ADSORPTION PRETREATMENT OF HYDROTHERMAL LIQUEFACTION AQUEOUS
STREAM FROM MIXED SLUDGE FOR ENHANCED BIOLOGICAL TREATMENT

by

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** ADSORPTION PRETREATMENT OF HYDROTHERMAL LIQUEFACTION AQUEOUS STREAM FROM MIXED SLUDGE FOR ENHANCED BIOLOGICAL TREATMENT **

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Abstract

Mixed sludge poses challenges due to its high volume, organic and inorganic hazardous components. Anaerobic digestion (AD) has been a conventional method for treatment and methane recovery from sludge; however, it suffers from low organic conversion efficiency and demands significant investments. Hydrothermal liquefaction (HTL) is a technology for biocrude oil generation from organic waste. However, HTL produces secondary products, such as an aqueous phase, solid waste (hydrochar), and predominantly carbon dioxide gas. Proper management of the aqueous by-product is crucial for commercializing HTL technology for sludge treatment. Given the high volume and soluble organic compounds that accumulate in sludge-derived HTL aqueous, on-site biological treatment represents a low-cost downstream treatment. However, the aqueous contains HTL by-products of protein, carbohydrate, and lipids in feedstock (sludge) with significant inhibition potential to downstream biological treatment processes.

This thesis investigated adsorption as a pretreatment to remove inhibitory compounds in HTL aqueous by-product. The research is divided into two parts: 1) testing biochar, hydrochar from the HTL process, and granular activated carbon (GAC) to find an optimum adsorbent and adsorption conditions, and 2) assessing adsorption effect on downstream anaerobic and aerobic biological treatment processes. GAC demonstrated superior adsorption capability (184 mg/g) for chemical oxygen demand (COD), outperforming biochar (44 mg/g) and hydrochar (42 mg/g). The adsorption of COD onto adsorbents followed the Freundlich isotherm and pseudo-second-order kinetics. The adsorption process was favored by higher temperatures and a neutral pH environment. GAC exhibited high efficiency in removing potentially inhibitory compounds, including COD (up to 66%), total phenolics (up to 94%), and cyclic ketones (up to 95%). Simultaneously, it retained valuable volatile fatty acids for downstream methane recovery. At GAC concentrations of 5-100 g/L, compared to un-pretreated HTL aqueous, methane yield enhancements were 8-97% and 10-83% under mesophilic and thermophilic conditions,
respectively. As the GAC concentration increased from 5 to 100 g/L, the removal of biochemical oxygen demand (BOD) also increased from 11 to 50%. Furthermore, the BOD/COD ratio of HTL aqueous increased from 0.81 ± 0.01 to 0.93 ± 0.03. The results suggested that adsorption is a suitable pretreatment for HTL aqueous for enhanced biological treatment.
Mixed sludge is the term used for combining different types of waste slurries generated during the treatment of municipal wastewater at treatment plants. Disposing of this sludge, without treatment, can have negative effects on the environment. However, hydrothermal liquefaction is an alternative technology that allows extracting valuable products from sludge, such as biofuels and nutrients. This process is similar to the natural occurrence of petroleum but is faster and occurs in engineered reactors at high temperatures and pressures. However, there is a liquid waste stream from hydrothermal liquefaction, which also contains potentially hazardous compounds. This study focused on finding a suitable adsorbent and optimum conditions for removing hazardous compounds from the liquid stream of hydrothermal liquefaction. As adsorbent, the study assessed biochar, hydrochar, and granular activated carbon. The results have significant benefits for integrating the hydrothermal liquefaction process to the future municipal wastewater treatment plants for advanced resource recovery.
Preface

The author of this thesis was primarily responsible for the conception, designing the study, conducting experiments, analyzing the data, and preparing the thesis. Throughout the process, the author received assistance and feedback from the supervisor, Dr. Cigdem Eskicioglu. Additionally, Dr. Huan Liu, a previous Ph.D. student at the Bioreactor Technology Group (BTG), helped to run the bench-scale high temperature/pressure HTL reactor to generate aqueous phase and hydrochar. A Ph.D. candidate at BTG, Ibrahim Alper Basar, contributed to the biological assessment of HTL aqueous samples. Jacky Takeuchi, the research technician at BTG, helped with the analysis of organic compounds using GC-MS. The instrumental analysis of FT-IR, TGA, and BET for adsorbents was conducted by research staff at Akdeniz University (Turkiye) under the supervision of Dr. Meltem Asilturk Ersoy. This research has led to two peer-reviewed journal articles and a conference paper.

