satisfactory by the jury members.

Jury Members

Prof.Dr. Neslihan SEMERCİ
Marmara University .....(SIGN) .....

Prof. Barış ÇALLI (Jury Member)
Marmara University.....(SIGN) .....

Prof. Ebubekir YÜKSEL (Jury Member)
Gebze Technical University .....(SIGN) .....

Dr. Gül Gülenay HACIOSMANOĞLU (Jury Member)
Marmara University .....(SIGN) .....

Assoc. Prof Dr. Alper BAYRAKDAR (Jury Member)
İzmir Institute of Technology.....(SIGN) .....

APPROVAL

Marmara University Institute for Graduate Studies in Pure and Applied Sciences Executive Committee approves that Dilara SANCAR be granted the degree of Doctor of Philosophy in Department of Environmental Engineering, Environmental Engineering Program on ..….. , 2026. (Resolution no: ……).

Director of the Institute

Prof. Dr. Bülent MERTOĞLU

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ÖZET

ANAEROBİK ÇÜRÜTME ENTEGRELİ YÜKSEK HIZLI TEMAS STABİLİZASYON PROSESİNDE ORGANİK MİKROKİRLETİCİLERİN AKIBETİ

Bu çalışmada, organik mikrokirleticilerin (OMP) gerçek belediye atıksuyunu arıtan pilot ölçekli modifiye Yüksek Hızlı Temas Stabilizasyonu (HiCS) sistemi ve anaerobik çürütme ile entegre edilmiş bir proseste davranışı ve giderim mekanizmaları incelenmiştir. 11 seçilen OMP'nin giderim performansı, uzun dönemli izleme verileri, kütle dengesi analizi ve batch adsorpsiyon deneyleri kullanılarak değerlendirilmiştir.

Sonuçlar, HiCS sisteminde OMP gideriminin büyük ölçüde bileşiğe özgü olduğunu ve başta çamur yaşı (SRT) olmak üzere işletme koşulları ile bileşiklerin fizikokimyasal özelliklerinin birlikte etkisi altında gerçekleştiğini göstermiştir. Hidrofobik bileşiklerin ağırlıklı olarak çamur fazına adsorpsiyon yoluyla geçtiği, daha polar bileşiklerin ise biyotransformasyon ile uzaklaştırıldığı belirlenmiştir. Deneysel olarak belirlenen katı-sıvı dağılım katsayıları (Kd), giderim mekanizmalarının yorumlanmasını desteklemiş ve sorpsiyon davranışının yalnızca hidrofobiklik ile açıklanamayacağını ortaya koymuştur.

Ham atıksu ile stabilizasyon çamuru arasında bir ön temas bölgesi içeren modifiye HiCS konfigürasyonu, karbon yakalama performansını artırmış; ancak OMP giderimi üzerindeki etkisinin sınırlı kaldığı ve daha çok kirleticilerin giderim mekanizmalarının göreli katkısını değiştirdiği belirlenmiştir. Modifiye HiCS konfigürasyonu karbon yakalama performansını artırmış olmakla birlikte, OMP giderimi üzerindeki etkisinin sınırlı olduğu ve daha çok kirleticilerin faz dağılımını etkilediği belirlenmiştir. Anaerobik çürütme ile entegrasyon sonucunda ortalama 0.36 ± 0.10 L CH_4/g VS metan üretimi elde edilmiş ve enerji geri kazanımı açısından olumlu sonuçlar gözlenmiştir. Ancak OMP davranışının çürütme sürecinde de bileşiğe özgü olduğu, bazı bileşiklerin kalıcı olduğu, bazılarının ise dönüşüme uğradığı belirlenmiştir.

Genel olarak, yüksek hızlı arıtma sistemlerinin karbon yakalama yoluyla enerji geri kazanımını desteklediği, ancak mikrokirletici gideriminin bileşiğe bağlı olarak değiştiği

ortaya konmuştur. Bu çalışma, enerji odaklı proses tasarımı ile mikrokirletici yönetimi arasındaki dengeyi ortaya koyarak, döngüsel ekonomi yaklaşımı kapsamında sürdürülebilir atıksu arıtma sistemlerinin geliştirilmesine katkı sağlamaktadır.

ABSTRACT

THE FATE OF ORGANIC MICROPOLLUTANTS IN HIGH-RATE CONTACT STABILIZATION PROCESS INTEGRATED WITH ANAEROBIC DIGESTION

This study investigates the fate and removal mechanisms of organic micropollutants (OMPs) in a pilot-scale modified High-Rate Contact Stabilization (HiCS) system integrated with anaerobic digestion, a high-rate system designed for carbon capture rather than oxidation, treating real municipal wastewater under dynamic operational conditions. The removal performance and behaviour of eleven selected OMPs—including ibuprofen (IBU), diclofenac (DICLO), carbamazepine (CBZ), estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethinylestradiol (EE2), bisphenol A (BPA), nonylphenol (NP), octylphenol (OP), and ciprofloxacin (CIPRO)—were evaluated using long-term monitoring, mass balance analysis, and batch sorption experiments.

The results demonstrated that OMP removal in the HiCS system is strongly compound-specific and governed by the interplay between physicochemical properties and operational parameters. Readily removed compounds such as NP (72 ± 10%), IBU (74 ± 13%), and CIPRO (84 ± 18%) exhibited high overall removal efficiencies, whereas moderate removal was observed for BPA (59 ± 7%), OP (55 ± 15%), and DICLO (61 ± 18%). In contrast, persistent compounds such as CBZ (23 ± 21%) and EE2 (27 ± 17%) showed limited removal. Hydrophobic compounds predominantly associated with sludge solids through sorption, whereas more polar compounds were mainly removed via biotransformation. The experimentally determined sludge–water distribution coefficients (Kd) supported the mechanistic interpretation and confirmed that sorption behaviour could not be reliably predicted based solely on hydrophobicity. Additionally, within the HiCS system, shorter SRT conditions (1 day) consistently resulted in improved OMP removal compared to longer SRT (2 day).

The modified HiCS configuration, incorporating a pre-contact zone between influent wastewater and stabilization sludge, enhanced carbon capture performance; however, its impact on OMP removal remained limited, with only marginal increases (typically within

5–11%) and no statistically significant differences when considering error bars, indicating that process modification primarily influenced the relative contribution of removal pathways rather than overall removal efficiency.

Integration with anaerobic digestion enhanced methane production potential, with an average yield of 0.36 ± 0.10 L CH_4/g VS, while OMP behaviour during digestion remained compound-specific, with evidence of both persistence and transformation.

Overall, the findings indicate that high-rate treatment systems can effectively support energy recovery through carbon capture, while micropollutant removal remains inherently compound-dependent, highlighting a fundamental trade-off between energy-oriented process design and micropollutant management. This study provides important insights into the development of integrated and sustainable wastewater treatment strategies within a circular economy framework.

CLAIM OF ORIGINALITY

This doctoral thesis investigates the behaviour and removal mechanisms of organic micropollutants in a pilot-scale modified High-Rate Contact Stabilization (HiCS) system integrated with anaerobic digestion. While high-rate activated sludge systems have been widely studied for carbon capture and energy recovery, their role in micropollutant removal, particularly under pilot-scale conditions treating real municipal wastewater, remains insufficiently explored.

The originality of this study lies in the integrated evaluation of micropollutant fate and methane production within a pilot-scale treatment system integrating a modified HiCS process with anaerobic digestion. The application of a modified HiCS configuration, including pre-contact between influent wastewater and stabilization sludge, provides new insights into how process design influences the carbon capture behaviour of the system and the distribution of micropollutants between liquid and solid phases.

Furthermore, this study distinguishes between sorption and biotransformation pathways through combined mass balance analysis and batch sorption experiments, demonstrating that removal behaviour cannot be explained solely by hydrophobicity. In addition, the fate of micropollutants during anaerobic digestion is evaluated, linking sludge-phase accumulation with downstream energy recovery processes.

To the best of our knowledge, this is among the first pilot-scale studies investigating OMP behaviour in a modified HiCS system integrated with anaerobic digestion, providing a comprehensive understanding of micropollutant dynamics in high-rate, resource-oriented wastewater treatment systems.

Overall, this thesis contributes to the development of integrated process design strategies that consider energy recovery, micropollutant management, and sustainable wastewater treatment within a circular economy framework. In this context, the findings also support the development of energy-oriented wastewater treatment strategies, particularly under increasing water reuse practices.

SYMBOLS

Kd : Solid–water distribution coefficient (L/kg)

Log Kow : Octanol–water partition coefficient

$\mathbf{C_{inf}}$: Influent concentration of OMP (ng/L)

$\mathbf{C_{eff}}$: Effluent concentration of OMP (ng/L)

$\mathbf{C_{PS}}$: OMP concentration in primary sludge (ng/g)

$\mathbf{C_{WAS}}$: OMP concentration in waste activated sludge (ng/g)

$\mathbf{C_{inf,L}}$: Dissolved OMP concentration in influent (ng/L)

$\mathbf{C_{inf,ads}}$: Solid-phase OMP concentration in influent (ng/g)

$\mathbf{Q_{inf}}$: Influent flow rate (L/d)

$\mathbf{TSS_{inf}}$: Influent total suspended solids (g/L)

$\mathbf{M_{in}}$: Influent mass load of OMP (ng/d)

$\mathbf{M_{eff}}$: Effluent mass load of OMP (ng/d)

R : Removal efficiency (%)

$\mathbf{q_e}$: Sorbed amount at equilibrium (mg/g)

V : Volume of solution (L)

M : Mass of sludge solids (g)

ABBREVIATIONS

AD : Anaerobic Digestion
BPA : Bisphenol A
CAS : Conventional Activated Sludge
CBZ : Carbamazepine
CIPRO : Ciprofloxacin
COD : Chemical Oxygen Demand
DICLO : Diclofenac
DO : Dissolved Oxygen
EPS : Extracellular Polymeric Substances
E1 : Estrone
E2 : 17β-Estradiol
E3 : Estriol
EE2 : 17α-Ethinylestradiol
F/M : Food to Microorganism Ratio
GC–MS : Gas Chromatography–Mass Spectrometry
HiCS : High-rate Contact Stabilization
HRAS : High-rate Activated Sludge
HRT : Hydraulic Retention Time
IBU : Ibuprofen
LOQ : Limit of Quantification
NP : Nonylphenol
OLR : Organic Loading Rate
OMP : Organic Micropollutant
OP : Octylphenol
PS : Primary Sedimentation
SPE : Solid Phase Extraction
SRT : Sludge Retention Time
TSS : Total Suspended Solids
VFA : Volatile Fatty Acids

VS **:** Volatile Solids

WAS **:** Waste Activated Sludge

WWTP **:** Wastewater Treatment Plants

WRRF **:** Wastewater Resource Recovery Facilities.

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1. INTRODUCTION

Sustainable management of municipal wastewater has become an increasingly important priority in response to rapid urbanization, population growth, and the growing pressure placed on aquatic ecosystems. Wastewater treatment plants (WWTPs) have traditionally been designed to remove organic matter and nutrients in order to protect receiving water bodies and maintain acceptable water quality standards. However, in recent decades, the role of wastewater treatment has expanded beyond conventional pollution control. Increasing attention is now directed toward the overall environmental performance of treatment processes, including their energy demand, greenhouse gas emissions, and the economic and environmental implications of sludge production and disposal.

As observed in hyper-urbanized megacities like Istanbul, the limitations of Conventional Activated Sludge (CAS) systems have reached a critical threshold. The immense spatial footprint required for CAS infrastructure is no longer viable in densely populated areas where land is scarce and expanding infrastructure is economically prohibitive. Beyond spatial constraints, CAS systems present a fundamental "carbon paradox." These facilities effectively remove bulk organic matter but do so through energy-intensive aeration, which facilitates the mineralization of organic carbon into CO_2. In an era where carbon neutrality is a global mandate, the mere discharge of carbon into the atmosphere can no longer be considered a success; rather, it represents a loss of potential energy and a contribution to the global carbon footprint.

Within this emerging framework, wastewater is increasingly viewed not simply as a waste stream requiring treatment but as a potential source of valuable resources such as energy, nutrients, and reclaimed water. This paradigm shift is strongly reflected in environmental policies promoting circular economy principles in the water sector. In particular, the revised European Urban Wastewater Treatment Directive (UWWTD) emphasizes the need to reduce the environmental impacts of wastewater treatment while addressing emerging challenges associated with contaminants of emerging concern. The updated directive aligns with major European sustainability initiatives such as the European Green Deal, the Zero Pollution Action Plan, and the Circular Economy Action Plan, and requires enhanced removal of a wide spectrum of OMPs from urban wastewater

while encouraging the wastewater sector to move toward energy neutrality (Díaz-Gamboa et al., 2024). In addition, increasing water demand and water scarcity have led to a growing interest in water reuse applications, particularly for irrigation, highlighting the importance of addressing micropollutants that may persist in treated wastewater.

In parallel with these developments, WWTPs have increasingly been reconceptualized as water resource recovery facilities (WRRFs), where the organic carbon contained in wastewater is captured and converted into valuable products. Concepts such as the ZeroWasteWater framework proposed by Verstraete and Vlaeminck (2011) emphasize the recovery of energy and materials from sewage through integrated treatment strategies. Within this perspective, treatment processes are designed to retain organic carbon in the sludge phase, for example through primary treatment or high-rate activated sludge systems, thereby maximizing carbon capture efficiency. The concentrated sludge can subsequently be subjected to anaerobic digestion, where organic matter is converted into methane-rich biogas that can be used for heat and electricity production, improving the overall energy balance of wastewater treatment plants.

Despite the increasing focus on energy recovery and carbon capture, municipal wastewater also contains a wide range of OMPs including pharmaceuticals, hormones, pesticides, and industrial chemicals. These compounds are typically present at trace concentrations but may pose significant environmental and human health risks due to their persistence, biological activity, and potential to accumulate in aquatic ecosystems (Luo et al., 2014). Therefore, modern wastewater treatment strategies must address not only energy efficiency and resource recovery, but also the fate and removal of micropollutants.

High-Rate Activated Sludge (HRAS) systems, particularly the High-rate Contact Stabilization (HiCS) process, have emerged as a key approach within this framework. Unlike CAS, HiCS is designed to "harvest" rather than "oxidize" carbon.

By operating under high-rate conditions, characterized by short sludge retention times (SRT) and dynamic "feast-famine" regimes, microorganisms are steered toward sorption and intracellular storage of organic carbon rather than complete growth or mineralization.

This strategic redirection of carbon into the sludge phase significantly reduces the aeration energy demand, addressing the high costs typically associated with mixers and pumps in conventional systems. The resulting carbon-rich sludge serves as a high-quality substrate for Anaerobic Digestion (AD). While the final stage of AD does produce biogas (CH_4 and CO_2), this configuration allows for the recovery of carbon as a renewable energy source, offsetting the facility's external energy needs and creating a more favourable net carbon balance.

However, the increasing emphasis on energy efficiency and the valorization of wastewater streams also introduces additional environmental considerations. This strategic focus on organic matter recovery creates a critical intersection between resource efficiency and environmental safety. OMPs are continuously introduced into wastewater due to widespread usage and can be detected across a wide range of environments. As high-rate treatment processes aim to retain organic carbon in the sludge phase for enhanced methane production, these systems may also promote the partitioning of certain OMPs to solids.

Therefore, understanding the distribution behaviour of OMPs, particularly through parameters such as the solid–water distribution coefficient is essential for evaluating the potential implications of energy-oriented treatment strategies on micropollutant fate within circular wastewater management systems.

Despite the growing interest in HRAS systems for carbon capture and energy recovery, the fate and removal mechanisms of OMPs in such systems remain insufficiently understood. Most existing studies have focused on CAS processes operating at long sludge retention times, where biotransformation is considered the dominant removal pathway. In contrast, HiCS operates under short SRT conditions, where sorption-driven mechanisms are expected to play a more significant role, yet this shift in removal dynamics has not been systematically investigated. However, the implications of short SRT-driven carbon capture on micropollutant removal mechanisms, phase distribution, and their subsequent fate during anaerobic digestion remain insufficiently understood under realistic operational conditions.

Furthermore, the majority of available studies are based on laboratory-scale experiments or synthetic wastewater, limiting their applicability to real wastewater conditions. Pilot-scale investigations under realistic operational conditions are still scarce. In addition, the combined assessment of OMP partitioning between liquid and solid phases together with their subsequent fate during anaerobic digestion has received limited attention. Therefore, a comprehensive evaluation of OMP behaviour in integrated high-rate treatment and sludge valorization systems is still lacking in the literature. In this context, short SRT should not be regarded merely as an operational parameter, but as a defining factor that fundamentally reshapes the dominant removal pathways of OMPs in HiCS systems.

1.1. High-Rate Activated Sludge Systems

In recent years, high-rate activated sludge systems have gained increasing attention as energy-efficient alternatives to conventional activated sludge processes. These systems operate under short SRT, short hydraulic retention times (HRT), high food-to-microorganism (F/M) ratios, and relatively low dissolved oxygen (DO) concentrations, which promote the rapid capture of organic carbon rather than its complete biological oxidation. Under these conditions, microbial growth and extensive carbon mineralization are intentionally limited, while sorption-driven mechanisms such as bioflocculation and sorption become dominant (Jimenez et al., 2015; Meerburg et al., 2015). In contrast to conventional activated sludge systems, where long SRT promotes complete oxidation and biotransformation processes, HRAS systems operate under short SRT conditions that favour rapid carbon capture and sorption-driven mechanisms over extensive biological degradation.

As a result, organic matter is rapidly transferred from the aqueous phase to the sludge phase, enabling the efficient capture of particulate and colloidal fractions. This shift in process dynamics promotes the retention of organic carbon within the biomass rather than its oxidation to carbon dioxide. Consequently, HRAS systems produce carbon-rich sludge streams that can be further treated by anaerobic digestion, where the retained organic matter is converted into methane-rich biogas. According to Rahman et al. (2020),

this strategic redirection of carbon not only enhances energy recovery through anaerobic digestion but also optimizes the resource recovery potential of the facility by effectively capturing organic matter before it is lost to oxidation. Through this approach, HRAS systems contribute to improving the overall energy balance of WWTPs and support the transition toward energy-neutral or energy-positive wastewater treatment. Despite these advantages, the performance of HRAS systems is subject to certain limitations. The short SRT conditions restrict the development of slow-growing microbial communities, potentially limiting the biotransformation of some compounds. In addition, the enhanced transfer of organic matter to the sludge phase may lead to the accumulation of particulate-associated substances, which requires careful consideration in downstream treatment processes.

1.2. High-rate Contact Stabilization Process

Among the different HRAS configurations, the HiCS process has emerged as a particularly promising approach for enhancing carbon capture efficiency. The HiCS process is derived from the conventional contact stabilization (CS) system, which was originally developed as a biosorption-based treatment concept to handle high organic loads. The HiCS configuration builds upon this concept by applying high-rate operational conditions, including higher loading rates and shorter retention times, which impose a strong selective pressure on the microbial community toward rapid substrate uptake and storage (Meerburg et al., 2015) .

Structurally, the HiCS process consists of two main reactors: a contact tank and a stabilization tank, as shown in Figure 1.1. In the contact tank, incoming wastewater is rapidly mixed with activated sludge under conditions that favour sorption processes. During this phase, particulate and colloidal organic matter is captured through bioflocculation and sorption, while readily biodegradable substrates may also be rapidly taken up and temporarily stored within the microbial cells.

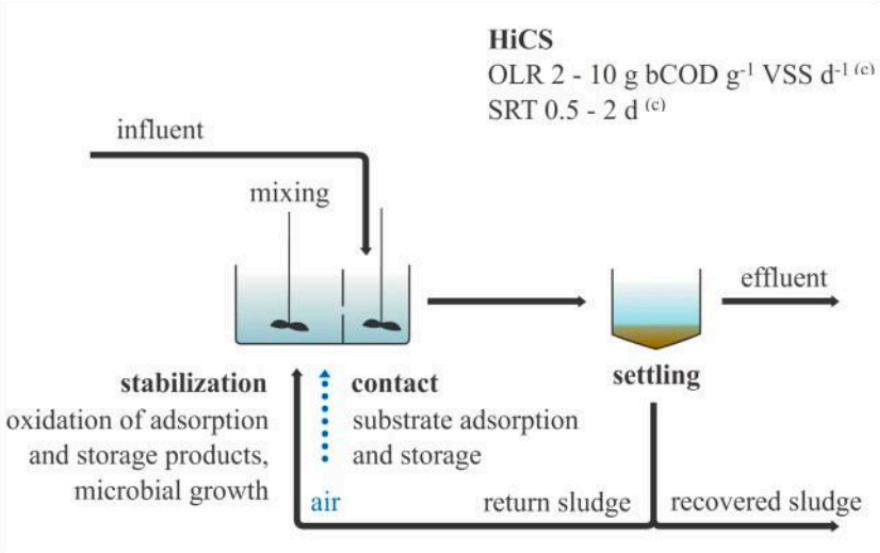


Figure 1.1. Schematic representation of the HiCS process (adapted from Meerburg et al., 2015)

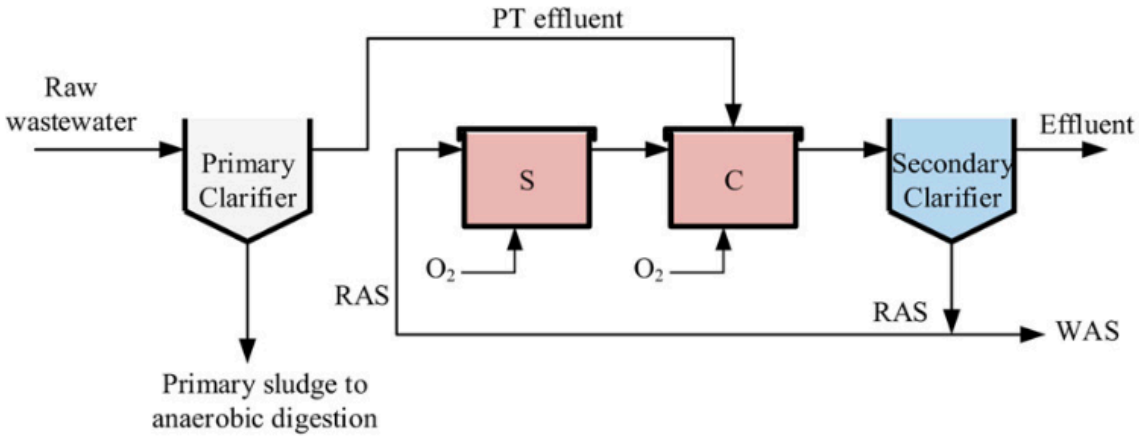


Figure 1.2. HiCS configuration integrated with Primary Sedimentation Tank (adapted from Rahman et al., 2020)

By enhancing the transfer of organic matter into the sludge phase, HiCS systems produce sludge that is rich in biodegradable organic carbon. When combined with primary sedimentation, the overall carbon capture efficiency can be further improved, as settleable organic matter is removed in the primary stage, while colloidal and soluble fractions are subsequently captured in the HiCS process through sorption-driven

mechanisms (Figure 1.2). As a result, HRAS-based systems can capture approximately 25–50% of influent chemical oxygen demand (COD) into sludge streams, significantly improving the potential for methane production during downstream anaerobic digestion (Demir et al., 2025; Jimenez et al., 2015).

Following the contact phase, the sludge is separated and transferred to the stabilization tank, where it is aerated. In this stage, the previously adsorbed and stored organic matter is partially oxidized, allowing the biomass to regenerate its sorption capacity before being recirculated back to the contact tank. This stabilization phase restores the availability of active binding sites by regenerating the surface properties and functional groups of the biomass, thereby enhancing its capacity for subsequent substrate and micropollutant capture.



Figure 1.3. Sketch of EPS Structure (adapted from Sheng et al. (2010))

A defining characteristic of the HiCS system is the presence of alternating feast and famine conditions. During the contact phase (feast), microorganisms are exposed to high substrate concentrations, which promotes rapid uptake and intracellular storage of organic compounds. In contrast, during the stabilization phase (famine), the absence or limitation of external substrate forces microorganisms to consume the stored compounds. This cyclic regime has been shown to enhance the production of storage polymers and extracellular polymeric substances (EPS), which in turn improve bioflocculation, sorption capacity and better settleability of primary sludge under low SRT conditions. (Meerburg et al., 2016; Rahman et al., 2017).

EPS are complex biopolymers mainly composed of proteins, polysaccharides, lipids, and nucleic acids that originate from microbial metabolism and cell lysis. EPS play a central

role in determining sludge properties by influencing surface charge, hydrophobicity, and sorption capacity. As illustrated in Figure 1.3, EPS form a structured matrix around microbial cells, consisting of tightly bound (TB-EPS) and loosely bound (LB-EPS) fractions. This matrix governs key physicochemical properties of sludge, including surface charge, hydrophobicity, and sorption capacity. Due to the presence of functional groups (e.g., –COOH, –OH, –C=O), EPS matrices can interact with organic compounds through multiple physicochemical mechanisms.

In the HiCS systems, enhanced EPS production strengthens bioflocculation and increases the availability of binding sites, thereby promoting the transfer of organic matter into the sludge matrix. Consequently, EPS are considered a key factor influencing both carbon capture efficiency and the association of organic matter with sludge solids. These interactions facilitate the transfer of organic matter into the sludge matrix, forming carbon-rich sludge streams suitable for subsequent energy recovery processes.

1.3. Primary Sedimentation

Primary sedimentation tanks (PS) are conventional physical separation units designed to remove settleable solids together with a fraction of suspended solids (SS) and associated organic matter from raw wastewater. In conventional treatment configurations, the primary function of the PS is to reduce the organic load entering the biological stage, thereby decreasing aeration demand. However, with the shift toward energy-oriented wastewater treatment, PSs are increasingly recognized as key units for carbon capture, enabling the redirection of particulate organic matter to the sludge line for subsequent energy recovery.

A common approach to enhance primary settling performance in CAS systems is the recirculation of waste activated sludge (WAS) to the PST inlet. This strategy increases solids concentration and promotes bioflocculation, improving the capture of colloidal and finely dispersed particles. Yetis and Tarlan (2002) demonstrated that the addition of activated sludge enhances floc formation through increased particle interactions, leading to higher suspended solids removal without the need for chemical coagulants.

From an energy recovery perspective, high-rate processes are designed to favour carbon capture rather than biological oxidation. Rahman et al. (2019) highlighted that high-rate contact stabilization (HiCS) systems, operating under short residence times and feast–famine conditions, effectively redirect organic carbon into the sludge phase. Under these conditions, carbon is preserved in a particulate and biodegradable form, making it suitable for downstream anaerobic digestion and energy recovery.

Building on this concept, Demir et al., (2025) investigated the integration of primary sedimentation with a pilot-scale HiCS system. The COD capture performance of this configuration has been previously evaluated and reported in detail by Demir, (2025). Their results demonstrated that the PS can simultaneously treat influent wastewater and retain recirculated biomass, thereby increasing the fraction of COD diverted to the sludge line and strengthening the basis for energy-neutral treatment.

The modified configuration was developed by extending the biosorption and bioflocculation principles of the contact tank to the primary treatment stage. In the conventional HiCS process, wastewater and activated sludge interact within the contact tank, where sorption and bioflocculation promote the capture of organic matter. In the modified configuration, stabilization sludge was recirculated upstream of the primary sedimentation tank and brought into direct contact with raw wastewater prior to settling. This additional biomass–substrate interaction step was intended to enhance bioflocculation, increase the capture of particulate and colloidal organic matter, and divert a larger fraction of organic carbon to the sludge line for subsequent anaerobic digestion and energy recovery.

The pilot-scale PS+HiCS system was operated over a two-year period treating real municipal wastewater under both conventional and modified configurations. During the operation of this pilot system, different aspects of process performance were investigated. While the carbon capture performance and COD recovery potential of the modified configuration were evaluated by Demir (2025), the present study, conducted simultaneously within the same pilot-scale system, focuses specifically on the fate, removal mechanisms, and phase distribution of OMPs under these operating conditions. Furthermore, the behaviour of OMPs during anaerobic digestion of the generated sludge

was investigated to assess the implications of enhanced carbon capture on sludge quality and energy recovery.

Despite the well-established benefits of carbon diversion for energy recovery, the fate of OMPs under conventional and modified configurations remains poorly understood. As carbon is increasingly transferred to the sludge phase, the dominant removal pathways of OMPs, particularly sorption-driven mechanisms, are expected to change. Therefore, this study aims to evaluate how enhanced carbon capture and process configuration influence the partitioning, removal mechanisms, and overall fate of OMPs under conventional and modified PS+HiCS configurations, and to assess the potential implications of these changes for downstream energy recovery processes.

1.4. Anaerobic Digestion

The enhanced capture of organic carbon in HRAS systems results in the production of sludge rich in biodegradable organic matter, which represents an ideal substrate for anaerobic digestion. During anaerobic digestion, complex organic compounds are biologically converted through a series of steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis, ultimately producing methane-rich biogas that can be used for energy generation. Compared to conventional sludge, the more readily biodegradable and concentrated organic matrix of HiCS sludge can lead to faster hydrolysis rates, potentially reducing the required retention time in anaerobic digesters.

The high particulate and adsorbed organic content of sludge derived from high-rate activated sludge systems enhances its biodegradability and increases its potential for methane production. In this context, AD is widely applied for both sludge stabilization and energy recovery in wastewater treatment plants (Jimenez et al., 2015; Meerburg et al., 2015). This indicates that the amount and quality of raw carbon captured in the upstream process directly influence methane production during anaerobic digestion.

HiCS systems can therefore be considered energy-efficient alternatives to conventional activated sludge processes, as they promote the capture of organic carbon in the sludge phase rather than its oxidation to carbon dioxide. The retained carbon can subsequently

be converted into methane through anaerobic digestion, enabling energy recovery while reducing aeration demand and associated CO_2 emissions. As a result, such integrated treatment configurations contribute to improving the overall energy balance and reducing the carbon footprint of wastewater treatment plants.

1.5. Organic Micropollutants

Integrated HiCS and anaerobic digestion systems are primarily designed to enhance carbon capture and energy recovery in wastewater treatment. However, improved energy efficiency alone does not necessarily ensure environmental sustainability. Within these systems, OMPs may be transferred, transformed, or retained within the sludge matrix, potentially influencing their overall environmental impact. Therefore, understanding the fate of OMPs across both treatment stages is essential to ensure that improvements in energy efficiency are not achieved at the expense of increased environmental and health risks.