- The findings in Chapter 3 have been published: Aktas, K., Liu, H. and Eskicioglu, C. Treatment of aqueous phase from hydrothermal liquefaction of municipal sludge by adsorption: Comparison of biochar, hydrochar, and granular activated carbon. Journal of Environmental management. 356 (2024) 120619.

- The findings in Chapter 4 are in the process of submission to a journal. Aktas, K., Liu, H., Basar, I. A. and Eskicioglu, C. Enhanced biological treatment of aqueous phase from hydrothermal liquefaction of mixed sludge by GAC adsorption.

The findings have been accepted for presentation at the upcoming conference:

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<tbody>
<tr>
<td>AD</td>
<td>Anaerobic digestion</td>
</tr>
<tr>
<td>ASCE</td>
<td>American society of civil engineers</td>
</tr>
<tr>
<td>ASTM</td>
<td>American society for testing and materials</td>
</tr>
<tr>
<td>BC</td>
<td>British Columbia</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer–Emmett–Teller</td>
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<tr>
<td>BJH</td>
<td>Barrett-Joyner-Halenda</td>
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<tr>
<td>BMP</td>
<td>Biochemical methane potential</td>
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<tr>
<td>BOD</td>
<td>Biochemical oxygen demand</td>
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<td>CAS</td>
<td>Conventional activated sludge</td>
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<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
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<tr>
<td>EPA</td>
<td>Environmental protection agency</td>
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<tr>
<td>EC</td>
<td>Electrical conductivity</td>
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<tr>
<td>FT-IR</td>
<td>Fourier-transform infrared spectroscopy</td>
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<td>GAC</td>
<td>Granular activated carbon</td>
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<tr>
<td>GC-FID</td>
<td>Gas chromatography with flame ionization detection</td>
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<tr>
<td>GC-MS</td>
<td>Gas chromatography with mass spectrometry</td>
</tr>
<tr>
<td>GC-TCD</td>
<td>Gas chromatography with thermal conductivity detector</td>
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<tr>
<td>HTL</td>
<td>Hydrothermal liquefaction</td>
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<tr>
<td>HTC</td>
<td>Hydrothermal carbonization</td>
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<td>HTG</td>
<td>Hydrothermal gasification</td>
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<tr>
<td>MSD</td>
<td>Mass-selective detection</td>
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<tr>
<td>NSERC</td>
<td>Natural Sciences and Engineering Research Council of Canada</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>STP</td>
<td>Standard temperature and pressure</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
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<td>TSS</td>
<td>Total suspended solids</td>
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<tr>
<td>UNEP</td>
<td>United Nations Environment Program</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VFAs</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solids</td>
</tr>
<tr>
<td>VSS</td>
<td>Volatile suspended solids</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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I would like to express my gratitude to Dr. Cigdem Eskicioglu, my research supervisor, for her support and valuable insights throughout my thesis. I would also like to extend my thanks to my committee members, Dr. Nicolas Peleato and Dr. Alyse Kiesser. I would like to thank Ibrahim Alper Basar, Dr. Huan Liu, Jacky Takeuchi, and other Bioreactor Technology Group (BTG) members and alumni for their help and support. I am thankful to Dr. Meltem Asilturk Ersoy for helping me with instrumental materials analysis, the School of Engineering for technical support, and Metro Vancouver for their partnership. Lastly, I wish to express my profound gratitude to my family and friends whose unwavering support and encouragement made this research possible.

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Dedication

To my grandfather.
Chapter 1: Introduction

This chapter lays a foundation for understanding the thesis topic of the effect of adsorption as a pretreatment option for downstream biological treatment of hydrothermal liquefaction (HTL) aqueous. The aqueous stream is generated from HTL of mixed sludge, which is a combination of primary and secondary sludge at municipal wastewater treatment plants (WWTPs). The chapter also discusses the background for this research, along with motivation and novelty. Thesis objectives are also presented at the end of the chapter.