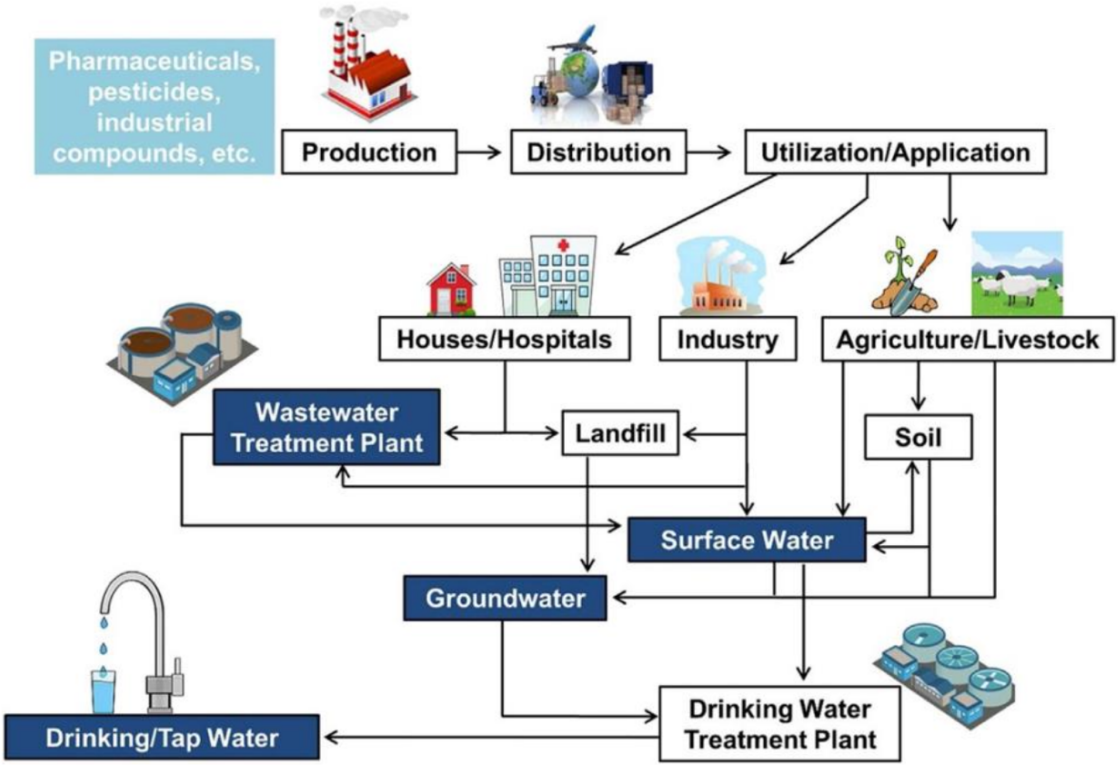


Figure 1.4. Micropollutants in the Environment (Barbosa et al., 2016)

In this context, OMPs represent a diverse group of trace organic compounds that are widely detected in municipal wastewater at concentrations typically ranging from ng/L to µg/L. This group includes pharmaceuticals, personal care products, endocrine-disrupting compounds, and various industrial chemicals. OMPs enter wastewater treatment plants (WWTPs) through domestic, hospital, and industrial discharges and are not specifically targeted by conventional treatment processes. As a result, their removal is often incomplete, leading to their continuous release into aquatic environments. The complex pathways through which these OMPs enter wastewater treatment plants and their subsequent distribution across different environmental compartments are illustrated in Figure 1.4.

A significant fraction of pharmaceuticals and other micropollutants is excreted from humans and animals in unmetabolized or partially metabolized forms, entering wastewater streams via urine and feces (Carballa et al., 2004). Domestic households represent the primary source of many pharmaceutical compounds, although hospitals and other healthcare-related facilities may also contribute substantially (Wick et al., 2009).

OMPs are of increasing environmental concern due to their persistence, bioaccumulation potential, and their potential to pose risks to both aquatic organisms and human health. These compounds are continuously introduced into aquatic environments and are typically detected at trace concentrations. WWTPs are therefore considered both barriers and secondary sources of OMPs, as many compounds are only partially removed and may be discharged into receiving waters (Luo et al., 2014). As treated wastewater is discharged into receiving water bodies or reused for applications such as irrigation, OMPs that are not fully removed during treatment can re-enter the environment and may ultimately contribute to human exposure. This continuous release may lead to their accumulation in aquatic and terrestrial ecosystems, resulting in chronic exposure and potential long-term impacts on ecosystem functioning.

Particular concern has been raised for endocrine-disrupting compounds (EDCs) and steroid hormones, which can induce adverse biological effects even at extremely low concentrations in the range of 0.1–0.5 ng/L (Clara et al., 2004a). These compounds have been associated with endocrine disruption in aquatic organisms, including alterations in

reproduction and development (Clara et al., 2005a). In addition, transformation products of certain micropollutants may retain or even enhance biological activity, contributing further to ecological risks (Loyo-Rosales et al., 2007).

Evidence from ecotoxicological studies also indicates that pharmaceuticals such as carbamazepine may affect aquatic organisms, including reductions in invertebrate abundance and reproductive impairments in fish species under environmentally relevant exposure conditions (Gagné et al., 2006; Oetken et al., 2005).

1.5.1. Removal Mechanisms of OMPs

Within wastewater treatment systems, OMPs are present both in the aqueous phase and associated with suspended solids. Due to sorption processes, their concentrations in sludge are often higher than in the liquid phase. This phase distribution directly influences the dominant removal pathways, as compounds associated with sludge are more likely to be removed through solid-phase separation, while those remaining in the aqueous phase depend primarily on biotransformation processes.

In wastewater treatment systems, the removal of OMPs is primarily governed by two key mechanisms: sorption and biotransformation. Other processes such as volatilization may contribute to the removal of certain compounds; however, for most OMPs typically present in municipal wastewater, volatilization is considered negligible due to their low volatility.

Sorption

Sorption plays a central role in determining the distribution of OMPs between the liquid phase and the sludge matrix. This partitioning behaviour is governed by the combined influence of compound-specific physicochemical properties and biomass characteristics (Alvarino et al., 2014; Carballa et al., 2008).

Mechanistically, sorption onto sludge particles occurs through two main pathways: (i) absorption, driven by hydrophobic interactions with the lipophilic cell membranes and lipid fractions of the sludge, and (ii) adsorption, governed by electrostatic interactions

between charged functional groups and microbial surfaces (Ternes et al., 2004; Siegrist et al., 2003). These mechanisms also explain differences in sorption affinity between primary and secondary sludge, which vary in lipid content and surface properties.

Hydrophobicity is generally considered the primary driver of sorption and is commonly represented by the octanol–water partition coefficient (log Kow). Compounds with higher lipophilicity, including various hormones and phenolic substances, tend to preferentially accumulate within sludge solids, largely independent of biomass morphology (Alvarino et al., 2014; Joss et al., 2005).

However, hydrophobic interactions alone cannot fully explain the sorption behaviour of all OMPs. For compounds that are ionized under typical wastewater conditions, electrostatic interactions may also play a significant role. In such cases, positively charged species can interact with negatively charged biomass surfaces, enhancing their retention within the sludge phase (Suárez et al., 2008). While log Kow provides a preliminary indicator of hydrophobic sorption potential, the solid–water distribution coefficient (Kd, L/kg) is widely used to describe this phase distribution, reflecting the ratio of compound concentrations in the solid and aqueous phases under equilibrium conditions (Hörsing et al., 2011).

Therefore, for polar and ionizable compounds, sorption behaviour cannot always be reliably predicted based solely on hydrophobicity (log Kow), emphasizing the need for experimentally determined Kd values as a quantitative measure of compound partitioning between sludge solids and the aqueous phase when interpreting removal mechanisms (Ternes et al., 2004).

Biotransformation

The biotransformation of OMPs within wastewater treatment systems is a complex process primarily governed by the metabolic and co-metabolic capabilities of the microbial community. Mechanistically, these transformations can be classified into three main categories based on the interaction between microorganisms and micropollutants.

Growth-linked biotransformation: In this pathway, specific microorganisms are able to utilize OMPs as a sole carbon and energy source. Although this mechanism has been reported in low-nutrient environments such as river sediments (Daughton and Ternes, 1999), its contribution in wastewater treatment systems is generally considered limited due to the presence of more readily biodegradable organic substrates (Joss et al., 2006).

Co-metabolism: Co-metabolic transformation is widely regarded as one of the dominant pathways for OMP degradation in conventional activated sludge systems. In this process, OMPs are non-specifically transformed by enzymes produced for the metabolism of primary substrates. In particular, ammonia monooxygenase (AMO), produced by nitrifying microorganisms, has been shown to play a key role in the transformation of a wide range of micropollutants without providing direct energy benefits to the microorganisms (Vader et al., 2000; Joss et al., 2006).

Mixed substrate utilization: This pathway involves the simultaneous degradation of OMPs in the presence of other organic carbon sources, which may enhance overall transformation rates and, in some cases, lead to partial or complete mineralization. However, the extent of this process is highly compound-specific and depends on both environmental conditions and microbial community structure (Verlicchi et al., 2012).

The efficiency of biotransformation is influenced by several factors, including operational parameters such as HRT, SRT, temperature, and the specific process technology employed (e.g., HiCS vs. CAS). Furthermore, the extent of biotransformation depends on the chemical structure of each OMP and its concentration in the influent. The concentration levels often dictate the metabolic pathway, determining whether the compound is degraded through primary metabolism or co-metabolism. Additionally, the type and metabolic activity of the biomass, which are largely governed by the prevailing redox conditions (aerobic, anoxic, or anaerobic), play a decisive role in the degree of OMP transformation. The relative contribution of sorption and biotransformation is influenced by operational and environmental factors including SRT, microbial activity, biomass characteristics, and treatment configuration (Verlicchi et al., 2012).

To understand how these biological and physical factors interact at the cellular level, the structural components of the biomass must be considered. Among these components, EPS play a role that extends beyond simple sorption processes. EPS provide a heterogeneous matrix rich in functional groups that can facilitate multiple interaction mechanisms, including hydrogen bonding, hydrophobic interactions, and other non-covalent associations. In addition, previous studies have suggested that EPS may also contribute to transformation processes through redox-active components, although such mechanisms are highly system-dependent (Wang et al., 2024). The conceptual role of EPS in micropollutant interactions is illustrated in Figure 1.5.

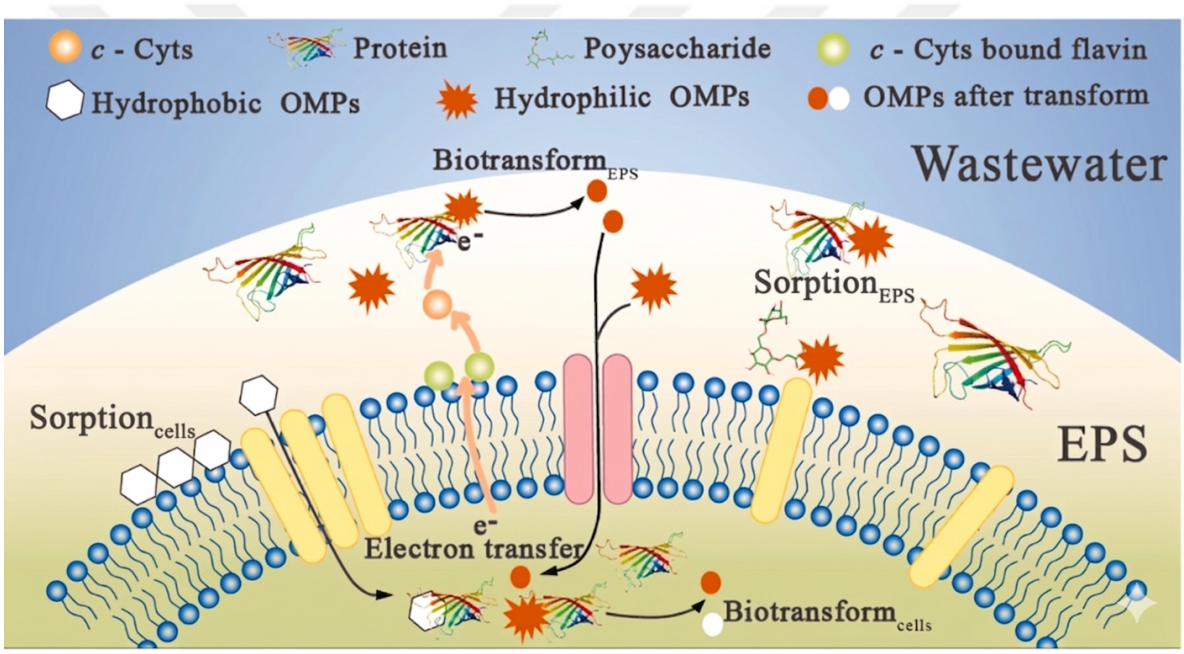


Figure 1.5. Conceptual illustration of EPS-OMPs interactions (adapted from Wang et al. (2024)).

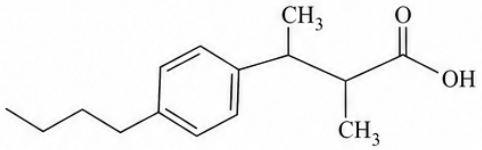
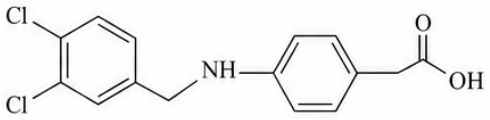
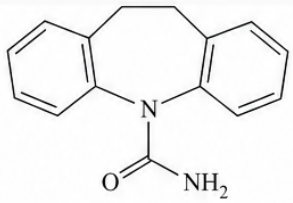
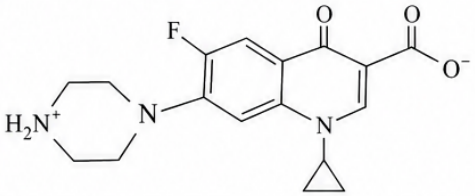
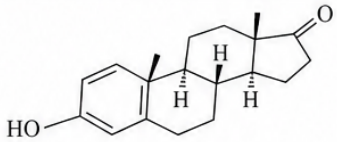
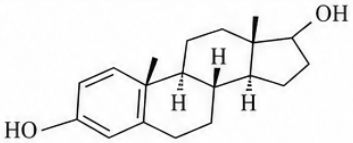
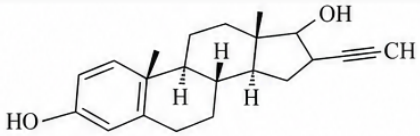
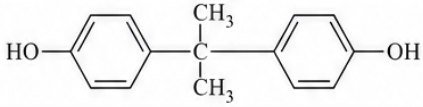
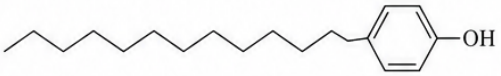
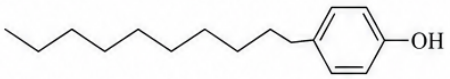
Importantly, these interactions are strongly compound-specific and governed by the physicochemical properties of the target micropollutants. As a result, different OMPs may follow distinct removal pathways, with varying contributions from sorption and biotransformation processes. Since primary sedimentation is based on solid–liquid separation, its contribution to OMP removal is limited. This is mainly associated with the fraction of OMPs bound to settleable solids, while the majority remains in the aqueous phase (Behera et al., 2011; Carballa et al., 2005, 2004).

In carbon-harvesting systems such as HiCS, where organic matter is intentionally redirected toward the sludge phase, the distribution and fate of OMPs may differ significantly from conventional systems. In such systems, short sludge retention times and high substrate availability may alter the balance between sorption and biotransformation processes, potentially enhancing the role of sludge-phase interactions. Despite increasing interest in energy-efficient treatment configurations, micropollutant partitioning between liquid and solid phases under such conditions and their subsequent fate during downstream sludge treatment remain insufficiently understood, particularly under short SRT conditions characteristic of HiCS systems. Therefore, a detailed evaluation of OMP fate across both liquid and sludge streams is essential to fully assess the environmental performance of carbon-harvesting wastewater treatment systems. In this framework, SRT emerges not merely as an operational parameter, but as a governing factor that fundamentally reshapes the dominant removal mechanisms of micropollutants in high-rate activated sludge systems.

In this study, a set of representative OMPs was selected, including pharmaceuticals (IBU, DICLO, CBZ, CIPRO), hormones (E1, E2, EE2), and industrial compounds (BPA, NP, OP). These compounds were selected to cover a broad range of physicochemical properties influencing their distribution and removal in wastewater treatment systems. In addition, the selection reflects current regulatory priorities, as these compounds represent key substance groups highlighted in the revised UWWTD and are frequently detected in municipal wastewater.

The selected OMPs exhibit diverse physicochemical properties and molecular characteristics that may influence their phase distribution and removal behaviour in wastewater treatment systems. The molecular formulas and representative structures of the investigated compounds are presented in Table 1.1. These parameters were used to interpret compound-specific behaviour and to distinguish between sorption and transformation-driven removal pathways within the HiCS system. By including compounds with contrasting behaviour, this selection enables a comprehensive evaluation of sorption and biotransformation mechanisms under high-rate treatment conditions.

Table 1.1. Molecular formulas and representative molecular structures of the selected OMPs.

Category	Compound	Molecular Formula	Molecular Structure
Pharmaceuticals	IBU	$C_{13}H_{18}O_2$	
	DICLO	$C_{14}H_{11}Cl_2NO_2$	
	CBZ	$C_{15}H_{12}N_2O$	
	CIPRO	$C_{17}H_{18}FN_3O_3$	
Hormones	E1	$C_{18}H_{22}O_2$	
	E2	$C_{18}H_{24}O_2$	
	EE2	$C_{20}H_{24}O_2$	
Industrial Compounds	BPA	$C_{15}H_{16}O_2$	
	NP	$C_{15}H_{24}O$	
	OP	$C_{15}H_{22}O$	

This study highlights an important limitation of energy-oriented wastewater treatment systems. While high-rate processes such as HiCS enhance carbon capture and support energy recovery through anaerobic digestion, they do not ensure complete removal of organic micropollutants. Instead, micropollutant behaviour varies depending on their physicochemical properties, and a significant fraction may persist or be redistributed within the system. Therefore, achieving both energy efficiency and effective micropollutant control requires integrated treatment strategies, where high-rate processes are complemented by additional treatment steps.

Following their partitioning to the sludge phase, the fate of OMPs during sludge treatment processes such as anaerobic digestion becomes a critical consideration. The characteristics of sludge subjected to anaerobic digestion can vary significantly depending on the upstream treatment configuration. In particular, sludge generated from HiCS differs from conventional activated sludge in terms of organic content and physicochemical properties, which can influence the behaviour of micropollutants.

While some compounds may undergo partial transformation under anaerobic conditions, others may persist or remain associated with the solid phase. However, the behaviour of OMPs during anaerobic digestion has been investigated mainly for conventional sludge, and information on sludge derived from HiCS systems remains limited.

1.5.2. Comparative Evaluation of OMP Fate: Operational Perspectives

Conventional activated sludge systems are widely applied in municipal wastewater treatment and are characterized by relatively long SRTs, typically ranging between 10 and 20 days. Under these conditions, organic matter is largely removed through complete biological oxidation, which requires continuous aeration and results in significant energy consumption. While such systems can support effective biotransformation of certain organic micropollutants due to extended microbial contact time, their high operational energy demand has raised concerns regarding overall process sustainability. These operational characteristics also influence the dominant removal mechanisms of OMPs, where extended SRT conditions favor biotransformation processes.

The removal of OMPs in CAS systems cannot be explained by a single dominant mechanism. As discussed by Joss et al. (2006), different compounds exhibit very different behaviours in activated sludge, and removal efficiencies can vary widely depending on the substance. While some compounds are readily biodegradable, others remain largely unaffected under similar conditions, indicating that removal is strongly compound-specific. More specifically, Fernandez-Fontaina et al. (2012) showed that under nitrifying conditions, increased biological activity can enhance biotransformation rates of certain compounds through co-metabolic processes. However, their results also demonstrated that activated sludge exhibits different affinities for each compound, leading to significant variability in removal efficiencies. In addition, compounds with slower or intermediate biotransformation kinetics were more sensitive to operational conditions such as hydraulic retention time and loading rates, resulting in reduced removal under high-rate conditions. Importantly, sorption behaviour remained largely unaffected by operational parameters, suggesting that biotransformation, rather than sorption, is the more condition-dependent pathway.

Activated sludge systems operated at long SRT are often associated with higher biotransformation efficiencies of certain micropollutants (Clara et al., 2005a). This indicates that SRT can partly explain the variability observed between compounds. However, this relationship is not universal, as removal remains strongly compound-specific and some substances show limited degradation even under extended SRT conditions.

Overall, these studies indicate that OMP removal in CAS systems should be interpreted on a compound-by-compound basis. Depending on the properties of the substance, removal may be governed by biotransformation, sorption, or may remain limited under typical treatment conditions.

The removal efficiency of OMPs in CAS systems is highly variable and is governed by both the physicochemical properties of the compounds and the operational conditions of the treatment process. OMP removal has been widely reported to range between 12.5% and 100%, depending on compound-specific biodegradability and system conditions (Luo et al., 2014). In general, compounds with high biodegradability, such as ibuprofen,

exhibit high removal efficiencies (>70%), whereas moderately biodegradable compounds such as diclofenac show intermediate removal (30–70%), and persistent compounds like carbamazepine remain poorly removed (<30%) (Luo et al., 2014). These variations are primarily attributed to differences in biotransformation potential and compound-specific physicochemical properties rather than process design alone.

In contrast to these CAS conditions, the transition toward energy-positive wastewater treatment through HRAS processes, such as HiCS, introduces distinct operational boundaries compared to CAS. While CAS systems typically operate at longer SRT (>10 days) and HRT (>6 h), high-rate configurations prioritize the redirection of organics under significantly shorter SRT and HRT conditions.

In a comprehensive laboratory-scale study, Koumaki et al. (2021) evaluated the fate of emerging contaminants in a HiCS system treating real municipal wastewater. The system was operated at a total SRT of approximately 2 days and a contact tank HRT of 0.25 h. Their results demonstrated that biotransformation processes were significantly limited under such conditions, and that for hydrophobic compounds, sorption onto sludge was the dominant removal pathway. This indicates a clear shift from degradation-dominated removal mechanisms, typically observed in CAS systems, toward partitioning-driven behaviour in high-rate configurations.

Similarly, Taboada-Santos et al. (2020) investigated OMP removal in a HRAS system operated under short SRT conditions (approximately 0.5 days) and an HRT of around 30 min. Their findings showed that while the system was highly effective in capturing particulate organic matter, the removal of moderately hydrophilic OMPs remained negligible. This suggests that the limited biological reaction time in high-rate systems constrains biotransformation processes and promotes compound-specific behaviour dominated by physicochemical interactions. However, the fate of OMPs in modified high-rate systems such as HiCS, particularly under dynamic operating conditions and real wastewater matrices, remains insufficiently understood, especially in terms of phase distribution and the relative contributions of sorption and biotransformation.

1.6. Regulatory Framework and Future Perspectives on Micropollutant Removal

In parallel with these scientific challenges, regulatory frameworks have also evolved to address the environmental risks associated with micropollutants. The management of OMPs has entered a transformative era with the recent revision of the European Urban Wastewater Treatment Directive (UWWTD). While the original Directive 91/271/EEC primarily targeted bulk organic matter and nutrients, the updated framework establishes a stringent mandate for the removal of pharmaceuticals and personal care products. Central to this regulation is the requirement for a minimum 80% removal efficiency for a defined set of indicator compounds, such as DICLO and CBZ, at large-scale facilities (Ianes et al., 2025). This regulatory shift acknowledges that conventional secondary treatment processes are fundamentally limited in their ability to mitigate the environmental risks posed by these trace contaminants.

To comply with these emerging standards, the integration of quaternary (fourth stage) treatment technologies has become a technical necessity. Recent evaluations indicate that advanced oxidation processes, particularly ozonation and activated carbon adsorption, are among the most reliable pathways to achieving the mandatory 80% removal threshold (Ianes et al., 2025). However, the implementation of such high-energy and capital-intensive infrastructure must be balanced against local conditions and economic feasibility. In this context, nature-based solutions are gaining renewed attention as potential quaternary alternatives. For instance, storage lagoons with extended hydraulic retention times have demonstrated a significant capacity for OMP attenuation through natural photolysis and microbial degradation, offering a lower-cost compliance strategy under specific regional conditions (Díaz-Gamboa et al., 2024).

Looking toward 2050, the evolution of wastewater treatment is characterized by a paradigm shift from simple “pollution control” to “resource recovery.” The future vision for the European water sector emphasizes the transition of treatment plants into circular economy hubs, where micropollutant removal is aligned with carbon neutrality goals, nutrient recovery, and energy-positive operations (Soares, 2020). Consequently, the challenge for modern environmental engineering lies not only in meeting the 80%

removal target but in achieving it through sustainable, low-carbon processes that align with the broader objectives of the European Green Deal.

Table 1.2. Ecotoxicological significance and regulatory status of target OMP

Compound Group	Target OMP	PNEC (ng/L)	General Environmental Profile	Ref.
Pharmaceuticals	DICLO	50	Regulatory concern: Associated with 80% removal target.	Figuière (2022) /Ianes (2025)
	CBZ	500	Highly persistent: Widely used as indicator compound.	Figuière (2022) / Ianes (2025)
	CIPRO	15.2 – 250	Potential ecological risk: Antibiotic active in aquatic systems.	Zhang et al. (2024)
	IBU	5.9 – 1000	Widely detected: Frequently observed in wastewater effluents.	Huang et al. (2018)
Alkylphenols	BPA	0.5 – 150	Endocrine-disruptor: Active at ultra-trace concentrations.	Wright-Walters (2011)
	OP	10	Persistent: Tendency to accumulate in aquatic environments.	Staniszewska (2015)
	NP	330	Industrial phenolic: Subject to environmental monitoring.	Staniszewska (2015)
Hormones	E1	1.0 – 2.0	Bioactive: Natural estrogenic compound.	Grzegorzek et al., (2024) / Liang et al., (2021)
	E2	0.4 – 1.0	High estrogenic potency: Active at very low concentrations.	Grzegorzek et al., (2024) / Liang et al., (2021)
	EE2	0.1	Top Priority: Synthetic hormone with extreme regulatory relevance.	Grzegorzek et al., (2024) / Liang et al., (2021)

1.7.Thesis Scope and Aim

A comprehensive understanding of the fate of organic micropollutants (OMPs) in energy-oriented wastewater treatment systems is essential to ensure the environmental sustainability of modern infrastructure. In this context, the present study investigates the fate and removal mechanisms of selected OMPs in a pilot-scale PS+HiCS system treating real municipal wastewater under conventional and modified configurations. The pilot system was operated under long-term conditions, during which different aspects of process performance were investigated simultaneously. While the carbon capture and COD recovery performance of the modified configuration were evaluated by Demir (2025), the present study focuses on the partitioning, removal behaviour, and dominant removal mechanisms of OMPs under these operating conditions. In addition, the subsequent behaviour of OMPs during anaerobic digestion of the generated sludge was investigated to evaluate the implications of carbon capture-oriented treatment on sludge quality and energy recovery.

The scope of the thesis further extends to the anaerobic digestion of the resulting PS+HiCS-derived sludge. This is of particular importance because the enhanced carbon capture characteristics of HiCS systems increase the transfer of biodegradable organic matter to the sludge phase, creating opportunities for energy recovery while simultaneously affecting the fate of OMPs during sludge treatment. Therefore, both methane production potential and the compound-specific behaviour of OMPs during anaerobic digestion were evaluated.

To provide a mechanistic understanding, mass balance calculations were employed to differentiate the relative contributions of sorption and biotransformation. In addition, experimentally determined solid–water distribution coefficients (Kd) were used to support the interpretation of selected compound-specific removal mechanisms.

By focusing on the pilot-scale treatment of real municipal wastewater under varying operational conditions, this study aims to provide new insights into the removal mechanisms and fate of micropollutants within carbon-harvesting and energy-recovery frameworks.

2. MATERIALS AND METHODS

2.1. Pilot Scale High-Rate Contact Stabilization

A pilot plant was operated at Paşaköy Advanced Biological Wastewater Treatment Plant. The pilot plant, shown in Figure 2.1, consisted of a primary settling tank, contact and stabilization tanks, and a secondary settling tank. The system was fed with municipal wastewater from the effluent of a grit chamber at a full-scale wastewater treatment plant, which treats wastewater from a significant portion of the Anatolian side of Istanbul, Turkey.



Figure 2.1. Pilot Plant

The pilot plant, as shown in Figure 2.2, consists of a primary clarifier (1.3 m^3), mixing tank (0.4 m^3), contact tank (6.3 m^3), stabilization tank (0.8 m^3), and final clarifier (1.1 m^3). Two centrifugal blowers (Proair A3TDX 226, SİSDOZ, Türkiye) are used for aeration of the contact and stabilization tanks, and two mechanical mixers are used for mixing. Sludge return and waste sludge removal are carried out by two pumps, and a

proportional valve is used to withdraw sludge from the primary clarifier (Figure 2.3). The plant was operated at a constant flow rate of 1 m^3/d.

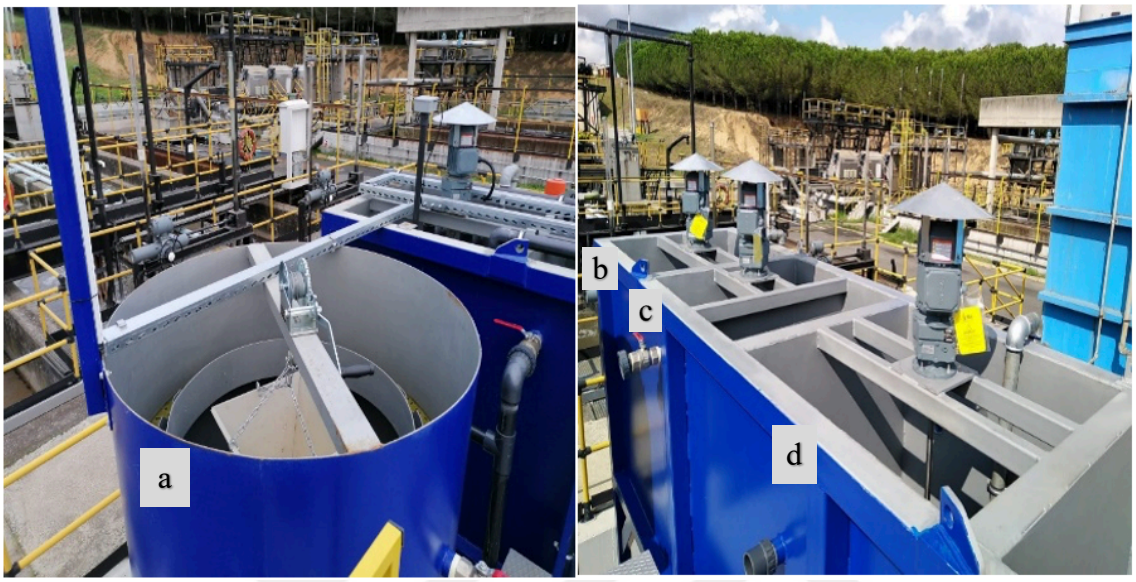


Figure 2.2. Primary Sedimentation tank(a) -Stabilization tank(b)- Contact tank(c) – Final Clarifier (d)



Figure 2.3. Flow in Pilot Plant

The pilot plant was operated in two distinct modes: a high-rate contact stabilization process and a modified high-rate contact/stabilization process, as depicted in Figure 2.4, with SRTs ranging from 0.5 to 2 days. The system was operated continuously over a two-

year period (January 2022–January 2024), during which significant seasonal fluctuations were observed in wastewater. Rather than maintaining fixed operational conditions, process parameters were intentionally varied to evaluate system performance under different scenarios. Therefore, the findings should be interpreted as representing system behaviour under dynamic, real wastewater conditions, instead of strictly controlled steady-state operation. These two-year operation period, routine monitoring parameters (such as pH, temperature, TS, TSS) and organic micropollutant (OMP) measurements were conducted on influent, effluent, and sludge samples collected from the pilot plant. The detailed analytical procedures are provided in Section 2.3.

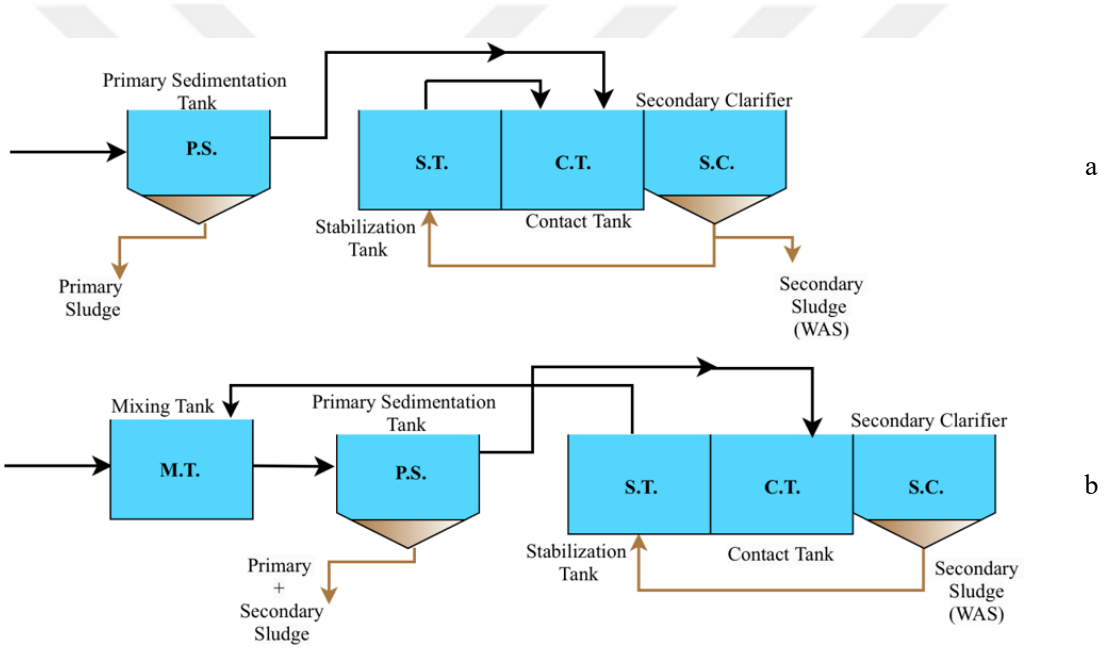


Figure 2.4. Flow diagram of Pilot Plant (a) conventional HiCS process (b) modified HiCS process

In this study, the pilot-scale plant was fed with 1 m^3/h wastewater throughout the study, and the HRTs of primary sedimentation, stabilization, contact, and secondary clarifier tanks were approximately 79, 72, 23, and 41 min, respectively. The blowers were operated to keep DO concentrations in the CT and ST at 1–3 mg/L. The MLSS concentration in the stabilization tank averaged 3400 mg/L, with values ranging from 1000 to 11200 mg/L.

Sludge management within the system was adjusted to maintain the targeted SRT. Return sludge from the secondary clarifier was directed to the stabilization tank at 67% of the influent flow rate. Excess sludge was removed daily, while primary sludge was discharged automatically at regular intervals, with increased discharge frequency during warmer periods.

In the modified HiCS configuration, the system was modified to enhance bioflocculation capacity by promoting the production of EPS, which are central to the functioning of the HiCS process. For this purpose, stabilization sludge was brought into contact with the influent prior to primary sedimentation. Previous studies (Nouha et al., 2018; Ye et al., 2011) have shown that short-term aeration of returned sludge in the stabilization tank can increase the available surface area of the biomass, thereby enhancing its biosorption capacity. During this phase, the production of EPS further supports both sorption and bioflocculation processes. Based on these mechanisms, the modified HiCS configuration was developed to improve the capture of organic carbon and to increase its subsequent recovery potential. In addition to carbon capture, the effect of this configuration on the fate and removal of OMPs was also evaluated. In this setup, primary and secondary sludge streams were discharged through a single outlet.

2.2.Sampling Points of the Pilot-Scale HiCS System

Sampling was conducted at key locations along the pilot-scale HiCS system in order to capture the distribution and biotransformation of OMPs across both liquid and sludge phases. The selected sampling points are illustrated in Figure 2.5.

Influent wastewater samples (1) were collected prior to primary sedimentation to determine the initial concentrations of OMPs entering the system. Effluent samples (2) were taken at the outlet of the secondary clarifier to assess overall removal performance. Influent and effluent samples were collected in duplicate to ensure analytical reliability. The samples were filtered immediately at the sampling site, transferred into amber glass bottles, and stored at 4 °C until further processing to minimize photolytic degradation and preserve sample integrity prior to analysis.

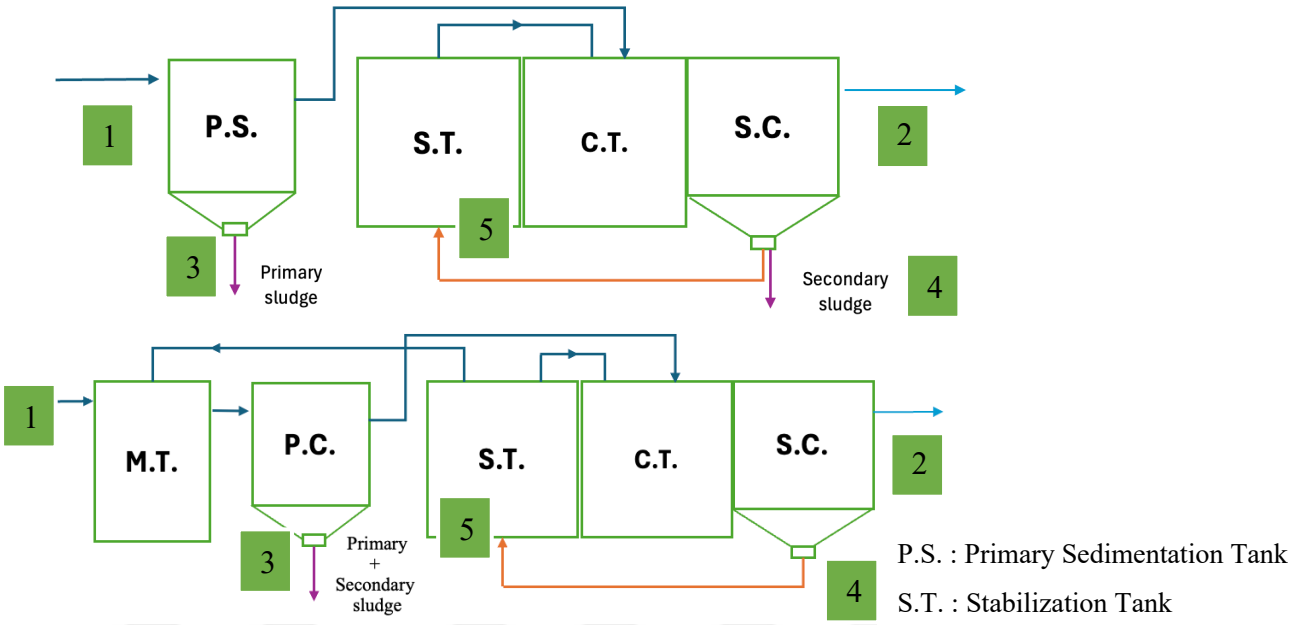


Figure 2.5. The collection points of samples of pilot-scale HiCS

To investigate the partitioning of OMPs into the sludge phase, sludge samples were collected from different stages of the process. A combined primary and secondary sludge sample (3) was obtained from the primary sedimentation unit, representing the fraction of OMPs associated with settleable solids. In addition, secondary sludge samples (4) were collected from the secondary clarifier to evaluate the distribution of OMPs within the biological sludge phase. Stabilization sludge samples (5) were taken from the stabilization tank to assess the transformation and potential accumulation of OMPs under stabilization conditions.

These sampling points enabled a comprehensive assessment of phase distribution and facilitated the identification of dominant removal pathways, including sorption and biotransformation, throughout the treatment process. As summarized in Table 2.1, the pilot-scale HiCS system was operated under different SRTs and process configurations throughout the study period. The operational periods represent the time intervals during which a given set of operating conditions was maintained.

Table 2.1. Operational periods and operating conditions of the pilot-scale HiCS system

Date	Operation Period	SRT (day)	Configuration
Dec. 21- Jan 22	12-60	2	Conventional
Feb. 22 - April 22	60-148	1	Conventional
May 22 - Jan 23	214- 422	1	Modified
Feb 23 - Apr. 23	422- 533	2	Conventional
May 23 -August 23	533-653	2	Modified
Sep. 23- Sep 23	653-673	0.75	Modified
Sep. 23- Oct 23	673-709	0.5	Modified
Nov. 23 - Jan 24	709- 793	2	Modified

2.3.Lab-Scale Anaerobic Reactor

Laboratory-scale continuously stirred anaerobic digester with a working volume of 15 L was operated for 380 days under mesophilic conditions (36 ± 1 °C), shown in Figure 2.6. For reactor start-up, anaerobic inoculum was obtained from an actively operating laboratory-scale mesophilic anaerobic digester to establish stable microbial activity under anaerobic conditions. Following the inoculation period, the reactor was fed daily with 1 L of HiCS + primary sludge (PS). The reactor was operated in semi-continuous mode and continuously mixed to ensure homogeneous conditions. The key operational parameters monitored during the operation of the lab-scale anaerobic digester are presented in Table 2.2.

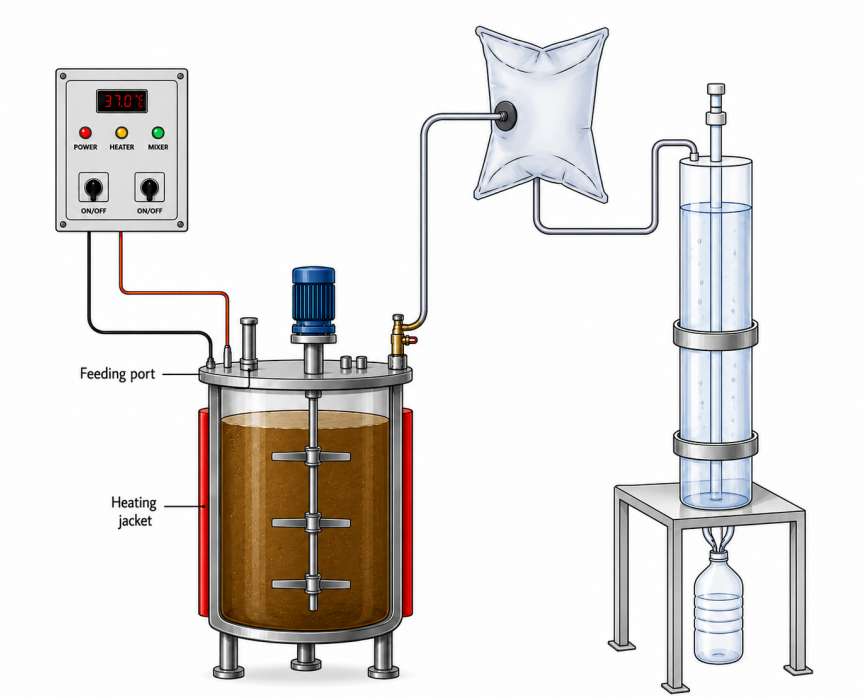


Figure 2.6. Lab-scale Anaerobic Digestion System

Table 2.2. Operational parameters in anaerobic digester

Temperature, ^{0}C	36 ± 1
SRT, days	15
VS/TS	71% ± 8
VS, g/L	26.4 ± 16
VFA, mg COD/L	175 ± 40
pH	7.3 ± 0.1
OLR, g VS/ L/d	1.59 ± 0.4
Alkalinity, mg/ L $CaCO_3$	5013 ± 1015

The feed sludge consisted of primary sludge (PS) and HiCS stabilization sludge obtained from the pilot-scale HiCS system. Sludge was not dewatered prior to feeding and was used in its original form. Since sludge was generated in a continuously operated pilot-

scale system treating real wastewater, its characteristics varied throughout the study period. Therefore, TS and VS concentrations were determined for each newly collected sludge batch and the daily feeding volume was adjusted accordingly to maintain a target OLR of approximately 1.5 g VS/L·d. During operation of the conventional HiCS configuration, primary sludge and stabilization sludge were collected separately and combined prior to feeding. Following implementation of the modified HiCS configuration, a combined sludge stream was collected from a single sampling point and used directly as digester feed.

Anaerobic digestion experiments were conducted in three separate 15-day experimental runs. During each set, the reactor was fed daily with the same mixture of HiCS sludge and primary sludge. The feed sludge was generated during operation of the pilot-scale HiCS system at SRTs of 2 and 0.5 days, thereby incorporating sludge produced under contrasting upstream operating conditions. Influent and effluent samples were collected on days 5, 10, and 15 of each set and analyzed for 10 OMPs using GC–MS.

Table 2.3. Characteristics of the sludge and operating conditions for the three anaerobic digestion sampling sets

Parameter	SET 1	SET 2	SET 3
Reactor sampling day	115	293	352
HiCS configuration	Conventional	Modified	Modified
HiCS SRT (d)	2	2	2
Sludge feeding strategy	Separate PS + WAS	Combined sludge	Combined sludge
Influent TS (g/L)	65.5	62.9	65.5
VS/TS	0.8	0.7	0.7
Total EPS-WAS (mg COD/g VS)	60.9	90.7	92.3

The characteristics of the sludge used during each experimental set are presented in Table 2.3. Although all sets were operated at a HiCS SRT of 2 days, the sludge originated from

different HiCS configurations and exhibited differences in solids content and EPS concentrations.

2.4. Organic Micropollutants Measurements Methods

Quantitative analyses of OMPs were performed using gas chromatography–mass spectrometry (GC–MS). Sample extracts were analyzed with a Shimadzu GCMS-QP2010 gas chromatograph coupled to a mass spectrometer (Shimadzu, Japan).

2.4.1. Liquid Samples Measurement

Before GC–MS analysis, the wastewater samples were subjected to pretreatment to purify and concentrate OMPs. For this purpose, the samples were processed using a solid-phase extraction (SPE) system (Thermo Scientific Dionex AutoTrace 280 Automated Large Volume SPE). Influent and effluent samples collected from the pilot plant were first passed through a coarse filter and then through a 0.45-µm PTFE membrane to remove suspended solids. This filtration step was essential, as particulate matter could otherwise clog both the SPE cartridges and the GC–MS column.

Sample preparation steps applied to the pilot plant influent and effluent wastewater samples;

- Filtered wastewater samples (500 mL) were subjected to SPE (Figure 2.7).



Figure 2.7. Solid Phase Extraction (SPE) Device

- Analytes retained on the SolEx C18 SPE cartridge were eluted with 10 mL of solvent, and the eluates were evaporated to complete dryness under a gentle stream of dry air (Figure 2.8).

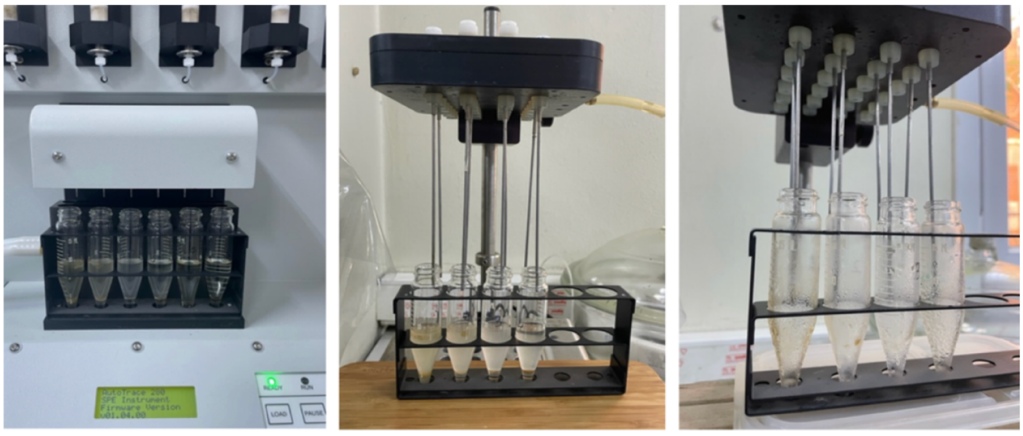


Figure 2.8. (a) Wastewater samples following SPE, (b) air-drying, and (c) dried samples.

- The dried extracts were reconstituted with 1 mL of acetone and sonicated for 15 minutes to ensure complete dissolution.
- After SPE, a surrogate standard solution (100 µL of anthracene-D10) was added to each sample, followed by incubation at 60 °C until dryness (Figure 2.9).

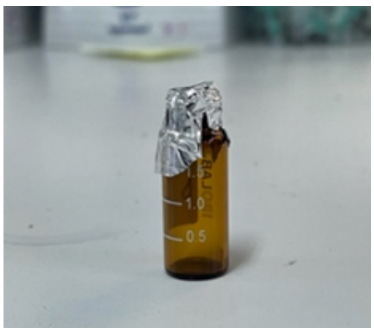


Figure 2.9. Reconstitution in 1 mL acetone followed by drying in a GC vial at 60°C.

- The dried residues were then reconstituted with 100 µL of acetone and sonicated for another 15 minutes in the GC vial to ensure full dissolution.
- Finally, 100 µL of the resulting acetone extract was transferred into a glass micro-insert and prepared for GC–MS analysis.

Wastewater Sample Preparation For GC-MS Analysis

500 mL pre-filtered wastewater sample is prepared for extraction.
Volume reduction is performed using a SPE device
Eluate is completely dried with dry air.
Sample volume is reduced to 10 mL using an SPE device, with methanol as the eluent.
Dried residue is reconstituted in 1 mL acetone and 100 µL surrogate solution (anthracene), followed by ultrasonic treatment for 15 minutes.
Sample is transferred to an amber GC vial and dried at 60°C.
Dried sample is reconstituted in 100 µL acetone, followed by ultrasonic treatment for 15 minutes.
Prepared sample is analyzed using GC-MS

2.4.2. Sludge Samples Measurement

To quantify micropollutant concentrations in the primary sludge, stabilization sludge, and secondary sludge of the pilot plant accurately and reliably, several sample-preparation methods were tested. Because sludge samples contain high levels of organic matter, SPE was also applied to remove organic matter from the matrix and improve the clarity of the extracts. After multiple trials, the most suitable procedure was selected, and the final steps used for sludge samples are described in detail below.

For the analysis of sludge, a two-step preparation procedure, solid–liquid extraction followed by SPE, was applied. Mixed liquor collected from the pilot plant was first placed in a beaker and allowed to settle for a short period, which was sufficient for solid–liquid separation. After removing the supernatant, the settled solids were prepared for subsequent GC–MS analysis.



Figure 2.10. Sludge sample for drying step

For the drying step, instead of freeze-drying (lyophilization), an alternative method reported in the literature was adopted. Following Samaras et al., (2011), the sludge samples (shown in Figure 2.10) were dried in an incubator at 60 °C for 48 hours. The dried sample is shown in Figure 2.11.

Following the drying step, 5 mL of methanol was added, and the sample was sonicated for 15 minutes in an ultrasonic bath. It was then centrifuged at 3000 rpm for 20 minutes, and this procedure was repeated three times. The methanol phase obtained from each cycle was collected in a 15 mL glass vial, and this extract was filtered through a 0.2 µm PTFE syringe filter to remove remaining particulates.



Figure 2.11. Dried Sample

The filtered methanol was completely evaporated at 60 °C, and the resulting residue was reconstituted in 50 mL of deionized water prior to SPE. Solid-phase extraction was then performed, after which the analytical workflow used for the liquid samples was applied.

Sludge Sample Preparation For GC-MS Analysis

1 L sludge sample is allowed to settle for 15 minutes.
Solid phase is separated and dried in an incubator at 60°C for 48 hours.
1.5 g of dried solid sample is sonicated in 5 mL methanol for 15 minutes in an ultrasonic bath
Sample is centrifuged at 3000 rpm for 20 minutes.
Liquid phase is collected, and the extraction is repeated three times with 5 mL methanol per cycle.
Combined liquid extract is filtered through a 0.2 µm PTFE syringe filter.
Filtrate is evaporated to dryness at 60°C in an incubator.
Dried residue is reconstituted in 50 mL deionized water and sonicated in an ultrasonic bath for 15 minutes.
Sample volume is reduced to 10 mL using an SPE device, with methanol as the eluent.
Sample is completely dried with dry air.
Dried residue is reconstituted in 1 mL acetone and 100 µL surrogate solution (anthracene), followed by ultrasonic treatment for 15 minutes.
Sample is transferred to an amber GC vial and dried at 60°C.
Residue is reconstituted in 100 µL acetone, followed by ultrasonic treatment for 15 minutes.
Prepared sample is analyzed using GC-MS.

2.4.3. Calibration and Validation of GCMS analysis

Analytical methods were developed for the quantitative determination of 11 OMPs in pilot-plant wastewater. The target compounds included IBU, DICLO, CBZ, E1, E2, E3,

EE2, BPA, NP, OP, CIPRO. Analyses were performed using a Shimadzu GCMS-QP2010 equipped with a TRB-5 MS capillary column. The oven temperature program was optimized for the separation of the selected OMPs, and the characteristic mass ions (m/z) used for quantification were identified. The optimized operating conditions of the Shimadzu GCMS-QP2010 system are presented in Table 2.2. The mass spectrometer was operated in selected ion monitoring (SIM) and scan modes, and the characteristic mass ions (m/z) determined for the target micropollutants are presented in Table 2.2.

Table 2.4. GC-MS Operating Conditions

GC	Column:	TRB-5MS (30 m x 0,25 mm x 0,25 µm)
	Injection temperature:	280 °C
	Injection mode:	Splitless
	Helium gas flow rate:	1.5 ml/min
	Sample volume:	1 µl
	Control mode:	Linear (37.5 cm/s)
	Oven temperature program	120 °C (1 min) → (10 °C /min) → 190 °C (1 min) → (5 °C /min) → 250 °C → (10 °C /min) → 290 °C (5 min)
	Injection unit:	250 kPa (1 min)
MS	Ion source temperature:	250 °C
	Interface temperature:	280 °C
	Measurement mode	4.00-4.50 min: Scan (0.50 s) 4.50-10.00 min: Scan (0.30 s) 4.50-10.00 min: SIM (0.20 s) m/z: 56; 106; 107; 119; 135; 161; 163; 178; 188; 206; 213; 220; 228; 245 10.00-18.00 min: Scan (0.30 s) 10.00-18.00 min: SIM (0.20 s) m/z: 56; 119; 141; 178; 188; 193; 163; 206; 213; 214; 228; 236; 242; 245; 250; 288; 296; 331; 332 18.00-29.00 min: Scan (0.30 s) 18.00-29.00 min: SIM (0.25 s) m/z: 141; 145; 146; 156; 160; 185; 206; 213; 214; 245; 250; 270; 272; 288; 296; 331; 332

Preparation of Calibration Standards and Calibration Curves

Reference standards of each organic micropollutant were obtained for use in the quantitative analyses. Appropriate amounts of the standards were dissolved in 10 mL of GC-grade acetone to prepare 1000 ppm stock solutions. These stock solutions were then diluted with GC-grade acetone to obtain calibration standards at the concentrations listed in Table 2.3. For the calibration procedure, injected mass values (picograms, pg) were used instead of solution concentrations. Expressing GC-MS responses in terms of injected mass simplifies the calculation of analyte concentrations in wastewater or sludge samples, particularly when extraction and concentration steps are applied.

Table 2.5. Calibration Standards

Extra-Low Calibration Standards (ng/mL)	OMP Mass in 1 µL (pg)	Low Calibration Standards (ng/mL)	OMP Mass in 1 µL (pg)	High Calibration Standards (ng/mL)	OMP Mass in 1 µL (pg)
10	50	500	500	2500	2500
25	250	1000	1000	5000	5000
50	500	2500	2500	10000	10000
100	1000	5000	5000	-	-

Calibration curves were plotted using the surrogate dilution technique. This approach is commonly used in methods involving several sample preparation steps, as it helps correct for potential analyte losses. For each calibration level, the ratio of the analyte peak area to the surrogate peak area was calculated and used for plotting the calibration curve. A constant amount of surrogate was added to all calibration standards, samples and blanks, ensuring that losses during sample preparation were directly accounted for in the final concentration calculations.

Calibration curves for low and high OMP mass ranges were obtained separately by performing separate injections. The calibration curves of the 11 OMPs quantified in the pilot-plant wastewater are presented below for the high (a) and low (b) concentration ranges in Appendix.

Analytical uncertainties related to solid-phase extraction (SPE) recovery and limits of quantification (LOQ) were considered in the interpretation of OMP concentrations. SPE recovery experiments were conducted to evaluate method performance by spiking target compounds into the sample matrix. SPE recovery represents the fraction of an analyte successfully extracted and detected after the extraction procedure relative to the amount initially introduced into the sample. Recovery was determined by comparing GC–MS responses of samples spiked prior to SPE extraction with those of samples spiked at the same concentration and directly analyzed without SPE. This approach provides an overall estimation of method performance, accounting for both extraction losses and matrix effects.

$$Recovery\ (\%) = \frac{Amount\ measured\ after\ SPE}{Initial\ amount\ in\ sample} \times 100 \qquad (1)$$

For recovery evaluation, duplicate samples were prepared both prior to and after SPE extraction, and each replicate was analyzed in duplicate by GC–MS. Recovery calculations were based on duplicate sample preparation and duplicate instrumental measurements and were evaluated using both absolute peak areas and area ratios to improve data reliability.

Table 2.6. SPE Recovery Parameters

SPE RECOVERY	
BPA	0.57
CIPRO	0.50
NP	0.59
OP	1.00
E1	0.87
E2	1.03
EE2	1.28
IBU	0.52
DICLO	0.47
CBZ	0.63

As shown in Table 2.4., compound-specific recovery values ranged between 0.47 and 1.28, indicating overall acceptable analytical performance across the target compounds. Recoveries slightly exceeding unity were attributed to matrix effects and analytical variability. Detailed analytical performance parameters, including limits of quantification (LOQ), are provided in Appendix.

2.5.Analytical Methods

VFAs were analyzed by gas chromatography, while pH, alkalinity, TS, and VS were measured according to Standard Methods (APHA, 2005). Biogas was collected in gas bags, and daily volume was recorded by water displacement method. As previously demonstrated by Mariné et al. (2012), gas bag collection provides a practical and reliable approach for biogas sampling, and its volume was measured daily using the water displacement method. The collected biogas was subsequently analyzed for its composition—particularly the methane (CH_4) percentage—using a gas chromatograph (Shimadzu GC-2014ATF, Shimadzu Corp., Kyoto, Japan) equipped with a thermal conductivity detector (TCD).

2.6. Spike-sorption experiments for Kd determination

Controlled laboratory batch sorption-spike experiments were conducted to determine the solid–liquid distribution coefficient (Kd) of selected OMPs. Batch reactors were prepared by spiking sludge suspensions at a constant solid concentration (0.5 g/L) with different initial concentrations of the target compounds in the range of 5–25 mg/L. For each concentration level, a corresponding blank sample without sludge was also prepared.

The reactors were mixed on an orbital shaker at 25 °C for two different contact times: 3.5 h, corresponding to the hydraulic retention time (HRT) of the HiCS system, and 24 h to represent equilibrium conditions. Following incubation, samples were filtered through 0.22 μm syringe filters to separate the aqueous phase. The residual OMP concentrations in the aqueous phase were quantified using GC–MS.

The amount of compound associated with sludge solids (q_e) was calculated according to:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (2)$$

where C_0 and C_e represent the initial and residual aqueous concentrations (mg/L), V is the solution volume (L), and m is the mass of sludge solids (g).

The sludge–water distribution coefficient (Kd) was determined from the linear relationship between the sorbed amount (q_e) and the aqueous concentration (C_e) according to:

$$q_e = K_d C_e \quad (3)$$

where K_d represents the solid–liquid distribution coefficient (L/g).

2.7. Calculation of Removal Efficiency and Mass Balance Analysis

The removal efficiency of each OMP was quantified on a mass-load basis, and the overall removal was determined using Equation 4.

$$R\ \% = \left(1 - \frac{M_{eff}}{M_{inf}}\right) \times 100 \quad (4)$$

where R % is the removal percentage of the target OMP, M_{inf} is the input mass flowrate of the OMP (ng/d), M_{eff} is the output mass flowrate of the OMP (ng/d).