1.1 Background

Treatment plants eliminate organic and inorganic matter, nutrients, and pathogens from wastewater. However, this process generates a significant amount of solids known as mixed sludge, which have to be treated and disposed of safely. In a treatment plant, primary treatment removes settleable solids, forming primary sludge, while secondary biological treatment breaks down remaining organic materials with microorganisms that settle as secondary sludge. In large cities, anaerobic digestion (AD) is frequently used to stabilize sludge, reduce organic matter and pathogens like fecal coliforms, and recover energy. Despite these treatments, the resulting digested sludge still poses disposal challenges due to its high volume and hazardous components, such as pathogens, heavy metals, antibiotics, etc. Managing sludge constitutes a significant portion of a treatment plant operating cost, accounting for 40-50% (Liu, 2023; Seiple et al., 2017). Current sludge management methods include land application of stabilized sludge, landfilling, and incineration. Developed countries often favor land application, but it has drawbacks, including long stabilization times, and public opposition due to residual pollutants. Incineration, practiced in certain European countries, is costly and not universally applicable due to high energy demands and infrastructure costs. HTL stands out as a promising solution as it converts mixed sludge into biocrude, a low-carbon fuel, using hot pressurized water without the
need for pre-drying while operating under subcritical conditions and a short residence time (Thomsen et al., 2020; Liu, 2023; Mathimani and Mallick, 2019; Marrone et al., 2018). Four distinct streams are generated through HTL of mixed sludge after phase separation. This thesis focuses on treatment of aqueous stream of HTL but also utilizes another HTL stream, hydrochar, as an adsorbent to remove pollutants in HTL aqueous.

1.2 Research motivation

Metro Vancouver, located on the coast of British Columbia, Canada, is experiencing rapid growth. Currently, it operates five WWTPs and produces around 55,000 tons of sludge every year. However, due to expansions, this production is projected to rise to 100,000 tons per year by 2026 and 150,000 tons per year by 2050 (Liu, 2023). Although AD is commonly used for sludge treatment, it is a slow (biological) process and has only 60% organics conversion efficiency. In response to this challenge, Metro Vancouver has been planning a pilot-scale HTL unit at Annacis Island WWTP.

HTL produces secondary products (aqueous and hydrochar) that need to be addressed. Especially, management of the HTL aqueous is critical for a large-scale implementation of HTL technology for mixed sludge treatment. Given the high volume and soluble organic compounds that accumulate in sludge-derived HTL aqueous, on-site biological treatment is necessary. Adsorption can be a feasible (economically) option to address these challenges. Since it is an established process for full-scale waste treatment application, implication of it for HTL aqueous stream may be applicable. Furthermore, using the hydrochar stream from the process itself to treat the aqueous stream can be very beneficial for circular economy. Lastly, literature on adsorption as a pretreatment option for HTL aqueous, derived from municipal sludge, is lacking. This study would fill the gap in the literature while providing valuable insights to wastewater treatment sector within and beyond Canada.
1.3 Objectives

Based on the research gaps that are identified, the objectives of the study were:

- **Objective 1**: Testing various adsorbents (biochar, HTL hydrochar and granular activated carbon) and finding their optimum conditions as a pretreatment for HTL aqueous, derived from mixed sludge.
- **Objective 2**: Assessing the aerobic and anaerobic biodegradability performance of un-pretreated and pretreated HTL aqueous of mixed sludge by adsorption under the optimum conditions identified in Objective 1.
Chapter 2: Literature Review

This chapter provides details about different stages of wastewater sludge treatment including hydrothermal liquefaction (HTL). Also, it gives insights on adsorption as a pretreatment to enhance biological treatment of HTL aqueous stream derived from mixed sludge. First, conventional wastewater and sludge treatment processes are described to set the background for HTL. Second, HTL is introduced with its advantages in sludge treatment and its bottleneck (HTL aqueous management) for full-scale application. Third, potential treatment approaches for HTL aqueous are discussed. Finally, recent studies on the adsorption of HTL aqueous are reviewed.

2.1 Wastewater treatment

Wastewater comes from sources such as households, industries, and agriculture. It carries various types of pollutants that can be harmful to both our health and the environment (EPA, 1988). The treatment of wastewater is essential to remove particles, dissolved substances, nutrients, and other impurities before it is discharged into the surroundings. This treatment process (Figure 2.1) generally consists of stages including primary, secondary and tertiary treatments. Primary and secondary sludges generated are treated in sludge treatment processes.