The pilot-scale system was supplied with effluent from the grit chamber. While this pretreatment stage efficiently eliminates larger and heavier particles, finer materials such as silt, clay, and organic matter remain suspended in the wastewater. Consequently, OMPs entered the system in two forms: dissolved in the liquid phase and sorbed onto particulate matter. Despite the focus on liquid-phase analysis for influent characterization, the presence of suspended solids in the raw wastewater introduced a

significant OMP fraction that remained unaccounted for in the aqueous measurements. Consequently, the dissolved concentration ($C_{inf,L}$) provided an incomplete representation of the actual pollutant influx. To achieve a more rigorous quantification of the total influent concentration (C_{inf}), it was necessary to integrate the mass of OMPs partitioned onto the solid matrix. Therefore, the total influent mass load for each target compound was determined by applying Equation 5:

$$M_{inf} = Q_{inf} \times C_{inf,L} + Q_{inf} \times C_{inf,ads} \times TSS_{inf} \quad (5)$$

where Qinf is the influent wastewater flow rate in L/d, $C_{inf,L}$ is the liquid phase concentration of OMP in ng/L as quantified after GC-MS analysis, $C_{inf,ads}$ is the OMP concentration in the solid phase of the influent (ng/g), TSSinf is the total suspended solids content of the influent (g/L).

A critical filtration step using 0.45-µm membrane filters was performed to prepare the liquid matrices for OMP analysis. This pretreatment was essential to eliminate suspended matter that could otherwise interfere with the Solid Phase Extraction (SPE) process or compromise the sensitivity of GC–MS measurements. Within this framework, the filtrate represents the soluble OMP fraction ($C_{inf,L}$), while the retained particles account for the solid-associated fraction ($C_{inf,ads}$).

In the pilot-scale configuration, the primary settling tank served as the collection point for both settleable and suspended solids. Given that these solids originate from the raw influent, the micropollutant concentrations detected in the primary sludge were considered a reliable proxy for the OMP fraction initially adsorbed onto the incoming particulate matter. Consequently, $C_{inf,ads}$ was substituted with C_{PS} (ng/g), representing the OMP concentration measured in the primary sludge. Based on this correlation, Equation 6 was adapted for the HiCS process as follows:

$$M_{inf} = Q_{inf} \times C_{inf,L} + Q_{inf} \times C_{PS} \times TSS_{inf} \quad (6)$$

where CPS is the OMP concentration in the solid phase of the primary sludge in ng/g.

To validate the theoretical assumptions used in the mass balance, a specific sampling campaign was conducted to observe the behaviour of OMPs within the primary settling tank. Influent samples were collected and followed by effluent sampling after a duration corresponding to the HRT of the unit.

The analytical results indicated no significant deviation in the liquid-phase concentrations between the influent and effluent of the primary settler. This experimental evidence confirms that the reduction of OMP mass within this stage is not driven by biotransformation in the aqueous phase. Instead, the removal is primarily attributed to the physical separation of OMPs adsorbed onto settleable and suspended solids. These findings justify the substitution of within the mass balance equations, as the primary sludge effectively captures the particulate-bound fraction without altering the dissolved OMP load.

Under the Modified HiCS configuration, the system operates with a unified sludge discharge, where primary and secondary sludges are removed together. In this setup, the stabilized sludge is recycled to the head of the works, merging with the raw wastewater in the mixing tank prior to entering the treatment process. This integration necessitates a further revision of Equation 6 to accurately quantify the total influent OMP concentration.

Since the sludge extracted from the primary sedimentation tank comprises a blend of primary solids and returned Waste Activated Sludge (WAS), a direct characterization of the pure primary sludge fraction is not feasible. To overcome this, OMP concentrations were measured in the WAS samples collected from the stabilization tank. For calculation purposes, it was assumed that the OMP concentrations in both the recycled WAS and the primary sludge remain constant, as defined in Equation 4. By performing a mass balance specifically around the primary settling tank, the concentration of the primary sludge component was derived using the empirical measurements of both the combined discharged sludge and the recycled WAS. Consequently, the influent OMP concentration for the modified process was established by integrating the primary sludge assumption from Equation 7 into Equation 8, as shown below:

$$C_{PS+WAS} * Q_{PS+WAS} * TSS_{PS+WAS} = C_{WAS} * Q_{WAS} * X_{stab} + C_{PS}{}^{*}Q_{inf}{}^{*}TSS_{inf} \quad (7)$$

$$M_{inf} = Q_{inf} * C_{inf,L} + C_{PS+WAS} * Q_{PS+WAS} * TSS_{PS+WAS} - C_{WAS} * Q_{WAS} * X_{stab} \quad (8)$$

where C_{PS+WAS}inf is the total OMP concentration of discharged sludge from primary sedimentation tank (ng/L), C_{WAS} are the OMP concentration in the secondary sludge (ng/g), X_{stab} is the suspended solids content of stabilization tank (g/L), TSS_{PS+WAS} is the total suspended solids content of discharged sludge from primary sedimentation tank (g/L).

Mass Balance Expressions in Pilot-Scale HiCS

The operational behaviour of OMPs within the HiCS process was evaluated through a systematic mass balance approach. By accounting for the measured concentrations across all relevant process streams, this framework facilitates a precise distribution of each compound between the aqueous and solid phases. This methodology allows for the isolation of specific removal pathways; biotransformation and sorption to sludge, providing a robust analytical basis for comparing the OMP fate across both original and modified system configurations. Sampling locations for the influent, effluent, and sludge streams are detailed in the HiCS process flow diagram. The overall fate of the target compounds was quantified by integrating these measured values into a mass balance, as formulated in Equation 9.

In the modified HiCS setup, the system is designed with a single outlet for the combined discharge of primary and secondary sludges. To ensure this operational change was correctly reflected, the mass balance was adjusted as shown in Equation 10. This version of the calculation incorporates OMP concentrations from the influent, effluent, and the combined sludge stream (primary plus waste activated sludge), providing a precise representation of sorption-related removal at the shared withdrawal point.

HiCS

$$M_{inf} = M_{eff} + Q_{WAS}C_{WAS}X_{stab} + Q_{PS}C_{PS}TSS_{PS} + MF_{bio} \quad (9)$$

Modified HiCS

$$M_{inf} = M_{eff} + Q_{PS+WAS}C_{PS+WAS}TSS_{PS+WAS} + MF_{bio} \quad (10)$$

where MFbio is the OMP concentration, removed by biotransformation.

OMP Mass Quantifications in Lab-Scale Anaerobic Digester

Micropollutant assessment in the anaerobic digester was conducted under semi-continuous operating conditions using a completely mixed laboratory-scale anaerobic reactor with a working volume of 15 L and an SRT of 15 days. During operation, 1 L of reactor content was withdrawn daily and replaced with 1 L of fresh feed sludge in order to maintain a constant reactor volume and solids retention time. The reactor operation and sampling strategy are illustrated in Figure 2.12.

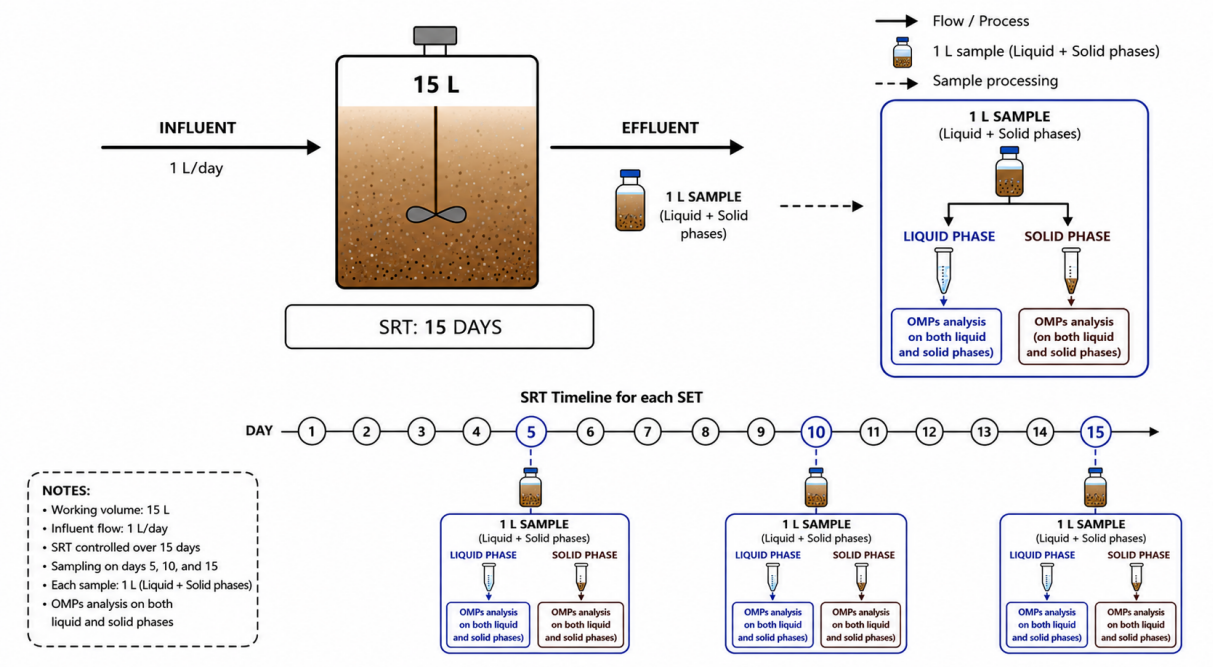


Figure 2.12. Schematic illustration of the anaerobic reactor operation and OMP sampling procedure

Direct comparison of a single influent sample with a single digestate sample may not adequately represent OMP behaviour in such systems, since the digestate composition reflects an integrated mixture of sludge entering the reactor over a period equivalent to the SRT rather than an instantaneous reactor response. Therefore, OMP evaluation was performed through three separate experimental sets, each covering a complete 15-day SRT period. During each set, the reactor was fed daily with the same sludge mixture, and samples were collected on days 0, 5, 10, and 15 to capture temporal variations in OMP behaviour during anaerobic digestion, as shown in Figure 2.12.

An apparent compound-specific mass balance approach was initially considered to evaluate potential OMP transformation within the anaerobic reactor. The general mass balance equation was expressed as:

$$M_{loss} = M_{reactor,0} + M_{in,total} - M_{out,total} - M_{reactor,15}$$

where $M_{reactor,0}$ is the initial OMP mass present in the reactor at the beginning of the experimental set (ng), $M_{in,total}$ is the cumulative OMP mass introduced during the experimental period (ng), $M_{out,total}$ is the cumulative OMP mass leaving the system during the experimental period (ng), and $M_{reactor,15}$ is the OMP mass remaining in the reactor at the end of the experimental period (ng).

The cumulative OMP mass leaving the reactor was estimated as:

$$M_{out,total} = = \sum_{t=1}^{15} M_{liquid,t} + M_{solid,t}$$

Direct measurements of OMP loads were available only on days 0, 5, 10, and 15. Therefore, estimation of cumulative output masses over the complete 15-day SRT period required assumptions regarding OMP behaviour during unsampled intervals. Intermediate daily OMP loads between sampling events were estimated by interpolation between adjacent measured values.

In addition, due to the semi-continuous completely mixed operation of the reactor, the reactor contents at the beginning of each experimental set reflected the cumulative

influence of sludge introduced during previous feeding cycles. Consequently, the initial reactor inventory and cumulative output masses may have been influenced not only by sludge introduced during the current experimental period but also by residual material retained from previous operational periods. Therefore, apparent mass balance calculations were considered exploratory and supportive rather than direct indicators of compound degradation.

Instead, OMP behaviour was primarily evaluated through a phase distribution approach based on measured loads in influent sludge, liquid phase, and solid phase.

Influent OMP loads were calculated as

$$M_{in} = C_{in} * TS_{in} * Q$$

where:

M_{in} = influent OMP load (ng/day)

C_{in} = OMP concentration in influent sludge (ng/g)

TS_{in} = total solids concentration of influent sludge (g/L)

Q = daily sludge feed rate (L/day)

OMP loads associated with the liquid phase were calculated as:

$$M_{liquid} = C_{eff,L} * Q * f_{liquid}$$

where:

M_{liquid} = OMP load associated with the liquid phase (ng/day)

$C_{eff,L}$ = OMP concentration in the liquid phase (ng/L)

f_{liquid} = liquid volume correction factor

OMP loads associated with the solid phase were calculated as:

$$M_{solid} = C_{eff,S} * TS_{eff} * Q$$

where:

M_{solid} = OMP load associated with the solid phase (ng/day)

$C_{eff,S}$ = OMP concentration in digestate solids (ng/g)

TS_{eff} = total solids concentration of digestate (g/L)

The relative distribution of OMPs between liquid and solid phases was subsequently evaluated to assess temporal changes in compound partitioning and solid-phase retention behaviour during anaerobic digestion. This approach enabled interpretation of OMP fate in terms of retention and redistribution between phases rather than direct degradation efficiencies.

3. RESULTS AND DISCUSSIONS

3.1. The Characterization of Influent wastewater

The characterization of influent wastewater was based on regular sampling conducted at the pilot-scale treatment plant. Wastewater samples were collected monthly from January 2022 to January 2024 for the analysis of OMPs. The influent wastewater characterization based on conventional parameters is summarized in Table 3.1.

Table 3.1. Characterization of wastewater

Parameter	***Range***	***Average***
COD (mg/l)	376 - 717	580
TSS (mg/l)	222 - 385	310
TN (mg/l)	41 - 76	60
TP(mg/l)	3 - 7	5
NH4-N(mg/l)	24 - 48	36
VSS (mg/l)	149 - 293	230
pH	7.5 - 7.8	7.6
Temperature (^{0}C)	12.2 -24.2	18.3

3.1.1. Influent Organic Micropollutant Characterization

The concentrations of OMPs in the influent wastewater exhibited considerable variability depending on compound type and seasonal conditions. Among the investigated compounds, IBU was the most abundant, with concentrations ranging from 456 to 4119 ng/L. In contrast, relatively lower concentrations were observed for E1 (106–259 ng/L), E2 (145–311 ng/L), and CBZ (89–334 ng/L).

The concentration ranges of the remaining compounds were as follows: BPA (112–586 ng/L), CIPRO (48–2296 ng/L), NP (96–4812 ng/L), OP (101–1644 ng/L), EE2 (200–901 ng/L), and DICLO (61–441 ng/L).

Seasonal variations in influent OMP concentrations are presented in Figure 3.1. Natural and synthetic estrogens showed relatively stable concentrations throughout the monitoring period. In contrast, pharmaceutical compounds exhibited pronounced seasonal variability, with IBU concentrations peaking during autumn and winter and decreasing in spring and summer. Alkylphenols and bisphenols showed fluctuating concentration patterns, which may reflect variations in industrial discharge and usage patterns.

The observed variability in influent composition is expected to influence the removal behaviour of OMPs in the HiCS system, particularly with respect to the relative contribution of sorption and biotransformation processes.

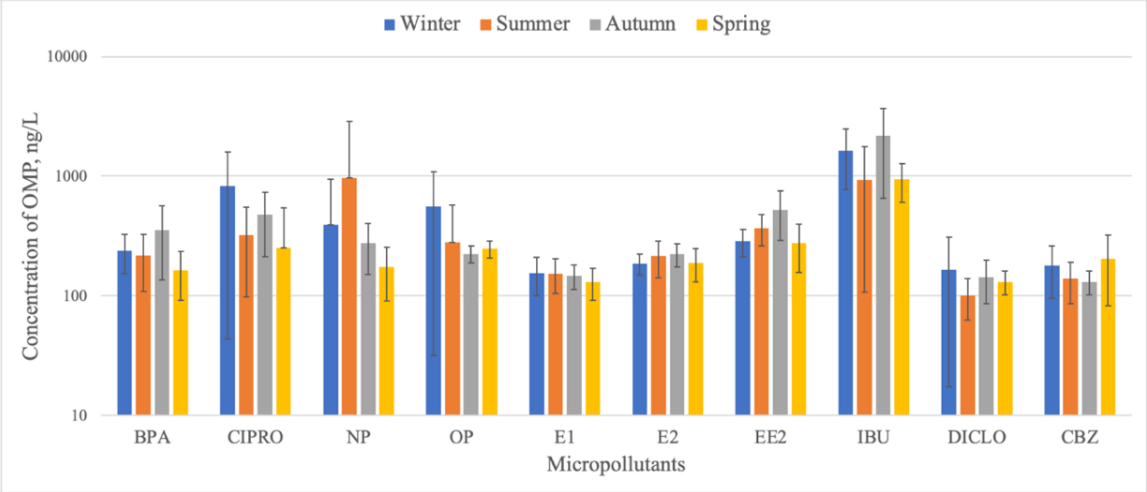


Figure 3.1. Seasonal variations in influent OMP concentrations

3.2. Performance of HiCS processes

The removal performance of the pilot-scale HiCS system was evaluated for selected OMPs under both conventional and modified operating conditions. During conventional HiCS operation, removal efficiencies varied depending on micropollutants and their physicochemical characteristics. Among phenolic compounds, NP showed the highest

removal efficiency with 72 ± 10%, while BPA and OP were removed at 59 ± 7% and 55 ± 15%, respectively. For natural and synthetic hormones, E1 and E2 exhibited similar removal efficiencies of 38 ± 16% and 38 ± 14%, whereas EE2 showed the lowest removal efficiency in this group, with 27 ± 17%. Among pharmaceuticals, CIPRO and IBU showed comparatively high removal efficiencies of 84 ± 18% and 74 ± 13%, respectively. DICLO was removed at 61 ± 18%, while CBZ exhibited the lowest removal efficiency among the pharmaceuticals, with 23 ± 21%.

Although E3 was monitored during the study, its concentrations were consistently below the limit of quantification (LOQ), and therefore it was not included in further evaluation.

In the modified HiCS configuration, sludge recirculation was introduced to the primary sedimentation tank. Under these conditions, phenolic compounds were removed at 44 ± 19% for BPA, 48 ± 22% for NP, and 52 ± 23% for OP. For hormones, the removal efficiencies were 45 ± 26% for E1, 34 ± 12% for E2, and 39 ± 19% for EE2. Among pharmaceuticals, CIPRO, IBU, DICLO, and CBZ showed removal efficiencies of 73 ± 28%, 55 ± 24%, 47 ± 18%, and 45 ± 18%, respectively. The removal efficiencies of the target OMPs under HiCS and modified HiCS configurations are presented in Figure 3.2.

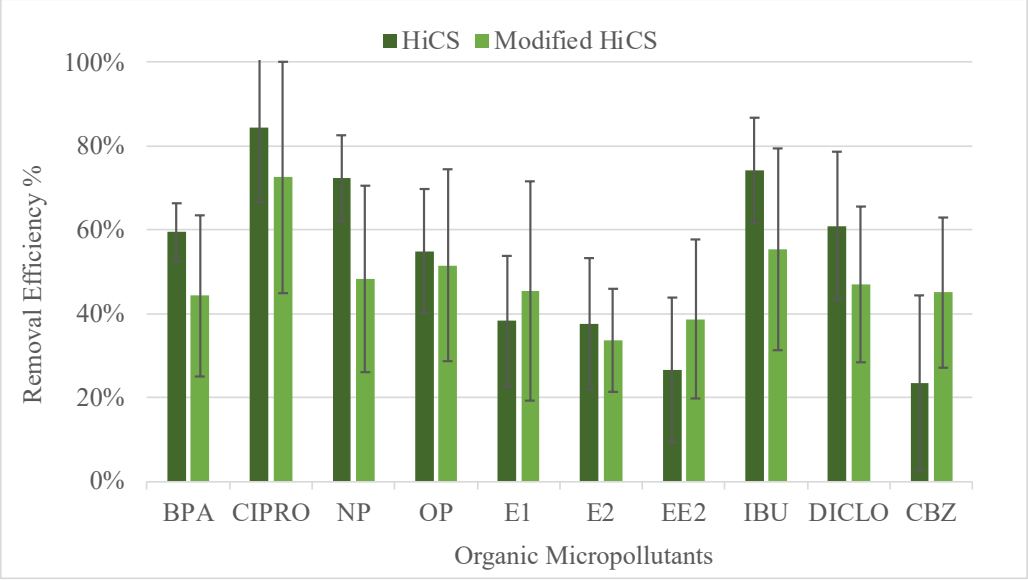


Figure 3.2. Removal Efficiency of OMPs in HiCS and Modified HiCS Processes

Overall, the results indicate that OMP removal in the pilot-scale HiCS system is compound-specific. Some compounds, such as CIPRO, IBU, and NP, exhibited higher removal efficiencies, whereas others, such as EE2 and CBZ, were only partially removed in both configurations. Furthermore, despite modifications to the HiCS process, removal efficiencies generally remained within a similar range. This indicates that, under high-rate conditions, OMP removal is not solely governed by overall system efficiency but is strongly influenced by compound-specific properties and dynamic operational conditions.

Over the two-year operational period of the pilot-scale system, variations in operational conditions, seasonal temperature fluctuations, and changes in influent wastewater composition were continuously observed. As a result, occasional negative removal efficiencies were detected for some OMPs, reflecting the non-steady-state nature of pilot-scale high-rate activated sludge operation. Seasonal variations in influent composition and temperature may contribute to this phenomenon. It should also be noted that seasonal variations may influence the influent composition and the initial distribution of OMPs entering the system. While temperature fluctuations can affect both microbial activity and physicochemical partitioning, the direct influence of temperature on OMP removal kinetics in HiCS systems remains an area that requires further long-term investigation

Temperature can influence sorption equilibria, microbial activity, and mass transfer processes, thereby affecting the partitioning and biotransformation behaviour of OMPs. Under such conditions, compounds that were previously associated with sludge solids may be partially released back into the aqueous phase, leading to temporary increases in measured effluent concentrations. These observations indicate that OMP concentrations in the effluent of high-rate systems cannot always be interpreted solely in terms of steady-state removal performance. Instead, they may reflect transient variations resulting from changes in influent characterization, operational conditions, and microbial activity. Consequently, the occasional negative removal efficiencies observed in this study were interpreted as manifestations of the dynamic interactions between sorption, desorption, and biotransformation processes within the system. Overall, the results suggest that OMP removal in the pilot-scale HiCS system was largely compound-specific and influenced by the operational characteristics of the high-rate process.

3.3. Mechanistic Assessment of OMP Removal: Sorption vs. Biotransformation

In the HiCS systems, the fate of OMPs is primarily governed by the relative contribution of sorption and biotransformation processes. The dominance of these mechanisms depends both on the operational characteristics of the treatment system and on compound-specific physicochemical properties. In particular, hydrophobicity, commonly expressed by log Kow, plays a key role in determining whether a compound remains in the aqueous phase or partitions onto sludge solids.

In this study, the dominant removal pathways were evaluated using the mass balance approach, detailed in Section 2.7, which enabled the distribution of OMPs between the aqueous phase and sludge solids to be quantified. The resulting phase distribution was subsequently interpreted in relation to the physicochemical properties of the investigated compounds, particularly hydrophobicity and aqueous solubility.

Table 3.2. Solubility and log K_{OW} of the target OMPs.

Micropollutant	Solubility (mg/L)	Log K_{OW}	Ref.
BPA	300[a]	3.4[a]	[a] (European Commission, 2021)
CIPRO	30000[b]	0.28[c]	[b] (Sodhi and Singh, 2021), [c] (Venegas et al., 2021)
NP	5.43[d]	4.48[e]	[d] (Ahel and Giger, 1993a), [e] (Ahel and Giger, 1993b)
OP	12.6[d]	4.12[e]	[d](Ahel and Giger, 1993a), [e] (Ahel and Giger, 1993b)
E1	13[f]	3.43[f]	[f] (Ying et al., 2002)
E2	13[f]	3.94[f]	[f] (Ying et al., 2002)
EE2	4.8[f]	4.15[f]	[f] (Ying et al., 2002)
IBU	173[g]	3.5[g]	[g] (Wu et al., 2023)
DICLO	17.8[h]	4.51[i]	[h] (Fini et al., 1999), [i] (Díaz-Gamboa et al., 2024)
CBZ	17.7[j]	2.25[i]	[i] (Díaz-Gamboa et al., 2024), [j] (Zhang et al., 2008)

The fate and transport of OMPs within treatment systems are strongly influenced by their partitioning behaviour between the aqueous and solid phases, often described by the log Kow. Compounds with log Kow values below 2.5 typically exhibit low sorption potential, values between 2.5 and 4 indicate moderate sorption potential, and compounds with log Kow values above 4 are generally considered strongly hydrophobic and therefore prone to partitioning into the solid phase (Luo et al., 2014). The solubility and log Kow values of the investigated compounds are summarized in Table 3.2.

The mass balance analysis indicated that micropollutants with log Kow > 4 (e.g., NP, OP, and DICLO, shown in Table 3.2.) were predominantly associated with the solid phase, highlighting the importance of sorption processes in high-rate treatment systems where HRTs are limited. Similar behaviour has been reported in HRAS systems. Taboada-Santos et al., (2020b) demonstrated that under low sludge retention times (0.5–2 d) biotransformation pathways are constrained, whereas the relatively high EPS content characteristic of HiCS biomass provides an effective matrix for the retention of hydrophobic micropollutants. In cases where both sorption potential and biotransformation rates are low, a common scenario for hydrophilic and recalcitrant compounds under short SRT conditions, the HiCS process exhibits limited removal may occur, resulting in the transport of these compounds through the system largely unchanged. Based on this framework, the removal mechanisms of the investigated OMPs were evaluated under two operational configurations of the pilot-scale system: HiCS operation and modified HiCS operation.

3.3.1. Behaviour of OMP removal under HiCS operation

The fate of OMPs in the HiCS system is illustrated in Figure 3.3a. The distribution of removal pathways indicates that the dominant mechanisms varied considerably among compound groups. In general, the low SRT characteristic of the HiCS process limits the establishment of specialized microbial communities responsible for the biotransformation of many micropollutants. As a result, removal mechanisms are largely influenced by compound-specific physicochemical properties, particularly hydrophobicity and aqueous solubility. Under such conditions, sorption to sludge solids

becomes a crucial removal pathway, while biotransformation remains significant only for compounds with high biodegradability.

The interpretation of OMP removal pathways was supported by compound-specific mass balance calculations. By quantifying the distribution of OMPs between the liquid phase and sludge solids, the relative contributions of sorption and biotransformation were distinguished, enabling a mechanistic interpretation of compound-specific behaviour under HiCS operation rather than relying solely on overall removal efficiencies.

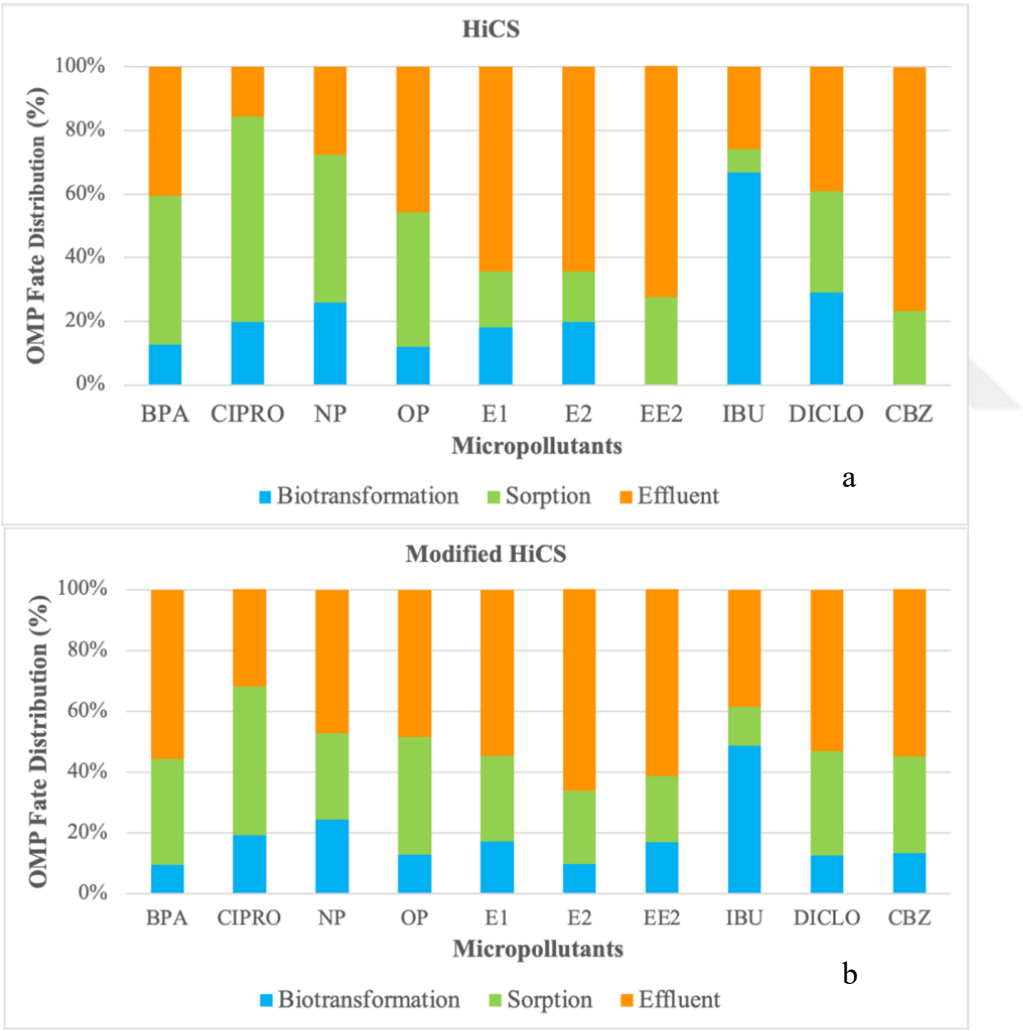


Figure 3.3. Contribution of Removal Pathways to Micropollutant Fate in a) HiCS and b) Modified HiCS

Phenolic compounds

Phenolic OMPs in this study included BPA, NP, and OP, which are widely used industrial chemicals. Their behaviour in the HiCS process reflected the combined influence of sorption and biotransformation potential. BPA has a log Kow value of 3.4 and is therefore considered moderately hydrophobic. In conventional wastewater treatment plants operating at long SRTs, BPA removal efficiencies of approximately 84% have been reported, largely attributed to biotransformation under nitrifying conditions (Melcer and Klečka, 2011). However, in high-rate systems such as HiCS, the short SRT limits the development of microbial populations capable of sustaining extensive metabolic pathways. Consequently, the approximately 60% removal observed in the present study was mainly associated with sorption processes. The alkylphenols NP and OP exhibited higher hydrophobicity and lower aqueous solubility (Table 3.2), which promotes their association with sludge solids. In the present study, average removal efficiencies of 72 ± 10% for NP and 55 ± 15% for OP were observed. Mass balance analysis indicated that sorption was the dominant removal pathway for OP, contributing approximately 46% of the total removal. In contrast, NP removal involved contributions from both sorption and biotransformation. Literature studies show that NP, as a hydrophobic pollutant, is primarily removed through sorption but can also undergo biotransformation, depending on oxygen supply and bioavailability. The fate of NP is significantly affected by environmental and chemical factors, especially aeration and temperature (Soares et al., 2008). Previous studies have reported variable NP removal efficiencies under different treatment configurations. For example, investigations on municipal wastewater treatment systems in Austria reported NP removals in the range of 50–80%, with biotransformation accounting for a considerable fraction of the overall removal (Clara et al., 2012). Similarly, Koumaki et al. (2021) observed an average NP removal of approximately 48% in a lab-scale HiCS system and identified biotransformation as a major removal mechanism. Compared with NP, OP removal has been less frequently investigated in wastewater treatment systems, limiting direct comparisons with literature data.

Pharmaceutical compounds

The pharmaceutical compounds investigated in this study displayed highly variable removal behaviour depending on their biodegradability and affinity for sludge solids. IBU showed the highest biotransformation potential among the investigated pharmaceuticals. Based on its physicochemical characteristics, IBU can be classified as a moderately hydrophobic compound. In this study, total IBU removal reached up to 96%, with an average removal efficiency of 74 ± 13%. Sorption contributed only marginally to this removal, accounting for approximately 1% in waste activated sludge and 5% in primary sludge. These results are consistent with previous studies indicating that IBU is readily biotransformed, with removal efficiencies typically exceeding 80% (Alvarino et al., 2014; Jia et al., 2020). The relatively high removal of IBU suggests that certain pharmaceuticals remain susceptible to rapid heterotrophic degradation even in high-rate treatment systems, possibly stimulated by the elevated F/M ratios characteristic of A-stage processes. In contrast, CBZ exhibited persistent behaviour. CBZ is characterized by relatively low-to-moderate hydrophobicity and moderate aqueous solubility, which are generally associated with weak sorption potential and limited biotransformation. Consistent with these properties, CBZ removal remained low, averaging 23 ± 21%, and was mainly attributed to sorption. DICLO showed readily removal behaviour with an overall efficiency of 61%, where 29% was attributed to biotransformation. Although DICLO is characterized by relatively high hydrophobicity (log Kow =4.51), its removal cannot be explained solely by sorption, indicating that partial biotransformation occurred even under HiCS process. This aligns with the broad range of DICLO removal efficiencies reported in the literature, which vary significantly from 0% to 80% across different systems (Zhang et al., 2008). The 29% biotransformation observed in this study is consistent with previous findings under HiCS operation; for instance, Koumaki et al., (2021) reported comparable biotransformation levels for DICLO in a pilot-scale HiCS system. These findings suggest that, although DICLO removal is inherently variable, the HiCS process can support measurable biotransformation beyond the limited removal typically expected under short SRT conditions.

The antibiotic ciprofloxacin (CIPRO) exhibited removal behaviour strongly dominated by sorption processes. Although CIPRO is highly soluble in water and relatively hydrophilic, it shows strong interactions with wastewater solids. This behaviour is attributed to electrostatic interactions between CIPRO molecules and EPS present in activated sludge. Since EPS matrices generally carry negative surface charges, the zwitterionic form of CIPRO at neutral pH can strongly interact with these sites (Zhang et al., 2018). As a result, CIPRO exhibited the highest removal efficiency in this study (84 ± 18%), primarily associated with sorption.

Hormonal compounds

Natural estrogens E1 and E2 are hydrophobic compounds and are typically removed through a combination of sorption and biotransformation. In conventional systems, removal often exceeds 90% via biotransformation (Joss et al., 2004; Andersen et al., 2005). However, under the short SRT conditions of the HiCS system, removal efficiencies were lower and more variable, with both E1 and E2 exhibiting average removals of approximately 38%. This behaviour can be attributed not only to the short SRT but also to the limited development of nitrifying bacteria, which play a key role in the biotransformation of many OMPs (Fernandez-Fontaina et al., 2016), as well as to the low HRT that kinetically constrains these processes (Taboada-Santos et al., 2020b).

Previous studies have shown that natural estrogens (E1 and E2) are removed via two mechanisms: sorption to sludge and biotransformation (Clara et al., 2004a). As a hydrophobic organic compound, its sorption onto sludge is expected to be a key removal mechanism (Silva et al., 2012). In CAS systems sorption is often considered an initial partitioning step that facilitates subsequent biotransformation within the biomass rather than a dominant final removal pathway (Clara et al., 2004a). In the HiCS process, sorption was initially expected to contribute to estrogen removal because of the physicochemical properties of these compounds. However, sorption behaviour in high-rate systems may differ from that observed in CAS systems because of differences in sludge characteristics and operating conditions, including lower MLSS concentrations and shorter contact times, while short SRT conditions may further restrict biotransformation processes. In addition, sorption is a dynamic and reversible process,

and desorption may occur depending on equilibrium conditions and environmental factors such as pH, temperature, and organic matter content (Silva et al., 2012). Consequently, both sorption and biotransformation may be kinetically constrained under HiCS conditions, contributing to the lower estrogen removal efficiencies observed in this study.

The synthetic estrogen, EE2, exhibits properties similar to other estrogens, as shown in Table 3.2. As a hydrophobic organic compound, its sorption onto sludge is expected to be a key removal mechanism (Ying et al., 2002). However, studies on removal of EE2 show conflicting results. Some research supports sorption as the dominant removal pathway for EE2 (Urase and Kikuta, 2005), while Andersen et al. (2005)found that biotransformation played a more important role in EE2 removal in sewage treatment plants. In this study, sorption was identified as the dominant removal mechanism for EE2, achieving a total removal efficiency of up to 23%. This behaviour is consistent with the short SRT conditions of the HiCS system, which limit biotransformation processes and increase the relative importance of sorption.

The results indicate that OMP removal in the HiCS process differs substantially from conventional activated sludge systems. While long SRT systems favour extensive biotransformation, the short SRT conditions characteristic of HiCS restrict such processes and increase the relative importance of sorption-driven removal pathways. This suggests that, under short SRT conditions characteristic of HiCS systems, sorption-driven mechanisms become increasingly dominant, while biotransformation remains limited to compounds with high biodegradability.

Overall, the findings of this study demonstrate clear differences in OMP removal behaviour in high-rate systems compared to conventional activated sludge and lab-scale studies. While CAS systems, with their long SRTs, rely heavily on biotransformation, the HiCS process tends to favour sorption-driven pathways due to its kinetic limitations, although this behaviour remains compound-specific. It is also important to note that the high variability and occasional negative removals observed here are often masked in idealized lab settings. These results suggest that while HiCS is effective for carbon redirection, its OMP removal performance is strongly influenced by the physicochemical

properties of the compounds, with biotransformation contributing to varying extents depending on compound characteristics. Consequently, the accumulation of OMPs in the sludge phase necessitates a detailed evaluation of their fate during subsequent sludge treatment processes.

3.3.2. Behaviour of OMP removal under modified HiCS operation

The modified HiCS configuration was implemented primarily to enhance carbon capture within the high-rate treatment stage, increasing the amount of organic matter diverted to sludge and thereby improving the potential for energy recovery during subsequent anaerobic digestion. To achieve this, secondary sludge was recirculated to the mixing tank, increasing both the suspended solids concentration and the available surface area for sorption processes. Although this modification was designed from a carbon diversion perspective, its influence on the removal behaviour of OMPs was also evaluated.

As shown in Figure 3.3b, the overall improvement in OMP removal efficiencies under the modified HiCS configuration remained limited. Sorption-based removal of E1, E2, IBU, DICLO, and CBZ increased moderately, with improvements ranging between 5% and 11%. Although differences in total removal efficiencies were observed for BPA, CIPRO, and NP between the HiCS and modified HiCS configurations, the considerable overlap of error bars presented in Figure 3.2 indicates that these differences were not statistically significant.

A notable observation was the increase in CBZ removal, despite the absence of a similar improvement for more hydrophobic compounds such as BPA and NP. This behaviour is likely associated with differences in EPS characteristics between the two operational configurations. The average total EPS concentrations measured in the HiCS and modified HiCS systems were comparable, at approximately 66.4 ± 10.3 mg L^{-1} and 67.6 ± 11.3 mg L^{-1}, respectively. EPS are complex macromolecular structures composed primarily of proteins and polysaccharides, and their composition strongly influences sludge properties such as floc structure, surface charge, and sorption capacity (Iorhemen et al., 2019).

Although the total EPS concentrations remained similar in both systems, differences were observed in the protein-to-polysaccharide ratio (PN/PS). In the HiCS configuration, the PN/PS ratio averaged 2.70 ± 0.51, whereas in the modified HiCS system this value increased to approximately 3.35 ± 0.68, indicating a relatively higher protein fraction in the EPS matrix. Changes in EPS composition may influence the interaction between sludge flocs and certain micropollutants. The potential role of EPS in OMP removal is discussed in more detail in the following section. In the modified HiCS configuration, approximately 7% of CBZ removal was attributed to biotransformation, while overall removal remained primarily governed by sorption processes.

For estrogenic compounds, operational parameters such as SRT and HRT are known to influence removal performance. In the modified HiCS configuration, SRT was maintained constant while a moderate increase in EE2 removal was observed. This improvement coincided with an increase in HRT, which may have increased the effective contact time between sludge and wastewater, thereby allowing more favourable conditions for sorption–desorption equilibrium and limited biotransformation.

It should also be noted that operation of a pilot-scale wastewater treatment plant inherently involves greater variability compared to laboratory-scale systems. Over the two-year operational period, seasonal fluctuations and changes in influent wastewater composition contributed to this variability, influencing both sorption and biotransformation processes. Therefore, the modified HiCS configuration is primarily evaluated from a carbon capture and energy recovery perspective, while OMP behaviour is interpreted as a secondary outcome rather than a direct optimization target.

In conclusion, the transition to the modified configuration did not lead to a clear improvement in OMP removal under the investigated conditions. While the modification enhanced carbon capture, its effect on micropollutant removal remained limited, and removal behaviour continued to be governed by compound-specific properties and system dynamics. Although total removal efficiencies remained similar, a shift in removal behaviour towards sorption was observed. This suggests that improvements in carbon redirection do not necessarily enhance overall OMP removal but rather result in a partitioning shift from the aqueous phase to the sludge phase. Consequently, the role of

downstream processes, such as anaerobic digestion, becomes critical in determining the ultimate environmental fate of these compounds. This indicates that process modifications aimed at improving carbon capture do not necessarily enhance overall micropollutant removal but rather influence the distribution of compounds between liquid and solid phases.

3.4. Sludge –water distribution coefficients and their implications for OMP

To support the interpretation of compound-specific removal mechanisms observed in the pilot-scale HiCS system, batch sorption experiments were conducted to determine the sludge–water distribution coefficients (Kd) of selected micropollutants. Based on the mass balance results, BPA and IBU were selected as representative compounds exhibiting contrasting removal behaviour. BPA was predominantly associated with sludge solids, whereas IBU showed high overall removal with limited solid-phase accumulation, in agreement with the mass balance results indicating the dominance of sorption and biotransformation, respectively. Accordingly, these observations suggested that sorption dominated the removal of BPA, whereas biotransformation played a more significant role in the removal of IBU.

Physicochemical parameters such as the octanol–water partition coefficient (log Kow) are commonly used to estimate the sorption potential of organic compounds. However, these relationships may not always adequately describe compound behaviour in biological treatment systems. Therefore, the experimental determination of sludge–water distribution coefficients provides additional insight into sorption processes under the operational conditions of the HiCS system.

Batch sorption experiments showed clear linear relationships between the sorbed amount (qe) and aqueous concentration for both compounds (Figure 3.4- 3.5), allowing the estimation of the sludge–water distribution coefficients (Kd).

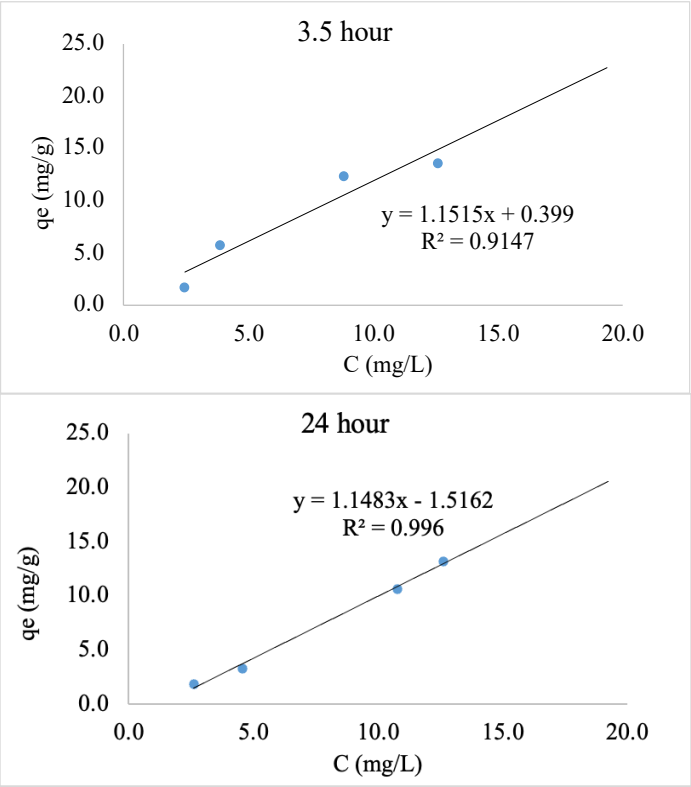


Figure 3.4. Linear sorption isotherms for BPA at contact times of 3.5 h and 24 h

For BPA, the calculated Kd values were 1151.5 L/kg at 3.5 h and 1148.3 L/kg at 24 h (Figure 3.4). The similarity between these values indicates that sorption equilibrium is approached rapidly, suggesting that the contact time corresponding to the hydraulic retention time (HRT) of the HiCS system is sufficient for effective partitioning of BPA onto sludge solids. The experimentally determined log Kd value for BPA (≈3.06) is also close to its reported hydrophobicity (log Kow ≈3.32), supporting the tendency of BPA to partition into the solid phase of sludge flocs. This indicates that the short contact times characteristic of high-rate activated sludge systems do not necessarily limit sorption-driven removal of hydrophobic micropollutants.

The experimentally determined BPA Kd value (1151 L/kg) was consistent with the adsorption coefficients reported by Clara et al. (2004a), who observed Kd values in the order of 1000 L/kg for BPA using batch adsorption experiments with sewage sludge. Similar partitioning behaviour has also been reported for high-rate activated sludge systems, where Koumaki et al. (2021) reported BPA Kd values of 1409 ± 813 and 648 ±

115 L/kgSS in HRAS and HiCS configurations, respectively. The agreement between the present results and previous studies further supports the strong tendency of BPA to partition onto sludge solids under both conventional and high-rate operating conditions.

Although the batch sorption experiments demonstrated a strong tendency of BPA to partition onto sludge solids, the overall removal observed in the pilot-scale HiCS system remained around 60%. This indicates that sorption potential determined under controlled laboratory conditions does not directly translate into removal under continuous-flow operation. In the pilot-scale system, dynamic hydraulic conditions, fluctuations in influent composition, competition for sorption sites with other wastewater constituents, and possible desorption processes may reduce the effective removal of BPA. Therefore, while sorption clearly contributes to the fate of BPA in the HiCS system, the net removal observed at pilot scale reflects the combined influence of process hydrodynamics and wastewater matrix effects.

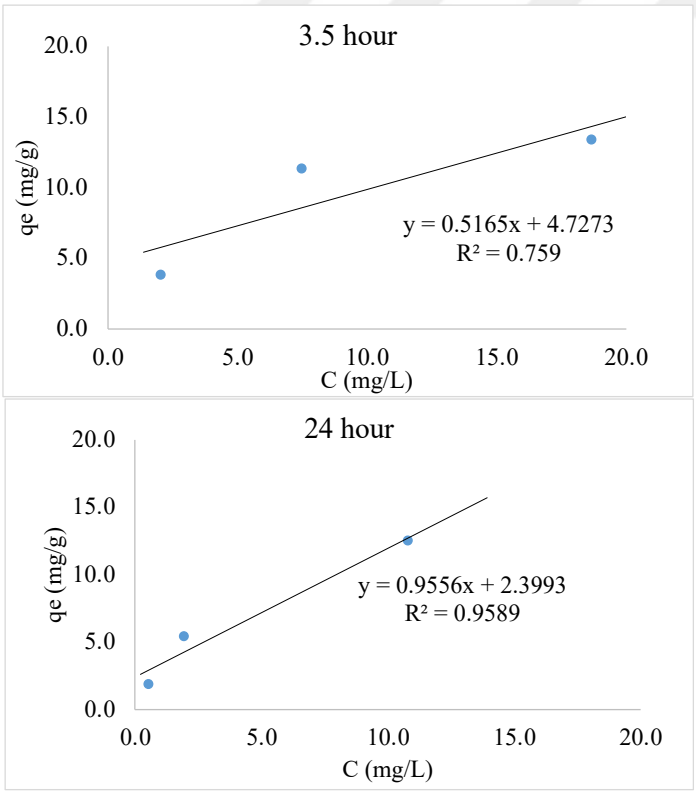


Figure 3.5. Linear sorption isotherms for IBU at contact times of 3.5 h and 24 h

In contrast, IBU exhibited lower sorption affinity toward sludge solids. As highlighted by Ternes et al. (2004), pharmaceuticals are often polar or ionizable compounds and therefore their solid–water distribution behaviour cannot always be reliably predicted solely on the basis of hydrophobicity relationships. Consistent with this observation, the sorption relationships were evaluated to determine the partitioning behaviour of IBU. As illustrated in Figure 3.5, the batch sorption experiments showed a linear relationship between the sorbed and aqueous concentrations. Based on these experimental data, the calculated Kd value for IBU at 3.5 h (516.5 L/kg) was substantially lower than that observed for BPA, although it increased to 955.6 L/kg at 24 h (Figure 3.5).

The experimentally determined IBU Kd values (516–956 L/kg) were substantially higher than those typically reported for conventional activated sludge systems. Ternes et al. (2004) reported an IBU Kd value of 7.1 ± 2.0 L/kg, while Joss et al. (2006) adopted a similar value (~7 L/kg), indicating limited sorption and suggesting biodegradation as the dominant removal pathway in conventional activated sludge systems. In contrast, Koumaki et al. (2021) reported considerably higher IBU Kd values of 243 ± 97 and 211 ± 106 L/kgSS in HRAS and HiCS systems, respectively. Although the Kd values obtained in the present study were still higher than those reported by Koumaki et al. (2021), all high-rate systems exhibited substantially greater partitioning of IBU than conventional activated sludge systems. This behaviour may be attributed to the distinct characteristics of high-rate sludge, including its younger biomass, higher organic content, and different floc and EPS structure, which may provide additional sorption sites for ionizable compounds such as ibuprofen.

Nevertheless, the pilot-scale system achieved an overall removal efficiency of approximately 70% for IBU. This suggests that sorption alone cannot fully explain the observed removal and that biotransformation likely represents the dominant removal pathway for IBU in the HiCS process. Despite the relatively high experimentally determined Kd values for IBU, its substantial removal in the HiCS system further supports the importance of biotransformation processes, as discussed in Section 3.3.1. The increase in Kd for IBU at 24 h may indicate a gradual partitioning behaviour; however, considering the short HRT of the pilot-scale HiCS system, the Kd value obtained at 3.5 h is more representative of the effective sorption occurring under

continuous-flow conditions. This highlights the importance of considering contact time when interpreting sorption parameters derived from batch experiments in relation to continuous-flow systems.

Overall, the results confirm the compound-specific nature of micropollutant removal mechanisms in the HiCS system. The experimentally determined Kd values support the removal behaviour identified through mass balance analysis, demonstrating that hydrophobic compounds such as BPA tend to partition rapidly onto sludge solids, whereas more polar pharmaceuticals such as IBU are primarily removed through biotransformation. The experimentally determined Kd values therefore provide mechanistic insight into the relative contributions of sorption and biotransformation under HiCS process. These findings confirm that sorption behaviour in high-rate systems cannot be fully predicted by hydrophobicity alone, highlighting the importance of experimentally derived parameters such as Kd for mechanistic interpretation.

3.5.Influence of Sludge Retention Time on the Removal of OMPs in HiCS Systems

The influence of sludge retention time SRT on micropollutant removal was evaluated under the operational conditions of the pilot-scale HiCS system. The pilot-scale system was operated at SRTs of 2, 1, 0.75 and 0.5 days, with the most systematic comparison based on the 1- and 2-day operating conditions (Figure 3.6). The results indicated that lower SRTs were generally associated with higher overall removal efficiencies for several of the investigated OMPs. This behaviour contrasts with the trend typically observed in conventional wastewater treatment plants, where longer SRTs often enhance micropollutant removal due to the development of specialized microbial communities. In the HiCS system, however, shorter biomass ages appeared to favour the removal of several target compounds.

A more detailed comparison was possible between SRT values of 1 and 2 days, for which sufficient monitoring data were available. The results showed that the removal of several compounds was higher at an SRT of 1 day compared to 2 days with differences of up to approximately 20–25% observed for certain compounds. These findings suggest that

operational conditions associated with lower biomass age may provide more favourable conditions for the removal of certain OMPs within the HiCS process.

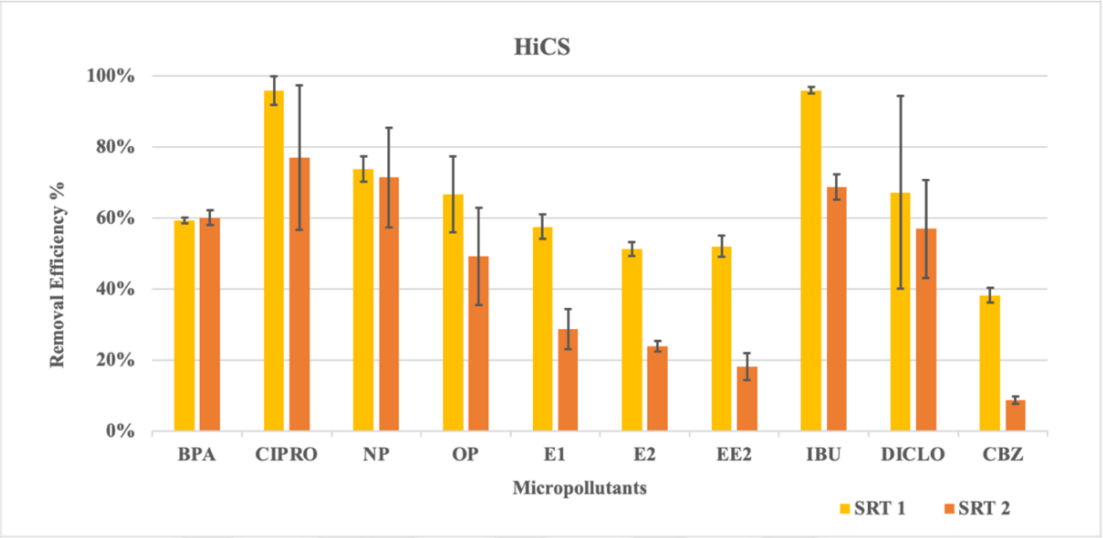


Figure 3.6. Micropollutant Removal Efficiencies in HiCS at SRTs of 1 and 2 Days

In addition to these two main operating conditions, short operational periods at SRT values of 0.75 and 0.5 days were also investigated during the pilot-scale operation. While limited sampling renders the results at SRTs of 0.75 and 0.5 days primarily indicative, the observed consistency with the 1-day SRT trend suggests that further reductions in biomass age may fundamentally alter the removal kinetics of specific OMPs under high-rate conditions.

Beyond overall removal efficiencies, SRT was found to significantly influence the fate and distribution of OMPs within the system. A distinct shift in removal mechanisms was observed between SRT conditions; operation at SRT 1 day was associated with higher apparent contributions of sorption and biotransformation, whereas a larger fraction of certain compounds remained in the effluent at SRT 2 days. This shift in removal behaviour may be associated with the characteristics of biomass maintained under lower SRT conditions. At shorter sludge ages, younger and more metabolically active biomass may enhance OMP removal both by increasing the availability of biosorption sites as well as higher substrate availability and food-to-microorganism ratios characteristic of

high-rate operation, which may support rapid enzymatic biotransformation processes. In contrast, for compounds with low sorption affinity, the SRTs investigated may be insufficient to support effective biotransformation pathways, which may contribute to their persistence in the aqueous phase. This may indicate that SRT affects not only the extent of removal but also the phase in which OMPs are retained.

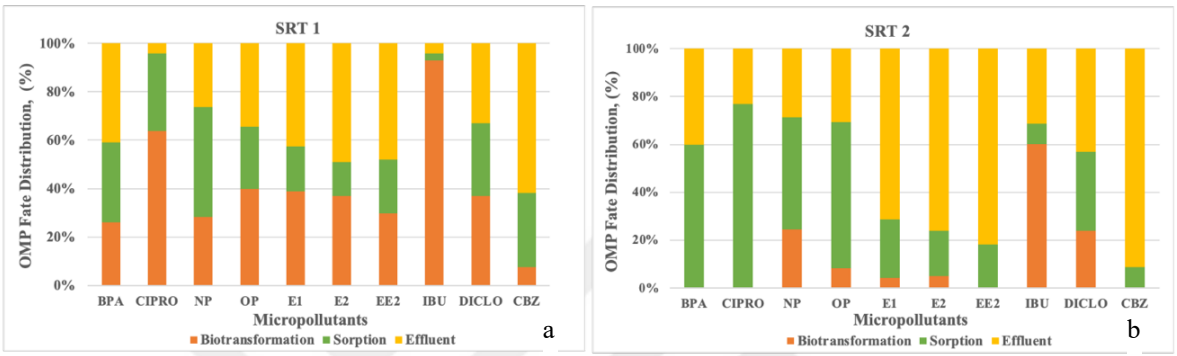


Figure 3.7. Contribution of Removal Pathways to Micropollutant Fate in HiCS process at SRT a) 1 day and b) 2 days

The impact of SRT on OMP fate was strongly compound-dependent. Hydrophobic compounds such as BPA and NP showed a dominant association with sludge solids, indicating sorption-driven removal. In contrast, compounds such as IBU were primarily removed through biotransformation, whereas more persistent compounds such as CBZ and EE2 remained largely in the aqueous phase, particularly at higher SRT.

These findings suggest that the effect of SRT in high-rate systems should be interpreted not only in terms of removal efficiency, but also in terms of the redistribution of micropollutants between aqueous and solid phases. This behaviour is consistent with the mechanisms discussed in Section 3.2, where both sorption and biotransformation were shown to govern OMP removal under HiCS conditions.

The observed relationship between SRT and micropollutant removal can be linked to the characteristics of the microbial community under high-rate operation. Lower SRT values favour fast-growing heterotrophic microorganisms and maintain higher food-to-microorganism ratios, potentially resulting in more metabolically active biomass. Under

such conditions, enzymatic transformation processes may be enhanced. Furthermore, the interplay between lower biomass concentrations and short SRT conditions may enhance removal through dual mechanisms: first, by fostering smaller and less dense floc morphologies that mitigate internal mass transfer limitations, and second, by optimizing the specific pollutant loading per unit of biomass. Together, these factors likely increase the effective accessibility of available sorption sites and facilitate more efficient interfacial contact between the OMPs and the microbial matrix.

Overall, the results highlight the compound-specific behaviour of micropollutants in the HiCS system and demonstrate that the relative importance of sorption and biotransformation is largely governed by the physicochemical properties of the investigated compounds. In HRAS systems, OMP removal appears to be influenced more strongly by operational conditions such as biomass age, substrate availability, and microbial activity than by the establishment of diverse specialized microbial communities typically associated with longer SRTs in conventional treatment systems.

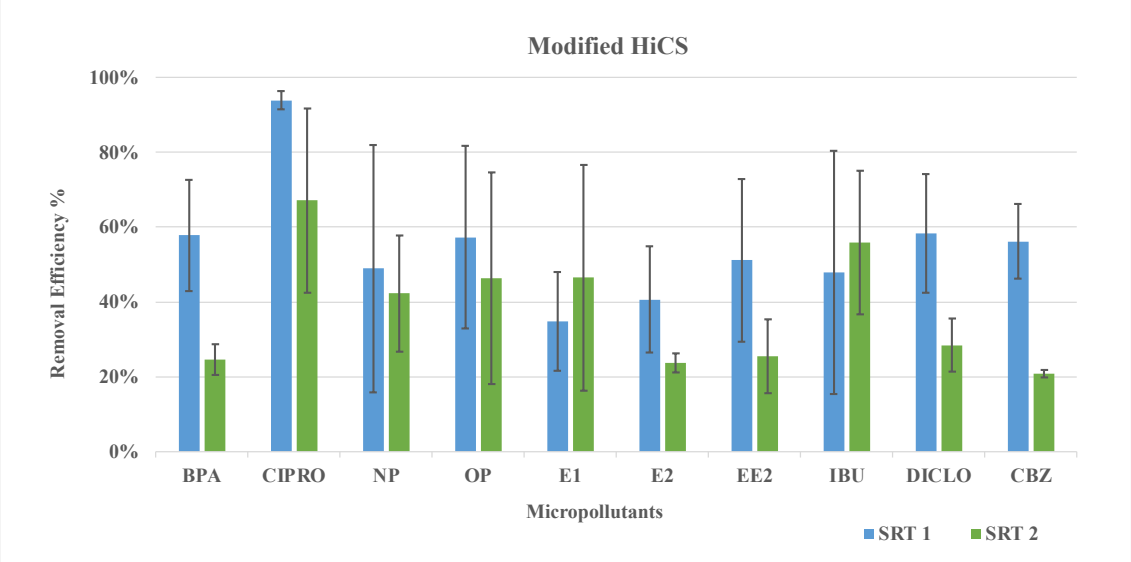


Figure 3.8. Micropollutant Removal Efficiencies in Modified HiCS at SRTs of 1 and 2 Days

A similar trend was also observed when the system was operated in the modified HiCS configuration. As shown in Figure 3.8, removal efficiencies obtained at an SRT of 1 day were generally higher than those observed at an SRT of 2 days. This consistency between the conventional and modified HiCS configurations suggests that the influence of

biomass age on OMP removal remains comparable under both operational strategies, indicating that shorter SRT conditions may favour micropollutant removal in high-rate contact stabilization systems.

Unlike CAS systems where OMP removal is driven by the establishment of slow-growing nitrifiers and specialized degraders at long SRTs, the HiCS process relies on the high metabolic turnover and rapid growth rates of heterotrophs. In this high-rate environment, decreasing the SRT may stimulate microbial growth and substrate uptake, which may contribute to enhanced biotransformation of OMPs. This suggests that, in high-rate systems, shorter sludge retention times may enhance micropollutant removal by promoting metabolically active biomass and improving mass transfer conditions.