![Figure 2.1. Flow diagram of Annacis Island WWTP (Metro Vancouver, 2024).](image-url)
During preliminary/primary treatment, coarse screens and settling tanks are utilized in wastewater treatment to eliminate particles and solid materials. Secondary treatment incorporates processes like conventional activated sludge (CAS), trickling filters or rotating biological contactors. Tertiary treatment involves steps, such as disinfection and filtration to decrease the levels of biochemical oxygen demand (BOD) and total suspended solids (TSS) and may also include biological nutrient removal (BNR). To destroy pathogens ultraviolet radiation, ozonation and chlorine can be employed. Once treated, the wastewater can be discharged into the environment and/or reused for other purposes like irrigation. The specific treatment process is determined by factors, such as the type and quantity of wastewater as well as the regulations set by the governing bodies for discharge or reuse (UNEP, 2016). The following sections provide details for the treatment plant unit processes.

2.1.1 Preliminary treatment process

This is the first step in wastewater treatment, which involves eliminating large, non-biodegradable materials like plastics, sticks, and stones through screening and/or sedimentation to prevent damage to downstream unit processes. This is done in several steps, including multiple stage of screens and a grit chamber. Trash racks and bypass screens have openings that exceed 36 millimeters (mm), while coarse screens have openings ranging from 6 to 36 mm. Coarse screens are often followed by fine screens that have openings of 0.5 to 6 mm (Droste, 1997). Grit chambers are placed after the screens and more critical for combined sewer systems where sand and grit from the streets can enter through storm drains. Grit and sand can cause damage to the plant's equipment and quickly settle once they reach the clarifier. Therefore, preliminary treatment is necessary for removing grit and sand, excluding any unwanted materials, which are typically transported to landfill. Also, grit chambers function by slowing the flow, blowing air to keep organic material in suspension, or creating a swirling motion to separate heavy grit from lighter material (Metcalf and Eddy, 2014).
2.1.2 Primary treatment process

The primary treatment, such as sedimentation or flotation, removes suspended inorganic and organic matter from the wastewater using physical process. This process aims to reduce the inorganic pollutant load and prevent clogging downstream (mainly biological) processes with inorganic solids, although suspended organics are also removed to a limited extent. Sedimentation tanks are typical primary treatment units. TSS and BOD removal efficiencies in primary sedimentation range from 50-70% and 25-40%, respectively (Metcalf and Eddy, 2014). The effluent from the primary sedimentation tank, with the remaining TSS and BOD, undergoes secondary treatment for biological treatment.

2.1.3 Secondary treatment process

In this stage, biological processes break down and remove mainly carbonaceous dissolved organic matter. This is achieved by microorganisms, such as bacteria and fungi, transforming organic matter into carbon dioxide, water, and other byproducts. During this process, the primary nutrients in wastewater (nitrogen and phosphorus) are also utilized for new cell growth. During the secondary stage of treatment, approximately 85% of the organic content is eliminated. Traditionally, secondary treatment methods mainly include trickling filter and CAS. In a trickling filter system, the wastewater is trickled through a bed of filter media, typically 3 to 6 feet deep (Metcalf and Eddy, 2014). The media has a large surface area for bacteria to grow and multiply, enabling them to utilize organic matter. In the past, natural media, such as rocks or gravel, were used as the media, however the modern trickling filters employ synthetic media with much higher surface area, such as interlocking plastic pieces. The treated wastewater then flows out through pipes for further processing. Following treatment in a trickling filter, the treated wastewater is directed to secondary clarifiers to settle any bacteria sloughed off during operation (Grady et al., 2011; Metcalf and Eddy, 2014). In some cases, trickling filters can be utilized as the
first-stage of a dual-stage secondary treatment processes (Muralikrishna and Manickam, 2017). Annacis and Lulu Island WWTPs operated by Metro Vancouver are good examples of this configuration.

Although trickling filters were popular in the past, after the 1970s, the CAS processes became more popular due to their ability to meet more stringent secondary treatment standards. In CAS, wastewater first goes to primary treatment and after that it is directed to aeration. In aeration tank, aerobic bacteria, (mixed liquor suspended solids, MLSS) and wastewater are mixed with pressurized air for several hours. During this period, the MLSS decomposes organic matter into harmless byproducts (EPA, 1998; Grady et al., 2011). Treated wastewater containing MLSS is sent to a secondary clarifier to separate bacteria from the treatment effluent before final discharge. Secondary treatment processes are evaluated based on their ability to remove BOD and TSS from wastewater. A properly designed and operated secondary treatment process can typically remove between 85% and 95% of the influent BOD and TSS, while it can remove around 65% of the influent COD (Metcalf and Eddy, 2014).

2.1.4 Tertiary treatment process

This stage targets persistent pollutants that were not eliminated by secondary treatment. It removes remaining pollutants, such as trace metals, remaining nutrients, and/or pathogens, to meet specific discharge or reuse standards. Physical and/or chemical treatment methods like reverse osmosis, ion exchange, adsorption and disinfection may be used in tertiary treatment (Mareddy, 2017). The selection of the tertiary treatment depends on the quality standards of the receiving water body. Typically, chlorine is added to the water to eliminate harmful bacteria and reduce odors. Chlorination can eliminate over 99% of the pathogenic bacteria before discharge. However, some states may require dichlorination (EPA, 1998; Metcalf and Eddy, 2014). In cases where the use of chlorine in treated wastewater effluent may be harmful to aquatic life, alternatives such as ultraviolet light or ozone disinfection are utilized (Droste, 1997). Tertiary treatment
methods may also involve enhancing biological treatment to further stabilize the oxygen consuming substances in wastewater or to eliminate nitrogen and phosphorus. Excessive amounts of nitrogen and phosphorus being discharged into water bodies can speed up the growth of vegetation such as algae blooms that can harm fish. This can even lead to a lake undergoing a transformation into a swamp. Hence it is necessary to remove nitrogen and phosphorus before releasing treated water into the environment (ASCE, 2018).

2.2 Sludge treatment

After going through unit treatment processes a significant amount of solid substances are separated into primary, secondary, and occasionally tertiary sludge respectively. These solids contain organic matter, nutrients and pathogens that need to be treated and disposed of. Sludge can undergo degradation through digestion and composting methods while incineration and landfilling have also been employed for its disposal. The digested sludge, once dewatered and dried, can be reused to enhance soil quality. After dewatering, the liquid portion is returned back to the beginning of the wastewater treatment process (Metcalf and Eddy, 2014). There is a motivation to convert waste sludge streams into liquid or gas fuels due to the high-level of energy embedded to them and also to lower/eliminate final disposal cost.

Current sludge treatment options have several drawbacks compared to HTL. Incineration has no final product and high energy demand. Composting, land application and landfill has emerging contaminant issue and anaerobic digestion generates significant amount biosolids that have hazardous contents. More details about these conversion methods will be provided in the following sections.

2.2.1 Chemical sludge treatment

At WWTPs, after the sludge thickening process the next step usually involves its stabilization that is necessary for final disposal or valorization. To achieve chemical stabilization,
lime is added to increase the pH value to more than 11 (Rorat, et al., 2019). Once stabilization is finished the sludge undergoes dewatering, which is commonly done using filter presses or centrifuges. Since efficient dewatering plays a role in disposal of sewage sludge, an additional sludge preconditioning step often becomes necessary. This step includes using conditioners, like polymers or metal ions, to coagulate colloids in the sludge and speed up the dewatering process. Chemical stabilization helps pathogen reduction, volume reduction, odor minimization and dewatering. On the other hand, it has chemical costs, environmental impact due to potential residuals (Novak, 2007; Rorat, et al., 2019).

2.2.2 Biochemical sludge treatment

Biochemical conversion refers to the process of breaking down organic matter in sludge by bacteria and/or enzymes. Unlike thermochemical conversion, this method is slower and does not require a significant amount of energy. There are three main biochemical pathways for organic conversion; digestion, which can be either anaerobic or aerobic, fermentation, and enzymatic or acid hydrolysis.

In aerobic digestion, microorganisms consume cellular material without being provided with any soluble substrate that can be easily broken down. This process is similar to CAS, except that the excess biomass (MLSS) from secondary treatment undergoes endogenous respiration. When primary sludge is introduced into the digester, it serves as another (more readily biodegradable) source of substrate. This helps delay the respiration process and extends the solid retention time (SRT). In aerobic digesters, microbial growth is typically rapid, leading to shorter retention times compared to anaerobic digesters with biogas production (Ohimain and Izah, 2017; Gunay and Karadag, 2015). Various aerobic methods, including aerobic batch reactors and sludge systems, have been employed to manage organic effluents. While aerobic reactors are relatively easy to operate and can reduce volatile solids (VS) by 38-50%, they tend to be more expensive due to the requirements of aeration and total alkalinity (Metcalf and Eddy, 2014).
Additionally, the sludge produced during digestion poses difficulties in dewatering. Furthermore, it does not yield biofuel as a byproduct (Kalat and Yuceer, 2017; Shabbir et al., 2021).