3.6. Evaluation of EPS Variation and Its Impact on OMP Removal

High-rate activated sludge systems are designed to enhance carbon capture primarily through sorption-driven processes, in which extracellular polymeric substances play a key structural role by promoting floc formation and providing binding sites for organic matter. Through their complex matrix composed of proteins and polysaccharides, EPS are known to contribute to floc structure and provide potential binding sites for organic matter and micropollutants through anionic, cationic and apolar functional groups (Wunder et al., 2011; Zhang et al., 2018; Burzio et al., 2022). Sorption processes have also been reported to depend on factors such as surface characteristics, specific surface area and EPS composition (Burzio et al., 2022).

In the present study, the potential influence of EPS on the removal behaviour of OMPs was evaluated using EPS data obtained from the same pilot-scale system. These EPS measurements and their interactions with COD removal processes were monitored and conducted by E.K. Demir within the scope of the overarching research project. EPS concentrations were reported to vary within a relatively narrow range of approximately 30–90 mg COD g/VSS under different operational conditions (Demir, 2025).

Comparison of OMP removal efficiencies across these EPS levels did not reveal a consistent relationship between EPS concentration and micropollutant removal. As

shown in Figure 3.9, no clear increasing or decreasing trend in removal efficiency was observed with increasing EPS concentration. Although moderate variations were observed for certain compounds, these changes did not follow a clear trend that could be directly attributed to EPS levels. This indicates that the observed variations in EPS within the investigated range were not sufficient to exert a measurable influence on OMP removal behaviour. Instead, differences in removal efficiencies are more likely associated with compound-specific physicochemical properties and sludge surface interactions, as well as fluctuations in influent composition and operational conditions.

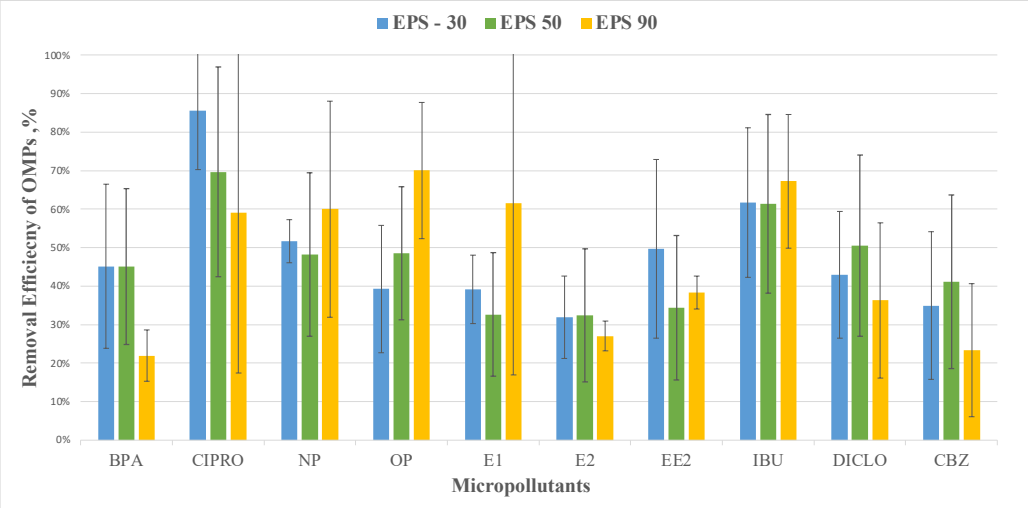


Figure 3.9. Influence of EPS concentration on OMP removal efficiency in the HiCS system

Despite operating within the same experimental framework as Demir (2025), where EPS levels were observed to correlate with COD removal, our findings regarding micropollutants diverged, showing no significant relationship between EPS concentrations and OMP removal. This suggests that while EPS concentrations within the 30–90 mg COD / g VSS range are sufficient for bioflocculation and bulk organic matter capture, their variation does not appear to be a primary factor governing the sorption of the investigated micropollutants under the studied conditions.

It should also be noted that the relatively narrow range of EPS concentrations limited the ability to establish a robust correlation between EPS characteristics and OMP removal.

A wider operational window with more pronounced variations in EPS production would be required to more definitively assess its role as a driver for sorption processes. In addition, previous studies have suggested that not only EPS quantity but also EPS composition, particularly the protein and polysaccharide content, may influence micropollutant partitioning and sorption behaviour (Khunjar and Love, 2011; Xu et al., 2013; Zhang et al., 2018).

3.7. Resource Recovery and Micropollutant Fate during Anaerobic Digestion

In this study, sludge produced in the pilot-scale HiCS system was evaluated as a feedstock for anaerobic digestion to assess its potential for energy recovery through methane production. The digester was fed with sludge generated under different HiCS operating conditions, including conventional and modified configurations as well as target SRTs of 2 and 0.5 days. In addition, the behaviour of OMPs during anaerobic digestion was investigated.

3.7.1. Methane Production

Methane production in the lab-scale anaerobic digester was monitored throughout the experimental period. Temporal variations were observed, reflecting differences in sludge biodegradability. In parallel, the concentrations of selected OMPs were monitored to evaluate their behaviour during anaerobic digestion. Table 2.1 presents the main operational parameters and sludge characteristics of the anaerobic digester.

The mesophilic laboratory-scale digester was operated continuously for 380 days, and methane production was monitored throughout the experimental period. Methane production was calculated based on biogas volume and methane composition determined by gas chromatography. The methane content of the produced biogas ranged between 60 and 70% during the experimental period. The specific methane yield ranged from 0.20 to 0.71 L CH_4/ g VS, with an overall average of 0.36 ± 0.10 L CH_4 /g VS (Figure 3.10). Occasionally observed methane yields exceeding 0.55 L CH_4/g VS should be interpreted with caution. Such elevated values may be associated with the contribution of residual biodegradable organic matter retained in the reactor from previous feeding cycles and,

potentially, with deviations from ideal complete-mixing conditions. Since the digester was operated as a semi-continuous system with a sludge retention time of 15 days, methane production measured at a given time may not originate exclusively from the sludge fed on that particular day but may also reflect the degradation of biodegradable organic matter retained from previous feeding periods.

To facilitate interpretation of methane production trends, major operational changes during digester operation are indicated in Figure 3.10. On day 188, the sludge collection strategy changed from separate collection of primary sludge and stabilization sludge to collection of a combined sludge stream following implementation of the modified HiCS configuration. In addition, sludge generated under a target HiCS SRT of 0.5 days was fed between days 233 and 287, whereas sludge produced at a target SRT of 2 days was used during the remaining operational periods

Temporal variations in methane production were observed, indicating differences in sludge biodegradability. The organic loading rate (OLR) was initially maintained at approximately 1.5 g VS /Ld. However, adjustments in the feeding strategy were implemented during the experimental period due to variations in sludge availability from the pilot-scale HiCS system, and higher loading rates were applied during certain operational periods. A clear seasonal pattern was observed in methane production. During summer (days 125–232), the average methane yield decreased to 0.27 ± 0.06 L CH_4 /g VS, whereas higher methane yields were observed during winter, reaching an average of 0.39 ± 0.10 L CH_4 /g VS. These differences likely reflect seasonal variations in sludge characteristics and influent composition. Since the reactor was operated under controlled mesophilic conditions, these variations are likely associated with seasonal changes in sludge characteristics and influent composition rather than temperature effects on microbial activity. In addition to seasonal variations, the potential influence of operational parameters such as organic loading rate (OLR) and VS/TS ratios on methane production was evaluated. However, no consistent relationship between these parameters and methane yield was observed during the operational period. This suggests that fluctuations in methane production were more likely associated with changes in sludge characteristics rather than variations in OLR or solids composition.

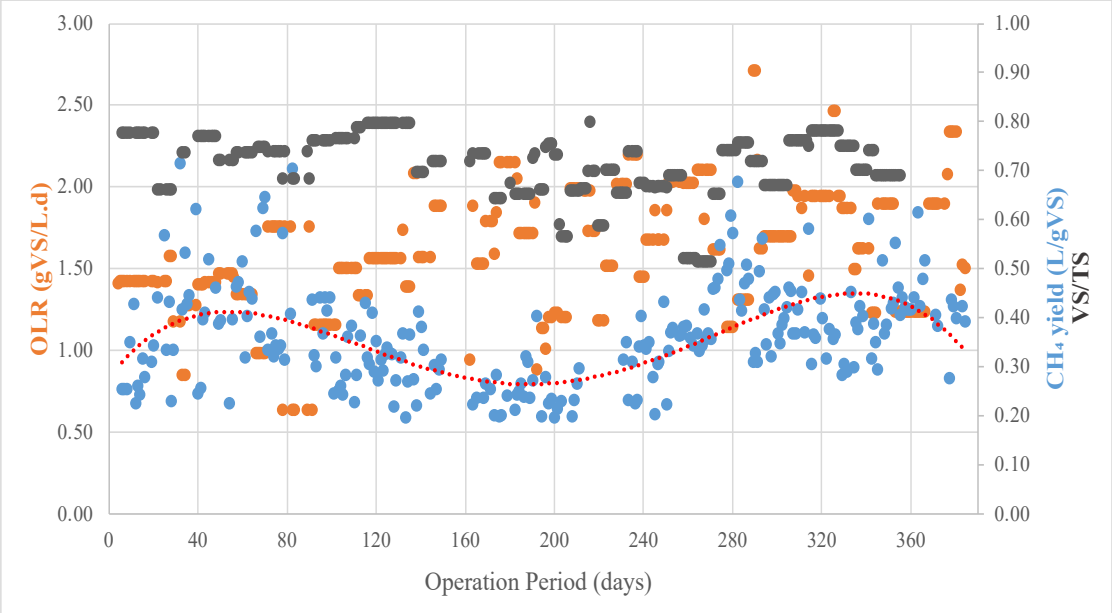


Figure 3.10. Temporal variation of OLR and CH_4 yield

Anaerobic digestion is widely applied for sewage sludge stabilization due to its ability to convert organic matter into biogas. In this study, the anaerobic digestion performance of HiCS-derived sludge was evaluated to assess its methane production potential. The produced biogas typically contains 60–70% methane, representing an important energy recovery pathway in wastewater treatment plants (Appels et al., 2008). The methane potential of sludge strongly depends on the upstream treatment process, particularly on the extent to which organic matter is oxidized or captured in the sludge phase. This is particularly relevant for high-rate systems such as HiCS, where a larger fraction of organic carbon is retained in the sludge.

The average specific methane yield obtained from HiCS sludge in this study (0.36 ± 0.10 L CH_4/gVS) was higher than the values reported for conventional waste activated sludge. For example, Sakurai et al. (2025) reported methane yields of 0.27–0.28 L CH_4/g VS for sludge produced in conventional activated sludge systems, whereas substantially higher values of 0.41–0.45 L CH_4/g VS were observed for sludge generated in the HiCS stage under comparable operational conditions. These methane potentials were determined using biochemical methane potential (BMP) tests, which evaluate the maximum methane production achievable from sludge under controlled anaerobic conditions. This enhanced energy potential is attributed to the high-rate operation and short SRT of the HiCS

process, which effectively redirects organic carbon to the sludge phase before extensive mineralization occurs. These findings are consistent with Ge et al. (2017), who reported that HRAS processes operated at very short SRTs can achieve anaerobic biodegradability fractions exceeding 80%. Assuming a theoretical methane potential of approximately 0.40–0.45 L CH_4 /g VS, this biodegradability corresponds to methane yields of roughly 0.32–0.36 L CH_4/g VS, which closely matches the values observed in this study.

The observed methane production trends indicate that the characteristics of sludge generated under short SRT conditions in the HiCS process play a dominant role in determining anaerobic digestion performance. In particular, the relatively high and stable methane yields obtained in this study suggest that the HiCS configuration effectively preserves readily biodegradable organic fractions within the sludge, which can be subsequently converted into methane. This highlights the functional linkage between upstream carbon capture and downstream energy recovery, demonstrating that process configurations promoting rapid sludge production without extensive oxidation can enhance the overall energy efficiency of wastewater treatment systems.

Overall, the high methane yields obtained from HiCS sludge demonstrate the effectiveness of high-rate processes in redirecting organic carbon to the sludge phase, thereby preserving biodegradable fractions and enhancing energy recovery through anaerobic digestion. These findings support the potential of integrated HiCS–AD configurations as energy-oriented alternatives to conventional activated sludge systems, as short SRT operation improves carbon capture in sludge and methane production.

3.7.2. Occurrence and Fate of OMPs during Anaerobic Sludge Digestion

The management of sewage sludge has transitioned from a paradigm of simple disposal to one of resource recovery, driven by the global imperative for circular economy practices within the wastewater sector. A central component of this shift is the deployment of high-rate carbon capture technologies, such as the HiCS system, which efficiently redirects organic matter from the aqueous phase to the solid phase for enhanced energy recovery via anaerobic digestion (Meerburg et al., 2016). While the HiCS process excels at capturing particulate and colloidal COD, it concurrently

concentrates a wide range of OMPs into the sludge matrix. The subsequent stabilization of this sludge in mesophilic anaerobic digesters represents a critical stage in the life cycle of these contaminants, determining whether they are mineralized, transformed into secondary metabolites, or sequestered within the final biosolid product (Fischer and Majewsky, 2014; Gonzalez-Gil et al., 2019). In this context, the behaviour of OMPs during anaerobic digestion was evaluated in a semi-continuous mesophilic digester operated at a SRT of 15 days. The system was investigated in three operational sets (Set 1: 115–130 day; Set 2: 293–308 day; Set 3: 352–367 day). Influent and effluent samples were collected on days 0, 5, 10, and 15 in order to represent one SRT period and to follow the temporal variation of OMP loads during the digestion cycle. This approach enabled the direct comparison of compound behaviour within a single digestion cycle using the same initial sludge matrix, thereby ensuring a consistent assessment of the operational sets. The experimental evaluation of ten representative OMPs (IBU, DICLO, CBZ, E1, E2, EE2, BPA, NP, OP, CIPRO) over 380 days of continuous operation provided detailed insight into the complex interactions governing OMP fate and behaviour within the anaerobic digestion environment.

The anaerobic digester was operated at a solids retention time (SRT) of 15 days and exhibited stable operational performance throughout the experimental period, as evidenced by consistent volatile solids (VS) destruction and sufficient alkalinity buffering capacity (Table 3.3). These stable operating conditions established a reliable and controlled platform for evaluating the compound-specific fate and transformation behaviors observed across the three operational sets.

The performance of the anaerobic digestion system is intrinsically linked to the characteristics of the HiCS-derived sludge. High-rate systems typically operate at very low SRTs (often below 1 day), which results in a sludge that is rich in easily degradable organic matter and EPS (Demir et al., 2025). Unlike conventional WAS, which has undergone significant endogenous respiration, HiCS sludge retains a high energy content.

During the 380-day study, the digester maintained a steady state with a mean pH of 7.3 ±0.1 and alkalinity exceeding 5000 mg/L as CaCO3. This stability is essential for the

evaluation of OMPs, as many of these compounds possess ionizable functional groups. For example, the pharmaceuticals IBU and DICLO have pKa values around 4.1 to 4.5, meaning that at the operating pH of 7.3, they exist almost entirely in their anionic form (Garcia-Ivars et al., 2017). Conversely, the antibiotic CIPRO is zwitterionic at neutral pH, which significantly influences its sorption behavior and interaction with the microbial community (Lima et al., 2025).

This operational stability and the lack of a pronounced response to OLR fluctuations are further supported by the residual VFA concentrations and methane production performance of the digester. The average VFA concentration remained at a relatively low level of 175 ± 40 mg COD/L (Table 3.3), indicating that intermediate fermentation products did not accumulate substantially during reactor operation. This low VFA accumulation, together with the stable specific methane yields observed throughout the 380-day operational period (Figure 3.10), suggests that the syntrophic relationship between acidogenic and methanogenic populations was effectively maintained. The continuous conversion of intermediate products into methane indicates that methanogenic activity remained functionally stable, despite the ongoing exposure to selected OMPs and variations in organic loading conditions. The sustained methane production further supports that no substantial inhibition of the anaerobic digestion process occurred during reactor operation. Such stable methanogenic conditions are particularly relevant because anaerobic biotransformation of several pharmaceuticals and endocrine-disrupting compounds has been reported to occur under active methanogenic conditions (Subramanian and Suresh, 2024).

3.7.2.1.General Behaviour and Controlling Mechanisms of OMPs

OMP behaviour in AD systems is governed by a complex interplay of biological, physicochemical, and operational factors. Accordingly, the interpretation of apparent removal based solely on concentration or load differences between influent and digested sludge should be approached with caution. Variations in influent OMP loads, reactor mixing, solids retention effects, and sludge mass reduction can all influence observed trends. Furthermore, decreases in the measured levels of a target compound do not

necessarily indicate complete mineralization or elimination of environmental relevance. Instead, such changes may reflect partial transformation into intermediate products or metabolites that are not captured by the applied analytical method.

Furthermore, a critical aspect of AD systems is the substantial transformation of the sludge matrix during stabilization. In conventional anaerobic digestion processes, approximately 40–60% of VS are converted into biogas, primarily CH_4 and CO_2, resulting in a major reduction in total solids mass (Appels et al., 2008; Metcalf & Eddy, 2014). This progressive loss of organic matter can significantly influence the apparent distribution of OMPs within the digester. For recalcitrant compounds, the absolute mass of the molecule may remain relatively unchanged while the surrounding sludge matrix decreases. This substantial reduction in total solids mass may lead to an apparent enrichment of recalcitrant OMPs within the remaining sludge matrix. Consequently, concentration- or load-based interpretations may become distorted by what can be described as a matrix enrichment or matrix redistribution effect.

The interpretation of OMP fate is further complicated by the hydraulic and operational behaviour of anaerobic digesters. Most AD systems operate as completely stirred tank reactors (CSTRs) with relatively long SRTs. Due to continuous mixing and extended residence times, the digested sludge withdrawn at a given sampling event does not directly correspond to the influent sludge entering the reactor at that same moment. Instead, the effluent represents a stabilized and mixed composite derived from multiple historical influent cycles. This temporal decoupling limits the validity of direct point-to-point comparisons between influent and digested sludge loads. Consequently, observed differences in OMP loads should be interpreted within the framework of long-term steady-state behavior rather than instantaneous removal efficiencies. Therefore, the evaluation of OMP fate in this study is based on a comprehensive assessment of phase distribution, mass load trends, and potential transformation pathways, rather than on apparent removal efficiencies alone.

A critical observation derived from the graphical data presented in Figure 3.11, together with the associated experimental findings, was the dominant distribution of the investigated OMPs within the solid phase of the anaerobic digester. Liquid phase OMP

loads remained consistently low for all compounds throughout the operational period. This behaviour indicates that the fate of OMPs during the anaerobic digestion of HiCS sludge was governed predominantly by solid-phase association and sorption-related mechanisms rather than by persistence in the aqueous phase.

The consistently low liquid phase OMP loads further demonstrated stable reactor operation and suggested that the majority of the investigated compounds remained strongly associated with sludge solids during digestion. Consequently, the evaluation of OMP behaviour within the anaerobic digestion system was primarily based on comparisons between daily OMP loads determined in the influent sludge and those detected in the digested sludge (solid phase).

The predominance of OMPs in the solid phase has significant implications for their bioavailability. While sequestration on sludge particles prevents the release of these compounds into the liquid effluent, it may also shield them from microbial attack. The high organic content of HiCS sludge, while beneficial for methane production, provides a vast surface area for sorption, potentially reducing the aqueous concentration of OMPs below the threshold required to induce the expression of specific degradative enzymes. Consequently, OMP removal in anaerobic digesters may be constrained by the limited bioavailability of sorbed compounds, as microbial transformation depends on the accessibility of OMPs to active microbial communities (Carballa et al., 2007; Rios-Miguel et al., 2023).

Based on mass load trends between influent and digested sludge, the investigated OMPs can be grouped into distinct behavioural categories reflecting their fate during AD. Compounds such as CBZ and CIPRO showed limited removal and remained largely associated with the sludge phase, indicating persistence under anaerobic conditions. DICLO, however, exhibited consistent reductions in mass loads, suggesting the occurrence of transformation processes within the anaerobic matrix. Compounds such as IBU and BPA demonstrated partial decreases, indicating moderate and condition-dependent biodegradation. The behaviour of E1, E2, and EE2 was primarily governed by transformation processes, particularly interconversion reactions, rather than net removal. Meanwhile, NP and OP exhibited apparent accumulation trends, largely influenced by in

situ formation from precursor compounds. Overall, these results indicate that OMP behaviour during AD is governed mainly by sorption-driven retention and compound-specific biotransformation processes rather than complete mineralization.

3.7.2.2.Compound-Specific Fate during AD

Building on the above categorization, a more detailed evaluation of individual compound behaviour is required to elucidate the underlying mechanisms governing their fate during AD. While the grouping provides an overall framework, it does not fully capture the compound-specific interactions between physicochemical properties, microbial processes, and reactor conditions. Therefore, the following sections present a systematic discussion of each OMP separately, focusing on their phase distribution, temporal trends, and potential transformation pathways within the anaerobic system. This approach enables a more comprehensive interpretation of the observed patterns and supports a mechanistic understanding of OMP behaviour during sludge digestion.

CBZ exhibited persistent behaviour during anaerobic digestion with only limited variations observed between influent and digested sludge loads across most operational periods. This behaviour is well documented in the literature and is primarily attributed to CBZ's recalcitrant molecular structure, which includes a fused tricyclic ring system that resists microbial enzymatic attack under both aerobic and anaerobic conditions. In addition, CBZ has moderate hydrophobicity (log Kow ≈ 2.25), which leads to partial sorption to sludge solids but does not significantly enhance its biodegradability. Previous studies consistently report near-zero removal of CBZ in anaerobic digestion systems. For instance, (Clara et al., 2004b) identified CBZ as a conservative tracer due to its persistence across wastewater treatment stages. Similarly, Carballa et al., 2007 and Phan et al., 2018 reported negligible degradation of CBZ under mesophilic anaerobic conditions, even at extended sludge retention times. This resistance is further supported by Vieno et al., 2007, who observed that CBZ passes through both biological treatment and sludge digestion largely unchanged.

A key observation from Figure 3.11 is that, in Set 2, influent sludge loads of CBZ were significantly higher than those measured in digested sludge. At first glance, this could be interpreted as an indication of CBZ removal. However, this discrepancy is more appropriately explained by process-related factors inherent to anaerobic digestion systems.

Anaerobic digesters operated under continuous or semi-continuous conditions behave as completely mixed reactors, where the effluent (digested sludge) represents an integrated mixture of sludge fed into the system over a period equivalent to the SRT, which in this study was 15 days. Consequently, the composition of digested sludge at any given sampling point reflects a time-averaged signal, rather than a direct or instantaneous representation of the influent sludge entering the reactor at that same moment. The elevated influent CBZ loads observed in Set 2 therefore likely reflect a temporal spike or fluctuation in upstream concentrations, which is not immediately mirrored in the digested sludge due to hydraulic and solids retention effects, which distribute the influent load over the SRT, dilution within the reactor volume, and phase lag between influent input and effluent output. This phenomenon can be described as a "non-synchronous sampling effect" or "snapshot limitation," where influent and effluent samples are not directly comparable on a one-to-one temporal basis. As a result, the lower CBZ loads in digested sludge during Set 2 do not indicate actual removal, but rather the buffering capacity of the digester and the temporal decoupling between input and output streams. Although CBZ is known to undergo minimal biodegradation, its interaction with sludge solids must also be considered. CBZ exhibits moderate sorption affinity, and some studies have suggested that minor reductions in aqueous concentration may occur due to partitioning into the solid phase (Phan et al., 2018). However, in sludge-based systems such as anaerobic digesters, where analysis focuses on the solid phase itself, sorption does not represent a true removal mechanism but rather a redistribution between phases.

Transformation of CBZ under anaerobic conditions is also extremely limited. Unlike some pharmaceuticals that undergo reductive or cometabolic degradation, CBZ shows no significant breakdown pathways in strictly anaerobic environments. While a few studies have reported the formation of minor transformation products under advanced

treatment conditions, such processes are generally absent in conventional mesophilic digestion (Alvarino et al., 2014; Phan et al., 2018).

Ciprofloxacin is an amphoteric compound that predominantly exists in a zwitterionic form under environmentally relevant pH conditions due to the presence of both a carboxylic acid group and a protonatable piperazinyl moiety (pKa ≈ 6.0 and 8.6, respectively), resulting in a net neutral but internally charged structure (Lima et al., 2025).

In Set 1, influent sludge loads of CIPRO were slightly higher than those observed in the digested sludge. While this might suggest removal, such an interpretation is not supported by the known persistence of CIPRO under anaerobic conditions. Instead, this difference is more plausibly attributed to reactor mixing and temporal averaging effects. Given the SRT of 15 days, the digested sludge represents a composite of previously introduced material and therefore may not directly reflect instantaneous influent conditions. Consequently, the lower loads observed in digested sludge likely result from dilution within the reactor, and integration of lower historical inputs, rather than actual degradation.

In Set 2, a substantial difference between influent and digested sludge loads was observed, with influent levels significantly exceeding those in the digested sludge. This pattern is indicative of a pulse input event, where elevated influent concentrations are not immediately reflected in the reactor output due to retention and mixing within the system. Such behaviour is typical of compounds with high sorption affinity, and limited mobility within the reactor.The strong binding of CIPRO to sludge solids further delays its redistribution, reinforcing the time-lag effect between influent loading and digested sludge composition. Therefore, the observed discrepancy should be interpreted as a consequence of reactor hydraulics and solids retention, rather than removal.

In Set 3, CIPRO loads in the digested sludge were markedly elevated relative to earlier sets and, in some cases, comparable to or exceeding influent levels. This trend reflects a cumulative enrichment effect, primarily driven by sludge mass reduction and persistent retention of CIPRO within the solid phase.

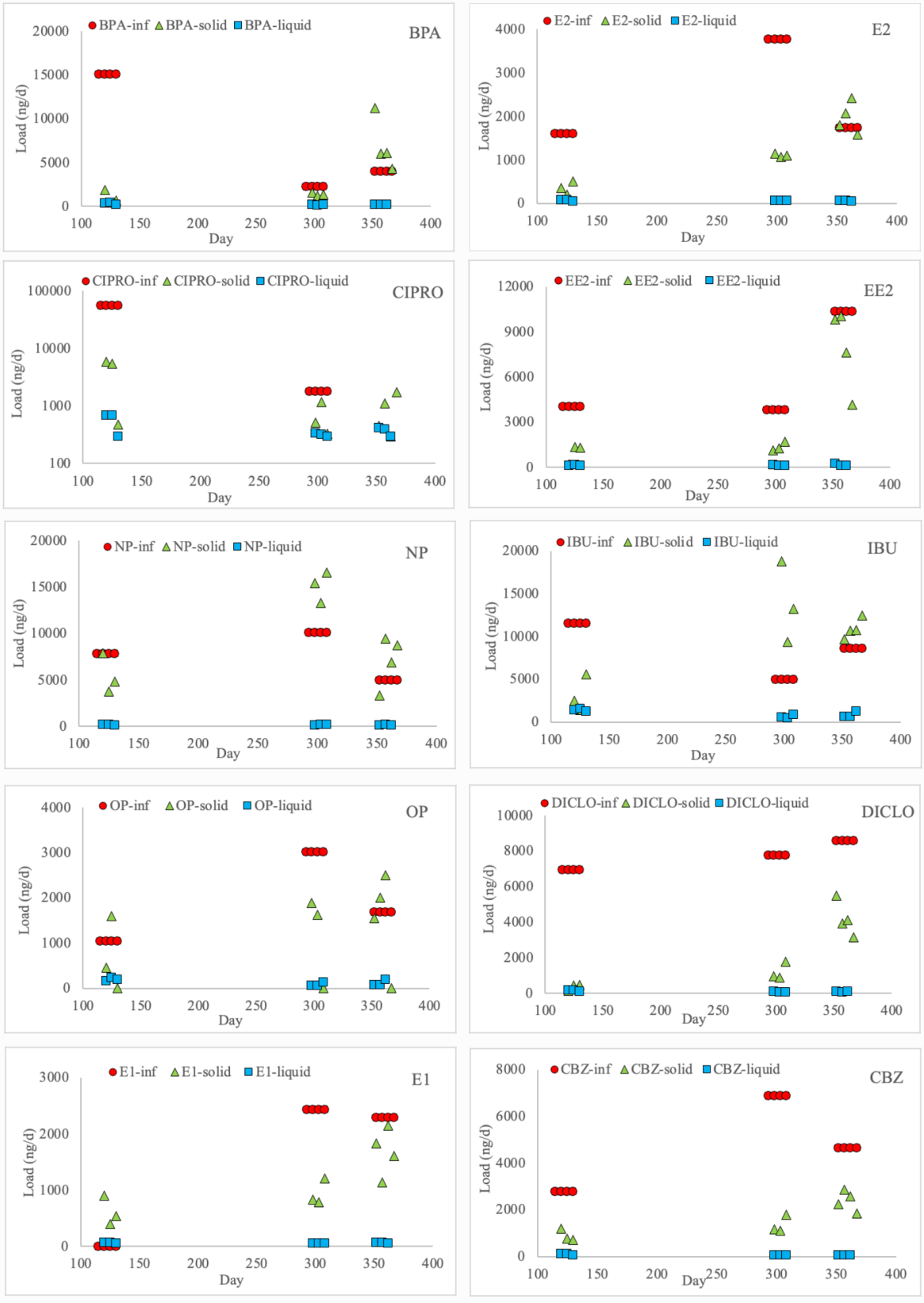


Figure 3.11. Distribution of Selected OMPs in Influent, Solid, and Liquid Phases during Anaerobic Digestion across Operational Sets

As AD progresses, the degradation of VS leads to a reduction in sludge mass, while strongly sorbed compounds such as CIPRO remain largely unaffected. This results in a progressive increase in concentration on a mass basis, particularly under conditions where biodegradation is negligible, and desorption processes are limited.

The magnitude of this effect is expected to be more pronounced for CIPRO than for less strongly sorbing compounds, due to its exceptionally high affinity for sludge solids.

The limited transformation of CIPRO in AD systems is closely linked to its low bioavailability. Strong sorption to sludge components restricts its accessibility to microbial communities, thereby inhibiting biodegradation. In addition, CIPRO's antimicrobial properties may further suppress microbial activity in its immediate vicinity, although such effects are typically localised and concentration-dependent (Trueba-Santiso et al., 2025).