Composting, which involves breaking down waste in the presence of oxygen, plays a role in recycling and preserving important nutrients found in sewage sludge within the soil. The collaboration between earthworms and microorganisms along with their metabolic activity leads to a reduction in volume (around 40–60%) increased availability for plants balanced C/N ratio and decreased presence of harmful contaminants like metals (Rorat, et.al., 2019; Suleiman, et al., 2017). While composting is widely recognized as a cost-effective method for converting sewage into compost, it does present some environmental challenges. The rapid breakdown of nitrogen matter can result in nitrogen losses and the release of greenhouse gases (Sanchez Monedero, et al., 2010).

Anaerobic digestion (AD) is an established technology that breaks down waste into sugars, amino acids, and fatty acids through microbial activity under oxygen free conditions (Li et al., 2011). The process involves four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Ultimately it results in biogas composed of methane and carbon dioxide. Biogas can be directly utilized for heating/energy generation or further refined into fuels and other valuable products (Ge et al., 2014). However, it also comes with drawbacks such as managing its residues. These residues contain water, nitrogen, phosphorus, pathogens, heavy metals and can pose challenges for the operation of AD plants as sludge is only partially degraded by digestion (Sheets et al., 2015; Li and Ge, 2018). The reactors are designed to maintain pressure to prevent oxygen inlet and to capture biogas. Biogas mainly consists of methane (60-70%) and carbon dioxide (30-40%) (Droste, 1997). While the AD process is endothermic in nature, most systems utilize biogas as fuel for heating the digester. The excess energy from biogas can efficiently heat nearby buildings. Alternatively, the quality of biogas can be enhanced by removing
impurities like carbon dioxide, hydrogen sulfide, ammonia siloxanes and moisture before injecting it into natural gas networks to utilize in residential communities (Metcalf and Eddy, 2014).

2.2.2.1 Operational parameters of anaerobic digestion

The most crucial microbial cultures in anaerobic systems are obligate anaerobes, meaning they can only live in an oxygen-free environment. This fundamental need has resulted in the utilization of enclosed reactors at the forefront of the advancements of high-speed anaerobic processes (Bajpai, 2000). The reaction rate is greatly influenced by temperature, considered one of the most significant environmental factors. Therefore, temperature control plays a crucial role in ensuring successful anaerobic processes. There are three recognized ranges for temperature in anaerobic processes: psychrophilic (5–15°C), mesophilic (35–40°C), thermophilic (50–55°C) (Droste, 1997).

Maintaining the ideal digester temperature and preventing heat loss are some of the AD challenges. The mesophilic and thermophilic ranges are optimal for anaerobic reactors at medium to large WWTPs. A general guideline states that the reaction rate doubles with every 10°C increase between 20-55°C (Bajpai, 2017). Most industrial-scale ADs operate within the mesophilic range, leading to faster waste stabilization and smaller digester sizes. The digesters’ contents can be heated using heat exchangers while the walls can be insulated with materials such as cork board or concrete. Since temperature influences the diversity of bacteria in a population it is crucial to maintain a temperature within ± 1°C in large scale industrial digesters, for effective wastewater treatment (Verma, 2002; Gerardi, 2003).

Anaerobic systems thrive best and stay stable in the pH range of 6.5-7.5 (Droste, 1997). Mixed cultures favor neutral pH. When the pH is low, it can slow down the activity of methanogens and cause a buildup of VFAs and hydrogen. This can severely inhibit bacteria that break down acid resulting in more accumulation of heavier VFAs, like propionic and butyric acids, which then
leads to an even lower pH and later inhibit the process (Labatut and Gooch, 2012). If the system does not have enough alkalinity, it can become acidic, and biogas production can decrease or even stop (Labatut and Gooch, 2012; Ostrem, 2004; Leitao et al., 2006). However, in a well-operated system, the effluent pH may slightly increase due to the alkalinity produced by organisms that consume organic matter rich in protein (Verma, 2002). Bicarbonate alkalinity helps to maintain a proper pH level. During the degradation of organic matter, carbon dioxide is released, and ammonium ions react with it to produce ammonium bicarbonate (Bajpai, 2017). Common toxic substances that can inhibit the growth of anaerobic bacteria include free ammonia, sulfur, light, heavy metal ions, and some organic compounds (Chen et al., 2008; Gerardi, 2003). In addition, heavy metals are harmful to anaerobic processes by blocking metabolism through enzymatic reactions (Malina and Pohland, 2002).