Experimental studies have consistently reported minimal removal of CIPRO during anaerobic treatment, with the compound largely persisting in the solid phase (Golet et al., 2003). This behaviour contrasts with more labile pharmaceuticals and reinforces the classification of CIPRO as a recalcitrant compound in AD systems.

DICLO exhibited a clear reduction in solid-phase loads during anaerobic digestion, particularly during the first and second operational sets, where digested sludge loads remained substantially lower than the corresponding influent sludge loads. In the third operational set, although digested sludge loads increased compared with the earlier sets, they still remained below influent sludge loads throughout the digestion cycle. Meanwhile, liquid-phase loads remained consistently very low, indicating that DICLO did not significantly partition into the aqueous phase during anaerobic treatment.

The absence of a substantial increase in liquid-phase concentrations further suggests that simple solubilization or desorption alone cannot explain the observed attenuation behavior. Instead, the simultaneous depletion observed in both phases indicates that the reduction of DICLO was not driven solely by phase redistribution but may point toward transformation processes occurring within the anaerobic matrix.

The persistence of DICLO in the solid phase is consistent with its relatively hydrophobic nature and affinity for particulate matter (log $K_o w \approx 4.5$), which favour its association with sludge solids (Luo et al., 2014). Since DICLO remained predominantly associated with sludge solids throughout the experimental period, the solid matrix likely represented the primary microenvironment for compound–biomass interactions and potential microbial transformation processes.

The existing literature regarding the anaerobic biotransformation of DICLO within sewage sludge matrices consistently highlights a high degree of recalcitrance. Comparative studies on standard mesophilic AD systems report that DICLO removal is typically negligible, with efficiency rates frequently failing to exceed 20% (Alvarino et al., 2014; Vieno and Sillanpää, 2014). This behaviour may be related to the complex molecular structure of DICLO, which consists of two benzene rings linked by an amine moiety, with two chlorine atoms on one ring and an acetate group on the other. Based on this structure, reductive dechlorination and decarboxylation of the phenylacetate moiety could theoretically be expected; however, DICLO is mostly considered persistent under strictly anaerobic conditions, partly because hydroxylation of the benzene ring is less likely in the absence of oxygen (Ghattas et al., 2017). However, more recent studies suggest that DICLO is not entirely resistant to anaerobic transformation and may undergo specific biotransformation reactions under suitable conditions. Proposed pathways include decarboxylation and phosphorylation reactions mediated by enzymes such as decarboxylase and acetate kinase, indicating that DICLO may be susceptible to microbial transformation under specific anaerobic conditions (Wang et al., 2024). Furthermore, molecular simulation analyses suggested that the carboxyl group of DICLO may interact with active sites (e.g., H-clusters) of key functional anaerobic enzymes such as [FeFe] hydrogenase, potentially leading to enzyme inactivation and altered microbial activity (Wang et al., 2024). This observation indicates a potentially complex interaction between DICLO and microbial metabolic processes during anaerobic digestion.

Although anaerobic digestion systems frequently report limited DICLO removal, the reduction in total solid-phase loads observed in the present study suggests that transformation processes may have contributed to the observed attenuation. Nevertheless, additional analyses, including metabolite profiling and targeted

identification of transformation products (TPs), would be necessary to clarify the exact mechanisms responsible for DICLO reduction under these specific anaerobic conditions.

As shown in Figure 3.11, IBU was primarily associated with the solid phase, although measurable concentrations were also detected in the liquid phase, reflecting its relatively lower hydrophobicity (log $K_ow \approx 4$) at neutral pH, but strongly pH-dependent due to its ionisable nature). Compared to more hydrophobic compounds, IBU exhibits weaker sorption affinity, and thus its distribution between solid and liquid phases is more dynamic (Siyal et al., 2025).

The removal of IBU in AD systems is primarily attributed to biotransformation, although this process is often less efficient under anaerobic conditions than under aerobic conditions. Previous studies have reported moderate and variable removal efficiencies for IBU in anaerobic systems, typically ranging from negligible to approximately 80%, depending on reactor configuration, SRT, and microbial adaptation (Alvarino et al., 2014; Carballa et al., 2007; Gonzalez-Gil et al., 2016; Samaras et al., 2013). This variability reflects the dependence of IBU degradation on microbial community structure and metabolic activity, which can fluctuate over time.

In Set 1, influent sludge loads of IBU were substantially higher than those measured in the digested sludge, indicating net mass load reduction during AD. This trend is consistent with partial anaerobic biodegradation, suggesting that the microbial community present in the reactor was capable of transforming IBU under the prevailing conditions.

IBU degradation under anaerobic conditions is typically associated with reductive transformation pathways, cometabolic processes, and syntrophic interactions within anaerobic consortia. The observed decrease in IBU loads may therefore reflect active biodegradation, potentially supported by favourable substrate availability or acclimated biomass (Carballa et al., 2007; Samaras et al., 2013). However, as with other semi-continuous systems, mixing and dilution effects may also contribute to the observed reduction.

In Set 2, IBU loads in the digested sludge were significantly higher than those in the influent sludge, indicating an apparent increase. This behaviour cannot be attributed to in situ formation, but rather reflects process-related effects, particularly those associated with sludge stabilization.

During AD, the conversion of VS into CH_4 and CO_2 results in a reduction in total solids mass, which can lead to the enrichment of residual compounds such as IBU in the digested sludge. This "concentration effect" is well documented for organic micropollutants and is particularly relevant for compounds that are only partially degraded (Carballa et al., 2007).

In addition, the apparent increase may be influenced by temporal variability in influent concentrations, and non-synchronous sampling between influent and digested sludge, given that the digested sludge represents a time-averaged mixture over the SRT (15 days). Therefore, elevated loads in the digested sludge may reflect prior influent loading events rather than current influent conditions.

In Set 3, IBU exhibited variable behaviour, with digested sludge loads generally comparable to or slightly higher than influent values. This pattern suggests that biodegradation was either limited or offset by solids reduction effects during this period. The reduced removal efficiency observed in this set may be attributed to changes in microbial activity or community composition, variations in substrate availability, or operational fluctuations affecting degradation kinetics.

As reported in the literature, IBU degradation under anaerobic conditions is highly sensitive to environmental conditions, and removal efficiency may decrease in methanogenic systems where metabolic pathways for its transformation are less favourable (Carballa et al., 2004).

The results demonstrate that IBU exhibits moderate but inconsistent removal during AD, with its fate strongly influenced by biodegradation processes and sludge matrix effects. While partial removal is evident under certain conditions (Set 1), apparent increases in load (Set 2 and partially Set 3) are primarily attributable to volatile solids reduction, and temporal decoupling between influent and digested sludge.

The behaviour of BPA during anaerobic digestion demonstrated clear set-dependent variability, reflecting the interplay between biotransformation, sorption processes, and sludge matrix transformation. In contrast to highly recalcitrant compounds such as carbamazepine, BPA is generally regarded as moderately biodegradable, although its degradation under anaerobic conditions is often incomplete and strongly influenced by operational parameters and microbial community dynamics (Limam et al., 2013; Stasinakis, 2012).

Consistent with the trends shown in Figure 3.11, BPA was predominantly associated with the solid phase, with negligible loads in the aqueous phase. This behaviour is in line with BPA's physicochemical properties (log Kow ≈ 3.4), which favour partitioning to sludge solids (Clara et al., 2005b; Staples' et al., 1998). However, unlike more hydrophobic and persistent compounds, BPA is not exclusively governed by sorption and may undergo anaerobic biotransformation, particularly under favourable microbial conditions.

Nevertheless, BPA degradation in anaerobic systems is widely reported to be variable and condition-dependent. The efficiency of its removal is strongly influenced by the structure and acclimation of the microbial community, as well as environmental parameters such as substrate availability and organic loading. Notably, under strictly methanogenic conditions, BPA removal is often limited or inconsistent, with several studies reporting negligible degradation and stable concentrations over time. This indicates that, in such systems, sorption may dominate over biodegradation, and overall removal efficiency may depend heavily on the characteristics of the inoculum and operational history (Hardegen et al., 2021).

In Set 1, influent sludge loads of BPA were significantly higher than those measured in the digested sludge, suggesting an apparent removal during AD. This behaviour is consistent with partial anaerobic biotransformation, which has been reported for BPA under mesophilic conditions. Biotransformation pathways are typically associated with reductive modification of phenolic groups, cometabolic processes, and syntrophic interactions within anaerobic microbial consortia (Ghattas et al., 2017; Yi et al., 2025).

The observed decrease may therefore reflect active microbial transformation, potentially supported by favourable reactor conditions or an acclimated biomass. However, it is important to recognise that such apparent removal may also be influenced by reactor dilution and mixing effects, particularly in semi-continuous systems.

In Set 2, BPA loads in influent and digested sludge were comparable, indicating negligible net removal. This observation aligns with the well-documented variability of BPA degradation under anaerobic conditions, particularly in methanogenic systems where biodegradation may be suppressed or inconsistent.

In addition, the reactor configuration (SRT = 15 days) introduces a temporal averaging effect, whereby the digested sludge represents a composite of sludge fed over the retention period. Consequently, short-term fluctuations in influent BPA loads may not be directly reflected in the digested sludge. The minimal difference observed in this set therefore likely results from a combination of limited biotransformation, and non-synchronous comparison between influent and digested sludge.

In Set 3, BPA loads in digested sludge were slightly higher than those measured in the influent sludge. This trend was directly verified by the analytical measurements of the samples collected from the reactor mixed liquor at the beginning of Set 3, which consistently revealed higher BPA levels within the reactor than in the feeding influent. This apparent increase does not indicate in situ formation of BPA, but rather reflects changes in sludge composition during anaerobic digestion, particularly the reduction of volatile solids (VS). During AD, a substantial fraction of organic matter is mineralised to methane (CH_4) and carbon dioxide (CO_2), resulting in a decrease in total sludge mass. As BPA is only partially degraded, the remaining fraction becomes enriched in the residual solids, leading to an apparent increase in concentration when expressed on a mass basis. This phenomenon, commonly referred to as a concentration or normalisation effect, has been widely reported for hydrophobic organic contaminants during sludge stabilisation processes (Carballa et al., 2004; Clara et al., 2005b).

Overall, BPA can be classified as a moderately persistent compound in anaerobic digestion, with its observed behaviour strongly influenced by operational conditions and sludge transformation processes, rather than solely by intrinsic biodegradability.

The behaviour of steroid estrogens (E1, E2, EE2) during AD exhibited compound-specific yet interrelated patterns, reflecting the combined influence of biotransformation, sorption, and interconversion processes. Unlike many other OMPs, these compounds cannot be evaluated independently, as reversible transformation reactions between E1 and E2 may occur under anaerobic conditions, thereby complicating the interpretation of apparent removal or formation trends.

Across all operational sets, E1, E2, and EE2 were predominantly detected in the solid phase, with only minor fractions present in the liquid phase. This distribution is consistent with their hydrophobic character (log Kow typically > 3.4), which favours sorption onto sludge solids. Previous studies have shown that estrogens exhibit strong partitioning to biosolids, limiting their presence in the aqueous phase during sludge treatment (Andersen et al., 2003; Carballa et al., 2004).

In anaerobic systems, the fate of natural estrogens (E1 and E2) is strongly influenced by microbially mediated redox reactions, particularly the reversible conversion:

$$E2 \rightleftharpoons E1$$

This interconversion is well documented and is governed by enzymatic oxidation–reduction mechanisms, with E2 being oxidized to E1 and, under certain conditions, E1 being reduced back to E2 ((Czajka and Londry, 2006). As a result, changes in the load of one compound cannot be interpreted as direct removal without considering transformation into its counterpart.

In contrast, EE2 is structurally more resistant due to its synthetic ethinyl group and therefore exhibits greater persistence and lower susceptibility to biodegradation.

In Set 1, both E1 and E2 exhibited notable differences between influent and digested sludge loads, suggesting active transformation processes during AD. The observed

patterns are consistent with interconversion between E1 and E2, rather than simple removal.

For example, reductions in E2 loads accompanied by elevated E1 levels may indicate oxidation of E2 to E1, a pathway commonly reported under anaerobic conditions. This transformation has been identified as a dominant mechanism controlling estrogen fate in sludge digestion systems (Czajka and Londry, 2006).

EE2, however, showed comparatively lower changes relative to influent levels, supporting its classification as a more recalcitrant compound. Any apparent decrease in EE2 may reflect limited biodegradation or dilution effects rather than complete mineralisation.

In Set 2, loads of E1, E2, and EE2 in digested sludge were generally comparable to influent levels, indicating limited net removal. This behaviour suggests that transformation and back-transformation processes reached a dynamic equilibrium, resulting in relatively stable concentrations. The absence of a clear removal trend may also be influenced by temporal variability in influent loading, and reactor mixing effects associated with SRT, which can obscure short-term transformation dynamics. Under such conditions, the system may maintain a pseudo-steady state, where production and consumption rates of E1 and E2 are balanced.

EE2 persistence in this set further supports previous findings that synthetic estrogens are less susceptible to anaerobic biodegradation, often remaining largely unchanged during AD (Carballa et al., 2004).

In Set 3, loads of E1, E2, and particularly EE2 in digested sludge were comparable to or higher than influent levels, indicating an apparent accumulation. This observation is best explained by sludge mass reduction during AD, rather than net production of these compounds.

As VS are converted to CH_4 and CO_2, the total mass of sludge decreases, resulting in the enrichment of residual micropollutants. For compounds such as EE2, which exhibit limited biodegradation, this effect is especially pronounced. Consequently, even if partial

transformation occurs, it may be offset by concentration effects associated with solids reduction.

For E1 and E2, the situation is further complicated by ongoing interconversion, which can redistribute mass between the two compounds without altering the total estrogenic load. Thus, increases in one compound may correspond to decreases in the other, rather than reflecting independent behaviour.

Overall, E1 and E2 exhibit dynamic but constrained behaviour, controlled largely by interconversion reactions, while EE2 behaves as a more persistent compound with minimal degradation. These findings highlight the importance of evaluating estrogen fate as a coupled system, rather than as independent compounds, and emphasize the need to consider transformation pathways and mass balance effects when interpreting AD performance.

As observed in Figure 3.11, NP was overwhelmingly associated with the solid phase, with only trace levels detected in the liquid fraction. This distribution is consistent with its high hydrophobicity (log Kow ≈ 4), which promotes strong sorption onto particulate organic matter. The affinity of NP for sludge solids is well established, and its partitioning behaviour significantly limits its mobility and bioavailability in aqueous environments (Ying et al., 2002)

In AD systems, NP fate is typically governed by a combination of adsorption to sludge solids, slow or incomplete anaerobic biodegradation, and formation from precursor compounds (e.g., ethoxylated surfactants). The latter pathway is particularly relevant, as anaerobic conditions can favour the de-ethoxylation of nonylphenol ethoxylates, leading to the formation of NP and thereby complicating the interpretation of concentration trends (Ahel et al., 1994).

In Set 1, NP loads in digested sludge were generally lower than or comparable to influent levels, suggesting no clear evidence of accumulation. This pattern may reflect a balance between ongoing formation from precursor compounds, and partial removal via sorption and limited biodegradation.

Given the complexity of NP transformation pathways, a reduction in measured NP loads does not necessarily imply net removal, but may instead indicate variations in precursor availability or transformation rates. Additionally, reactor mixing and the integration of sludge over the SRT may contribute to smoothing of load differences.

In Set 2, NP levels in the digested sludge were substantially higher than those in the influent sludge, indicating an apparent increase. This behaviour is consistent with two complementary mechanisms:

i. Formation from precursor compounds under anaerobic conditions, nonylphenol ethoxylates can undergo sequential shortening of ethoxy chains, ultimately yielding NP. This process has been widely reported in sludge treatment systems and can result in net production of NP, even in the absence of direct influent inputs (Ahel et al., 1994).

ii. Enrichment due to sludge mass reduction as AD progresses, the degradation of VS leads to a decrease in sludge mass, concentrating residual compounds such as NP. Given its resistance to rapid degradation, NP is particularly susceptible to this enrichment effect.

iii. Initial high reactor concentration (Carry-over effect): A baseline accumulation of residual NP within the reactor matrix from preceding periods may have also contributed to the elevated loads observed at the beginning of this set.

The combination of these mechanisms likely explains the pronounced increase observed in this set.

In Set 3, NP loads in digested sludge remained elevated relative to influent levels but showed greater variability compared to Set 2. This behaviour suggests that the system may have approached a more stable operational state, where precursor transformation rates, sorption equilibria, and sludge composition reached a relative balance. At this stage, the net NP load likely reflects the combined influence of residual formation and persistent retention, rather than active removal. Variations observed within this set may be linked to fluctuations in influent precursor concentrations, and minor changes in reactor conditions affecting transformation kinetics.

As shown in Figure 3.11, OP was primarily associated with the solid phase, with only negligible loads detected in the liquid phase. Paralleling the trends documented for NP, this distribution is consistent with its high hydrophobicity (log Kow > 4), which promotes strong binding to organic matter within sludge. Such behaviour limits its mobility and reduces its accessibility to microbial degradation processes (Ying et al., 2002).

Consistent with the trends observed for NP, the environmental fate of OP under anaerobic conditions is primarily governed by strong partitioning onto sludge solids and inherent metabolic recalcitrance. A critical factor in the anaerobic mass balance of OP—paralleling the behavior of NP—is the potential for secondary formation via the microbially mediated cleavage of parent polyethoxylate chains (Octylphenol ethoxylates). As established in previous studies on alkylphenolic compounds, anaerobic environments facilitate the sequential shortening of these ethoxylate units, which may result in an apparent increase in OP concentrations that complicates the interpretation of primary biodegradation rates (Ahel et al., 1994). Consequently, the observed mass load of OP in the digester must be evaluated not only as a function of its own degradation kinetics but also as a terminal metabolite of its precursor surfactants.

In Set 1, OP loads in digested sludge were generally lower than influent levels, indicating no clear evidence of accumulation. This pattern may reflect a balance between ongoing formation from precursor compounds, and retention within the sludge matrix.

The relatively moderate digested sludge loads observed suggest that OP dynamics during this period were influenced by variations in precursor availability, as well as reactor mixing effects that tend to homogenize concentration differences over the SRT.

In Set 2, OP loads in digested sludge were consistently higher than influent values, indicating an apparent increase. This behaviour can be attributed to two main mechanisms:

i. Transformation from precursor compounds the anaerobic degradation of octylphenol ethoxylates can result in the formation of OP, leading to net production within the digester.

ii. Concentration due to sludge mass reduction as VS are converted to CH_4 and CO_2, the overall sludge mass decreases, resulting in enrichment of persistent compounds such as OP.

The combined influence of these processes explains the elevated loads observed in this set.

In Set 3, OP loads in digested sludge were generally lower than those observed in Set 2, and in some cases approached influent levels. This behaviour suggests a shift toward more stable system conditions, where precursor transformation rates may have decreased, and the balance between formation and retention reached equilibrium.

Despite this reduction, OP remained present in the solid phase, indicating continued persistence and limited biodegradation. Variations within this set may be linked to fluctuations in influent composition, and minor operational differences affecting transformation pathways.

Combined Interpretation of NP and OP

The behaviours of NP and OP during AD exhibit strong similarities, reflecting their shared origin as alkylphenolic surfactant degradation products. Both compounds are characterised by: (Ying et al., 2002)

i. high hydrophobicity and strong sorption to sludge solids,
ii. limited biotransformation under anaerobic conditions, and

potential formation from ethoxylated precursors.

However, their observed load patterns suggest that in situ formation plays a particularly significant role, especially in operational periods where elevated levels in digested sludge cannot be explained solely by influent inputs or sludge mass reduction. The transformation of alkylphenol ethoxylates under anaerobic conditions is therefore a key pathway governing their fate.

Overall, NP and OP differ from many other OMPs in that their behaviour is not only controlled by persistence and partitioning, but also by ongoing generation within the system, which may lead to apparent accumulation even in the absence of external loading. These findings highlight the importance of considering precursor-product relationships when evaluating the fate of surfactant-derived compounds in AD systems.

Beyond the compound-specific mechanisms discussed above, a consistent pattern observed for several OMPs in Set 3 requires further interpretation. Although enrichment effects associated with VS destruction can theoretically occur throughout AD, their manifestation depends on the dynamic balance between sludge mass reduction and compound attenuation processes. In the earlier operational sets, this balance was likely influenced by simultaneous biodegradation, transformation reactions, and temporal variability in influent loading, which collectively offset the concentration effect and resulted in digested sludge loads that remained comparable to or lower than influent values.

In contrast, Set 3 appears to represent a more stabilised phase of digestion, in which reactor performance became more consistent and transient effects diminished. Under these conditions, persistent or only partially degradable OMPs remained associated with the solid phase, while the continued conversion of VS to CH_4 and CO_2 led to a progressive reduction in total sludge mass. As a result, the relative influence of sludge mass reduction became more pronounced than that of biodegradation, allowing the concentration effect to emerge more clearly.

Furthermore, due to the SRT, the digested sludge reflects a time-integrated mixture of previously introduced material, meaning that residual OMP mass from earlier operational periods may contribute to the observed loads. This temporal decoupling between influent and digested sludge further reinforces the apparent enrichment. Therefore, the slight increase in OMP loads observed in Set 3 does not indicate net formation of these compounds, but rather reflects the combined effects of sludge stabilization, persistent solid-phase retention, limited biodegradation, and cumulative system dynamics.

3.8. Engineering Implications and Sustainability Assessment

3.8.1. Technical Viability of Energy-Neutrality via HiCS–AD Integration

Conventional Activated Sludge processes are inherently energy-intensive due to the high aeration demand required for the mineralization of organic carbon. The findings of this study demonstrate that the HiCS configuration promotes carbon capture by diverting organic matter into the sludge phase rather than oxidizing it.

Energy Balance Perspective

Based on the specific methane yield values (L CH_4 g^{-1} VS) obtained from the anaerobic digestion experiments, the sludge generated from the HiCS system exhibited a higher energy recovery potential compared to conventional sludge streams. When combined with the reduced aeration demand associated with short SRT operation, these findings indicate that the HiCS–AD configuration can improve the overall energy balance of wastewater treatment systems, supporting a transition toward energy-neutrality.

Infrastructure Optimization

The stable operation of the HiCS system at low HRT (≈ 3.5 hour) and SRT (≈ 1 day), compared to CAS systems typically operating at SRTs of 13–15 days, indicates that effective carbon redirection can be achieved with limited biomass retention requirements. This allows more intensive use of reactor volumes and provides the potential to accommodate higher hydraulic loads within existing infrastructure, without proportional increases in footprint.

3.8.2. Strategic Trade-off: Carbon Capture Efficiency vs. OMP Management

From an engineering design perspective, a critical trade-off was identified between maximizing carbon capture for energy recovery and the management of OMPs**.**

Accumulation Dynamics

Experimental results demonstrated that hydrophobic OMPs—particularly BPA, NP, and ciprofloxacin—were predominantly removed via sorption and associated with the sludge phase. This indicates that increased carbon capture is intrinsically linked to the enrichment of specific micropollutants within the biomass. While this mechanism improves effluent quality, it simultaneously increases the micropollutant load in the sludge stream.

Design Implications

For facilities where treated sludge is intended for land application, this accumulation may represent an environmental and regulatory concern. Therefore, sludge management should be considered as an integral part of process design. In such cases, the integration of advanced stabilization processes, such as thermal hydrolysis or pyrolysis, may be required to reduce potential risks associated with micropollutant persistence.

3.8.3. Operational Road Map and Digital Control Strategies

The implementation of the HiCS–AD configuration requires precise operational control to maintain performance and stability.

Precision Control of SRT

Maintaining an SRT of approximately 1 day is critical for sustaining high carbon capture while preventing system instability. Achieving this in full-scale applications requires reliable monitoring and automation, including online turbidity or total suspended solids (TSS) sensors coupled with controlled sludge wastage strategies.

Process Adaptability

Seasonal variations in wastewater characteristics and methane production may influence system performance. Therefore, flexible operational strategies are required to adapt to fluctuating organic loads and support consistent energy recovery.

3.8.4. Integration with Advanced Treatment and Future Considerations

In alignment with emerging EU directives on quaternary treatment, the HiCS process can function as an efficient primary stage when integrated with advanced technologies for comprehensive OMP removal.

Water Reuse and Nutrient Recovery

When treated wastewater is intended for agricultural irrigation, the HiCS process preserves residual nitrogen and phosphorus. To meet reuse standards, post-treatment methods such as advanced oxidation, adsorption, or membrane filtration may be integrated to target remaining OMPs while maintaining nutrient recovery potential.

In addition to micropollutant removal, nutrient management in HiCS-based systems should be defined based on the discharge or reuse scenario. Since the HiCS process primarily focuses on carbon capture under short SRT conditions, nitrogen and phosphorus are not specifically targeted for removal within this stage.

When treated effluent is intended for discharge into receiving water bodies, compliance with regulatory limits requires the implementation of an additional nutrient removal stage. In such cases, biological nutrient removal processes or alternative nitrogen removal pathways should be integrated downstream of the HiCS system.

In contrast, for water reuse applications such as agricultural irrigation, the presence of residual nitrogen and phosphorus may be beneficial. Under these conditions, additional nutrient removal may not be necessary, provided that effluent quality meets reuse standards.

Therefore, the design of HiCS-based treatment systems should follow a scenario-based approach, where nutrient removal requirements are determined according to the intended end-use of the treated effluent.

Sludge Fate and Transformation:

As anaerobic digestion follows the HiCS process, the fate of sludge-associated OMPs, particularly their degradation, transformation, or persistence, remains an important consideration. Understanding these pathways is essential for balancing energy recovery with safe and sustainable sludge management.

4. CONCLUSION

This study investigated the behaviour and removal mechanisms of OMPs in a pilot-scale HiCS system and evaluated the methane production potential of the resulting sludge during anaerobic digestion. The results showed that the removal of the ten investigated OMPs in the HiCS process was strongly compound-specific and mainly governed by the physicochemical properties of the compounds and the operational characteristics of high-rate activated sludge systems.

Mass balance analysis revealed that both sorption and biotransformation contribute to OMP removal, with hydrophobic compounds predominantly associating with sludge solids, while more polar compounds are mainly removed through biotransformation. Batch sorption experiments further confirmed that sorption alone cannot fully explain the removal behaviour of all compounds.

Since OMPs may enter PS–HiCS systems in both dissolved and particulate-associated forms, representative estimation of influent OMP loads requires consideration of both fractions. The presence of primary sedimentation together with the different sludge withdrawal configurations in the conventional and modified PS–HiCS systems required the development of configuration-specific mass balance approaches. This approach enabled the quantification of OMP partitioning between aqueous and solid phases and provided mechanistic insight into the relative contributions of sorption and biotransformation.

Based on the observed removal efficiencies under conventional HiCS operation, the investigated OMPs could be classified into three groups according to their removal behaviour. Compounds exhibiting high removal efficiencies (>60%) included CIPRO (84 ± 18%), IBU (74 ± 13%), NP (72 ± 10%), and DICLO (61 ± 18%). Moderate removal efficiencies (30–60%) were observed for BPA (59 ± 7%), OP (55 ± 15%), E1 (38 ± 16%), and E2 (38 ± 14%). Persistent compounds (<30%) included EE2 (27 ± 17%) and CBZ (23 ± 21%), indicating limited removal under the short SRT conditions characteristic of the HiCS process.

Based on mass balance analysis under conventional HiCS operation, different removal pathways were identified for the investigated compounds. CIPRO, BPA, and OP were predominantly removed through sorption-driven mechanisms due to their strong association with sludge solids, whereas IBU removal was mainly attributed to biotransformation processes. NP, DICLO, E1, and E2 exhibited mixed behaviour, indicating that both sorption and biotransformation contributed to their removal. In contrast, CBZ and EE2 showed persistent behaviour, with limited evidence of either sorption- or biotransformation-driven removal under the investigated HiCS conditions.

Within the scope of this study, mass balance approaches were developed to evaluate OMP fate in the conventional and modified PS–HiCS configurations. The inclusion of primary sedimentation in the PS–HiCS system required the consideration of primary sludge within the mass balance framework, while the combined sludge withdrawal in the modified configuration necessitated further adaptation of the approach. The application of these approaches enabled the quantification of OMP partitioning between the aqueous and solid phases. The comparison of both configurations demonstrated that process modification affected not only overall removal efficiencies but also the distribution behaviour of several compounds, providing mechanistic insight into the relative contributions of sorption and biotransformation.

Operational conditions were found to play a key role in determining removal behaviour. In contrast to conventional activated sludge systems, shorter SRTs in the HiCS process were associated with enhanced removal of several compounds. This highlights the distinct removal dynamics of high-rate systems, where elevated substrate availability and metabolically active heterotrophic communities may support rapid transformation processes.

The potential influence of EPS on OMP removal was also evaluated; however, no consistent relationship between EPS levels and OMP removal was observed within the investigated range.

In addition to the conventional HiCS configuration, a modified system was evaluated in which the stabilization sludge was recirculated to the mixing tank prior to primary

sedimentation. This modification was developed to enhance carbon capture and increase the transfer of biodegradable organic matter to the sludge phase for subsequent energy recovery. The results demonstrated that the modified configuration successfully improved carbon capture within the system. However, its influence on OMP removal remained limited. Moderate increases in sorption-related removal were observed for E1, E2, IBU, DICLO, and CBZ, with improvements ranging between approximately 5 and 11%. In contrast, BPA, NP, and CIPRO did not show statistically significant changes in overall removal efficiency. These findings indicate that the modified configuration primarily influenced the distribution of OMPs between the liquid and solid phases rather than substantially improving their overall removal.

Anaerobic digestion experiments demonstrated that HiCS-derived sludge exhibited a relatively high methane production potential, with an average methane yield of 0.36 ± 0.10 L CH_4/g VS. This result indicates that high-rate treatment processes effectively capture organic carbon in the sludge phase and preserve biodegradable organic matter for downstream energy recovery.

OMP evaluation during anaerobic digestion required a different assessment approach because the completely mixed reactor operation and sludge retention time prevented direct influent–effluent comparisons. Therefore, OMP behaviour was evaluated based on phase distribution and temporal changes during complete SRT periods. The behaviour of OMPs during anaerobic digestion remained compound-specific. Most investigated compounds were predominantly associated with the solid phase, while liquid-phase concentrations remained consistently low. CIPRO, NP, and OP exhibited strong solid-phase retention, indicating limited bioavailability under anaerobic conditions. CBZ and EE2 showed persistent behaviour with limited evidence of degradation, whereas DICLO exhibited reduced solid-phase loads, suggesting the occurrence of transformation processes during digestion. BPA and IBU demonstrated variable behaviour among operational sets, reflecting the combined influence of partial biodegradation, sludge mass reduction, and reactor mixing effects. The natural estrogens E1 and E2 exhibited dynamic behaviour associated with reversible transformation processes. Overall, anaerobic digestion primarily altered the phase distribution and

transformation pathways of OMPs rather than achieving complete elimination of the investigated compounds.

Overall, the findings demonstrate that high-rate treatment systems such as HiCS can enhance carbon capture and support energy recovery, while micropollutant removal remains inherently compound-dependent. These results contribute to a better understanding of micropollutant fate in high-rate, resource-oriented wastewater treatment systems.

From an engineering perspective, the findings of this study highlight that HiCS-based systems should be designed as integrated treatment configurations, where carbon capture, energy recovery, and micropollutant management are considered simultaneously. While enhanced carbon capture improves energy recovery, it also promotes the transfer of certain micropollutants to the sludge phase, introducing a trade-off that must be addressed in sludge management strategies.

Furthermore, nutrient removal requirements should be defined based on the intended use of the treated effluent. For discharge into receiving water bodies, additional nutrient removal processes are required to meet regulatory limits. In contrast, for water reuse applications such as agricultural irrigation, the retention of nitrogen and phosphorus may be beneficial. In this context, the integration of quaternary treatment technologies may be necessary to achieve advanced micropollutant removal, particularly under strict discharge or reuse standards.

In addition, the operation of HiCS systems at short sludge retention times provides potential advantages in terms of reduced reactor volume requirements and increased treatment capacity within existing infrastructure.

Overall, these findings emphasize the importance of scenario-based design approaches to balance energy recovery, effluent quality, and environmental protection in modern wastewater treatment systems.

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APPENDIX A

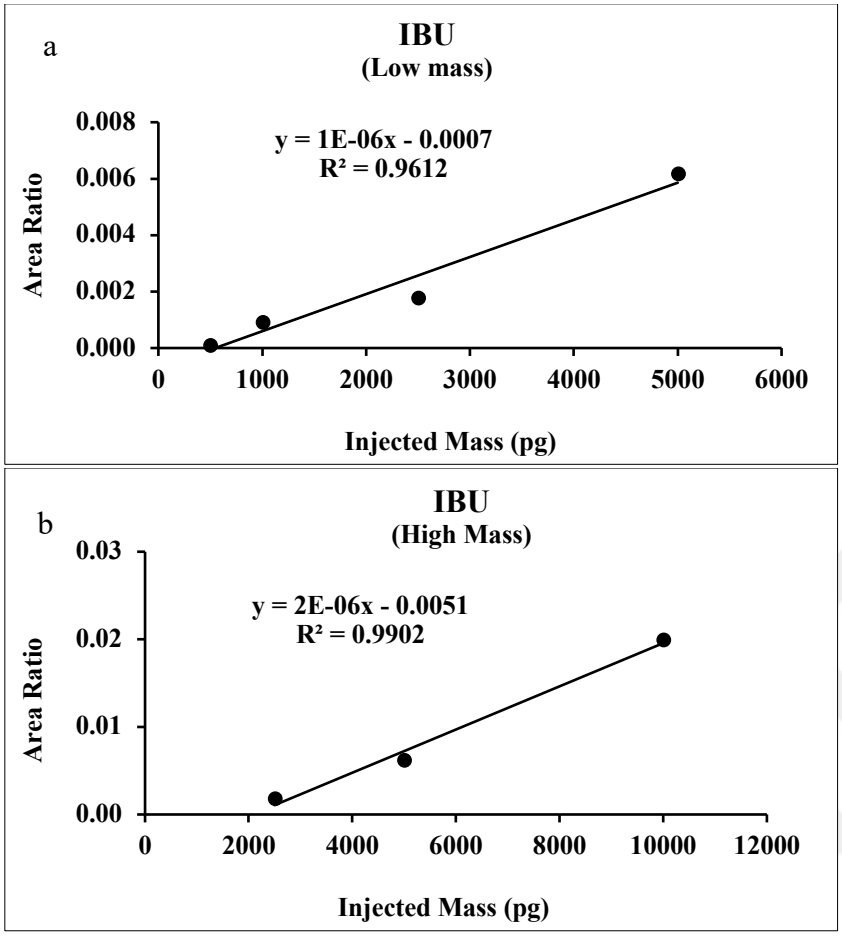
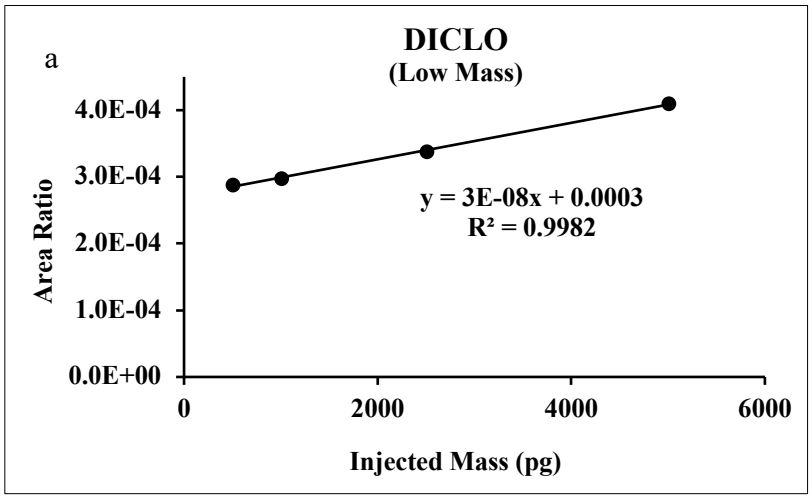


Figure A.1. IBU Calibration Curves: (a) Low Mass, (b) High Mass



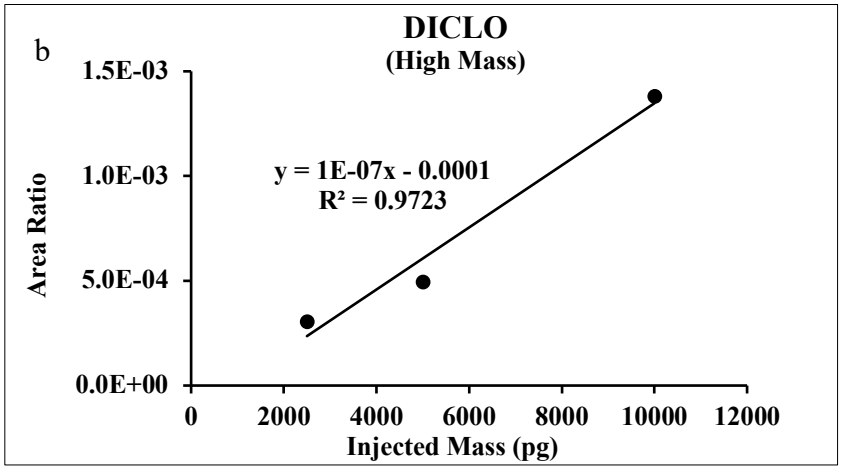


Figure A.2. DICLO Calibration Curves: (a) Low Mass, (b) High Mass

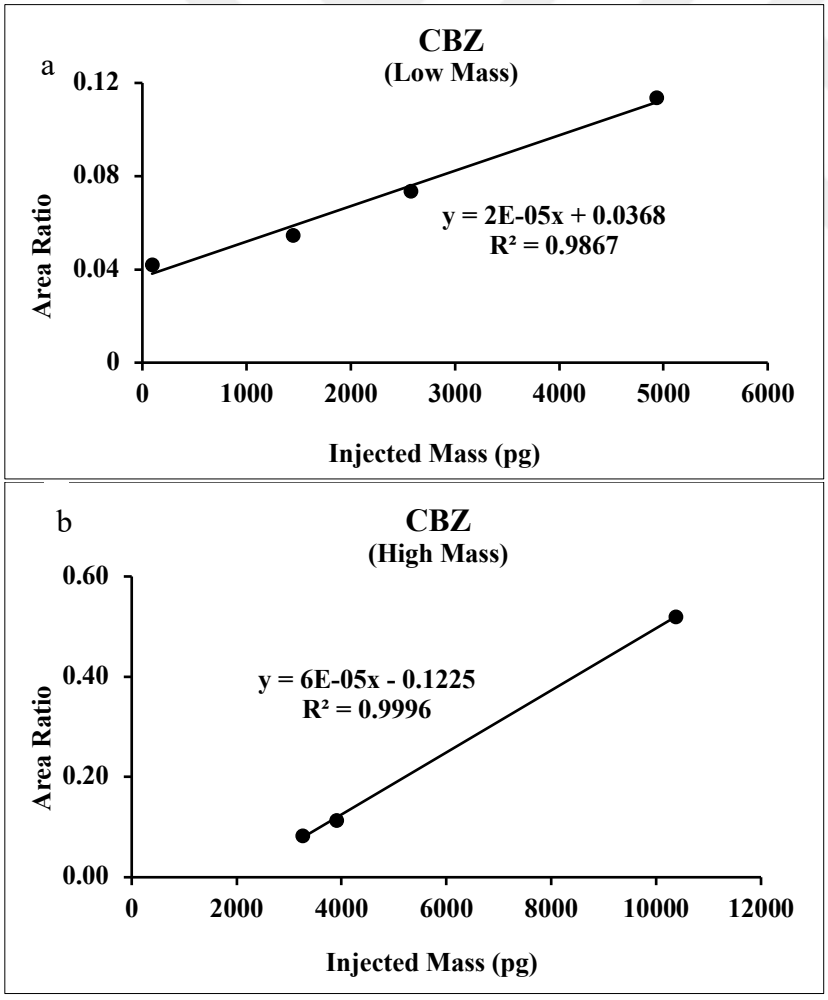


Figure A.3. CBZ Calibration Curves: (a) Low Mass, (b) High Mass

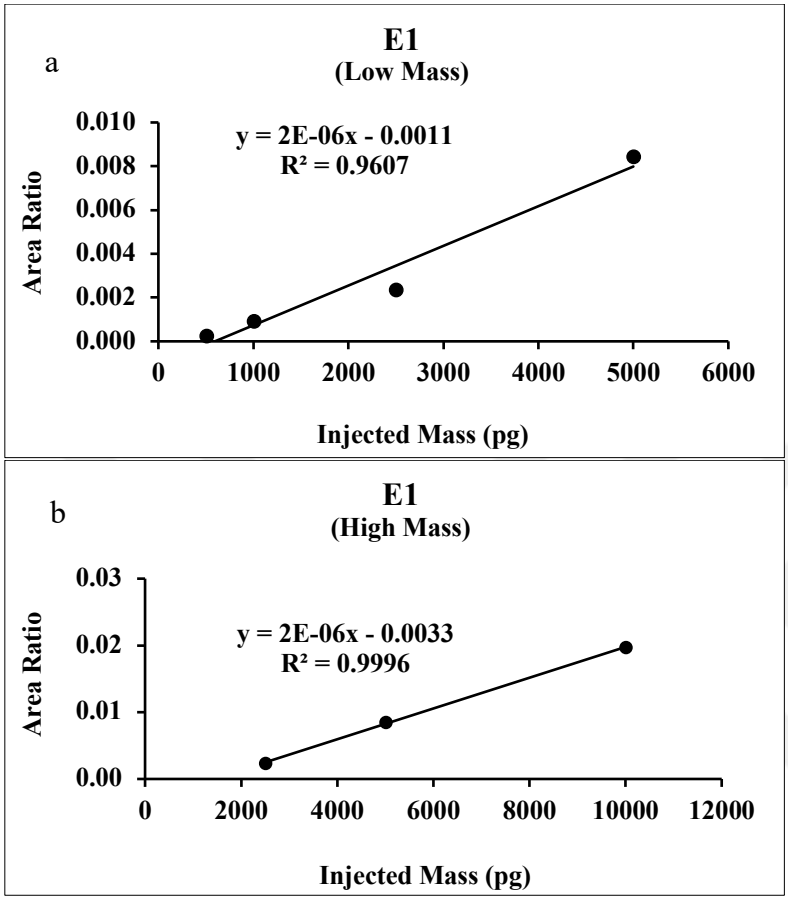
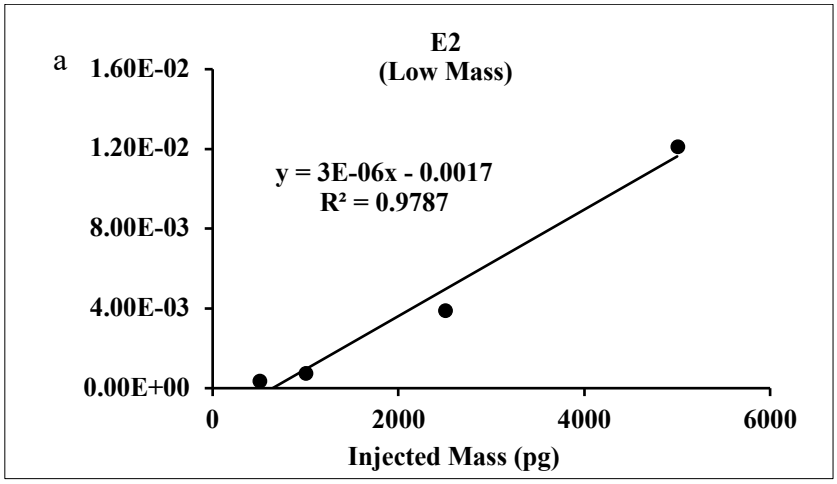


Figure A.4. E1 Calibration Curves: (a) Low Mass, (b) High Mass



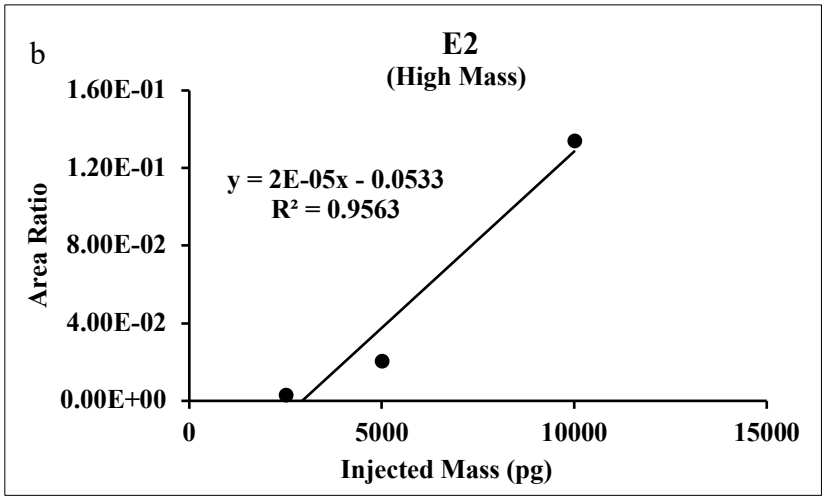


Figure A.5. E2 Calibration Curves: (a) Low Mass, (b) High Mass

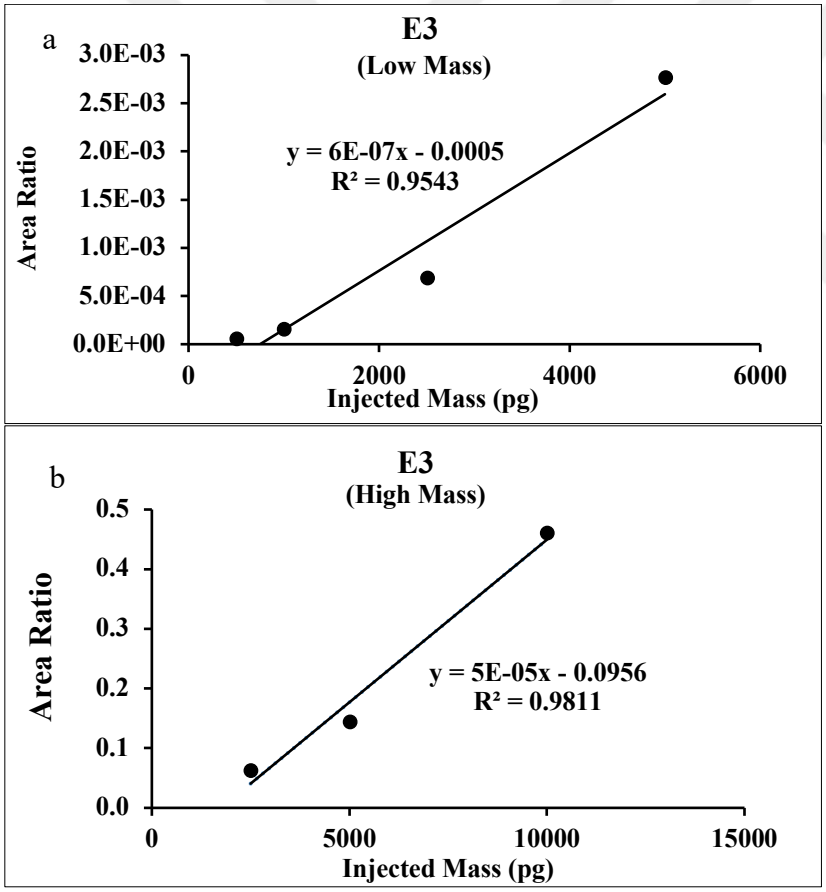


Figure A.6. E3 Calibration Curves: (a) Low Mass, (b) High Mass

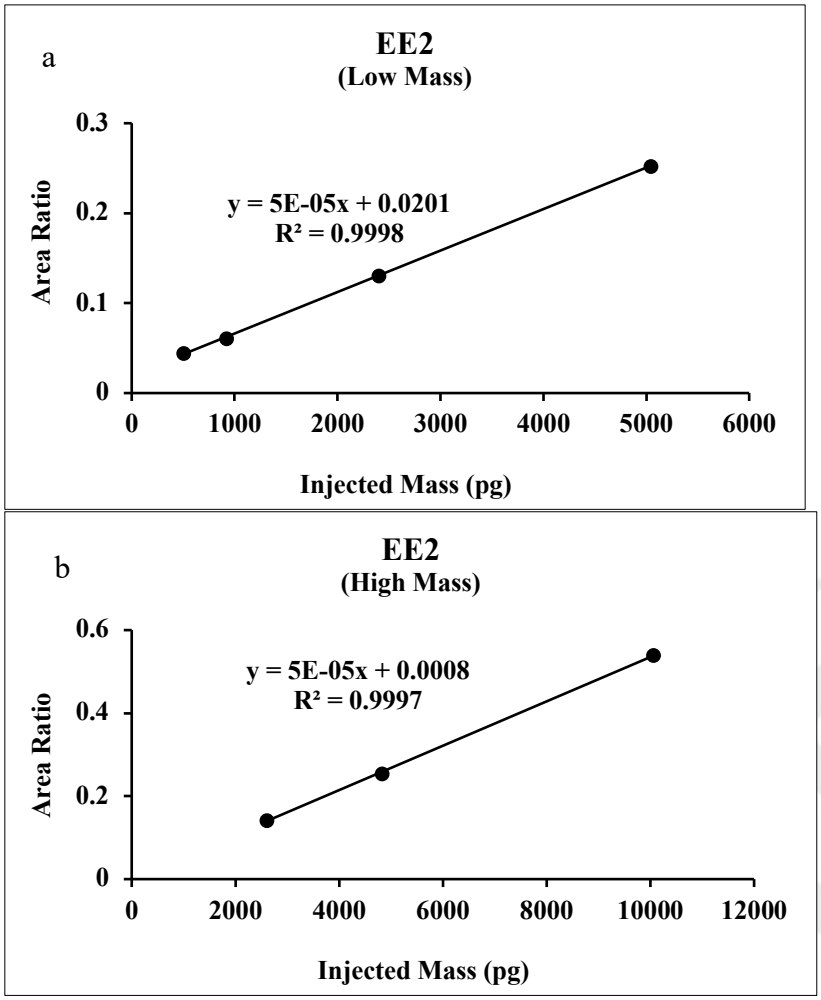
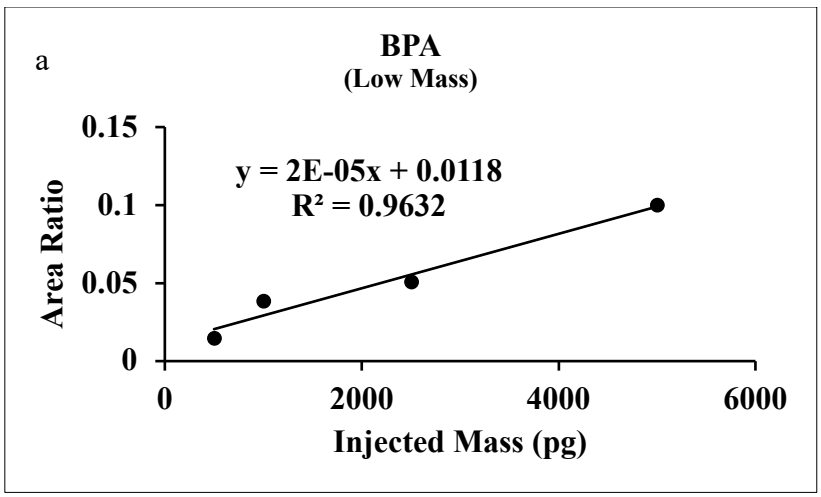


Figure A.7. EE2 Calibration Curves: (a) Low Mass, (b) High Mass



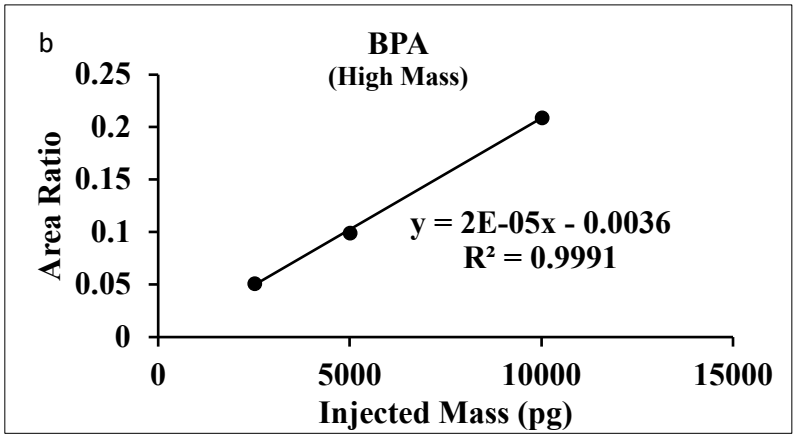


Figure A.8. BPA Calibration Curves: (a) Low Mass, (b) High Mass

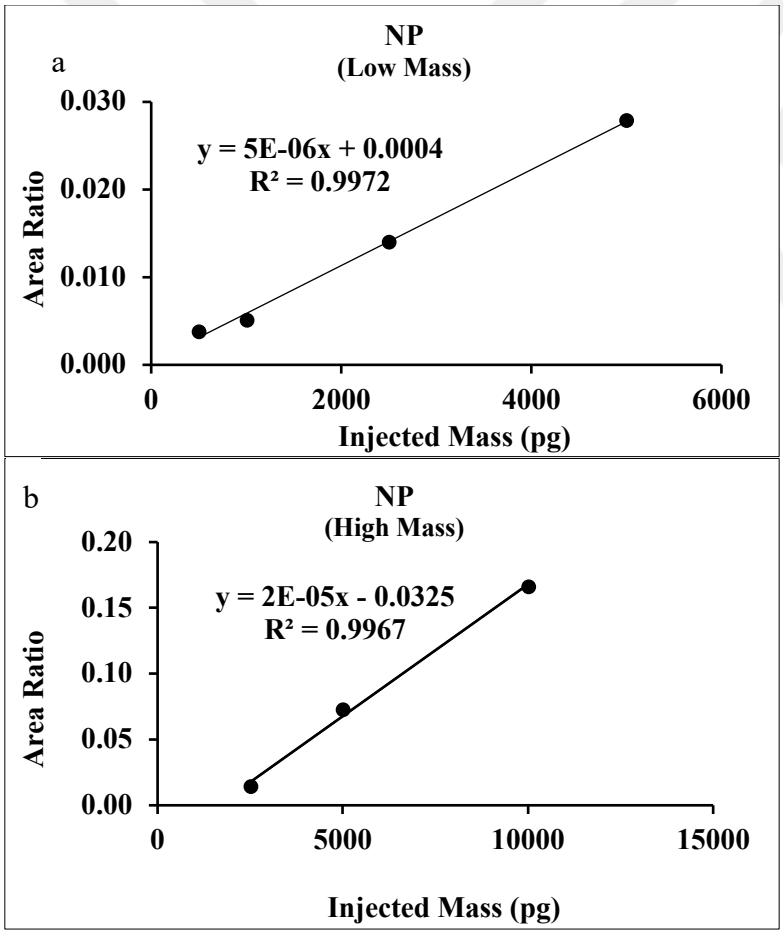


Figure A.9. NP Calibration Curves (a) Low Mass, (b) High Mass

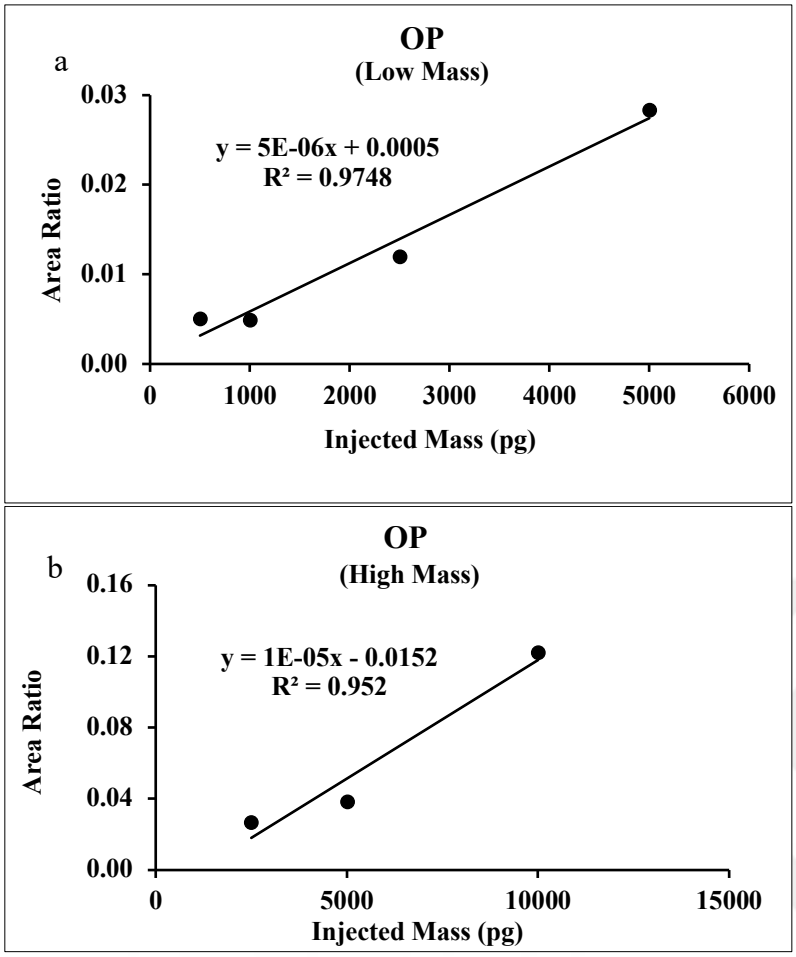


Figure A.10. OP Calibration Curves (a) Low Mass, (b) High Mass

Table A.1. Instrumental LOQ values determined from the lowest calibration level and corresponding signal-to-noise (S/N) ratios

Compound	Instrumental LOQ (pg injected)
BPA	492
CIPRO	199
NP	382
OP	519
E1	449
E2	570
E3	779
EE2	557
IBU	801
DICLO	440
CBZ	584

CURRICULUM VITAE

Name: Dilara SANCAR

Education

2021 – . **PhD in Environmental Engineering**

Marmara University, Istanbul, Türkiye

GPA: 3.67 / 4.00

The Fate of Organic Micropollutants in High-Rate Contact Stabilization Process Integrated with Anaerobic Digestion

2018 – 2019. **Master in Engineering Management**

Galatasaray University, Istanbul, Türkiye

GPA: 3.95 / 4.00

Application Of Analytic Hierarchy Process In A New Line Of Bus Selection For Public Transportation

2014 – 2017. **Master in Environmental Science and Engineering**

Istanbul Technical University, Istanbul, Türkiye

GPA: 3.75 / 4.00

Treatment Of Municipal Wastewater And Organic Waste By A Pilot Scale High Rate Activated Sludge System

2014 – (thesis phase) **Master in Hydraulic and Water Resources Engineering**

Istanbul Technical University, Istanbul, Türkiye

GPA: 3.56 / 4.00

2007 – 2012 **Bachelor in Environmental Engineering**

Marmara University, Istanbul, Türkiye

GPA: 3.13 / 4.00

Anammox- Zeolite Process

Projects

Innovative, Energy-Positive Wastewater Treatment Process for Removal of Macro and Micro Organic Pollutants - *TÜBİTAK Project, Marmara University (2021–2024)*

The energy-efficient disposal of organic solid waste in domestic wastewater treatment systems - *SAN-TEZ Project, Istanbul Technical University (2014–2017)*

In hydrological basins: low flows and drought -*TUJJB Project, Istanbul Technical University (2015–2016)*

Publications

Sancar, D., Hacıosmanoğlu, G.G., Can, Z.S., Çallı, B., Semerci, N. (2026)
Pilot-Scale Insights into Micropollutant Removal in Modified High-Rate Contact Stabilization Process Treating Municipal Wastewater. Bioresource Technology

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A pilot-scale high-rate contact stabilization process with primary sedimentation for energy-neutral wastewater treatment. Process Safety and Environmental Protection

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Presentations in International Conferences

Sancar, D., Hacıosmanoğlu, G.G., Can, Z.S., Semerci, N. (2025) -Evaluation of the effect of solid retention time on organic micropollutant removal efficiency in a pilot-scale high-rate contact stabilization system. Stockholm, Sweden

Demir, E.K., Sancar, D., Şahinkaya, E., Çallı, B., Semerci, N. (2022) - A pilot scale modified high-rate contact stabilization process for energy-neutral wastewater treatment. Istanbul, Türkiye

Aksoy, H., Sancar, D., Eriş, E. (2017)
Hydrology and sediment characteristics of Mediterranean watersheds in Turkey.Tunisia.