2.2.2.2 Biochemical methane potential assay

Biochemical methane potential (BMP) is a lab-scale assay that assesses the methane potential and biodegradability of organic waste through batch AD. The BMP assay is a quick and inexpensive way to assess the effect of a substance and inhibitory compounds on AD. The typical process for BMP involves combining anaerobic inoculum (seed) and organic substrate in serum bottles at an optimum substrate to inoculum ratio. This ratio is substrate specific. After adjusting the pH and alkalinity along with adding nutrients (if necessary), the bottles are purged with an inert gas to eliminate oxygen, sealed and incubated/mixed at the target temperature until they stop producing biogas. It is crucial to allow the inoculum to lose its VFAs and other readily biodegradable substrate to minimize biogas production from inoculum itself before starting the BMP experiment. This can be achieved by not feeding any substrate to the inoculum reactor for 4-5 days prior to setting up BMPs (Holliger et al., 2016; Filer et al., 2019; Angelidaki and Sanders, 2004).


2.2.3 Thermochemical sludge treatment

Thermochemical conversion focuses on thermal transformation of biomass into biofuels or chemicals. Table 2.1 compares major thermochemical methods for biomass conversion and provides their respective typical temperature ranges. The process of combustion takes place when there is high temperature oxidation, in conditions where oxygen is abundant, resulting in the production of hot flue gas. On the other hand, gasification occurs when chemical reactions happen in an environment with a supply of oxygen leading to the creation of gases. Pyrolysis involves heating without any presence of oxygen. Lastly liquefaction refers to the breakdown of molecules in wet feedstock into smaller liquid molecules. This process sometimes utilizes a catalyst and occurs at lower temperatures (Brown, 2019; Wang, 2008). The following sub-sections provide further details.

Table 2.1. Different temperatures and pressures for processes of thermochemical biomass conversion (adapted from Demirbas, 2009).

<table>
<thead>
<tr>
<th>Process</th>
<th>Temperature (°C)</th>
<th>Pressure (Bar)</th>
<th>Time (Hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquefaction</td>
<td>250-374</td>
<td>50-220</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>300-600</td>
<td>1-5</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td>Combustion</td>
<td>700-1400</td>
<td>≥ 1</td>
<td>-</td>
</tr>
<tr>
<td>Gasification</td>
<td>500-1300</td>
<td>≥ 1</td>
<td>&lt; 0.5*</td>
</tr>
</tbody>
</table>

*Depends heavily on feedstock characteristics and operation conditions.

2.2.3.1 Combustion

The process of combustion involves the reaction between oxygen and hydrocarbons in biomass, which results in the creation of compounds, water, and carbon dioxide. The release of heat from this reaction is the source of energy for more than 90% of the energy derived from biomass (Basu et al. 2011). Biomass serves as a resource for both heat and electricity production. It is widely used for cooking and heating purposes in some areas. Industrial and district heating systems rely on steam generated by burning biomass in boilers. Furthermore, biomass combustion is a method for generating electricity, which is essential for modern economic
activities. This is typically achieved by burning biomass in a boiler to produce steam that drives a turbine to generate electricity. Biomass can be utilized either as a fuel or in combination with fuels, within a boiler system. The latter approach has become increasingly popular as it offers a cost-effective means to reduce carbon dioxide emissions from existing fossil fuel plants (Basu et al. 2011).

2.2.3.2 Pyrolysis

Unlike the process of combustion, pyrolysis occurs without the presence of oxygen unless there is a need for combustion to generate the heat. In this method, biomass is rapidly heated to temperatures ranging from 300°C to 650°C causing it to break down into gas, liquid, and solid components through decomposition (Basu, 2018). During pyrolysis the long hydrocarbon molecules found in biomass are converted into smaller molecules. Fast pyrolysis primarily produces fuel called bio-oil, while slow pyrolysis results in gas and solid charcoal. Charcoal is a fuel that was historically used for heating and extracting metals before coal was discovered. Pyrolysis shows potential for converting waste biomass into fuels and it does not release heat as a byproduct (Basu, 2018; Demirbas, 2000).

2.2.3.3 Gasification

Gasification is a process that transforms both fossil and non-fossil fuels, regardless of their physical state, into useful gases. This process relies on mediums such as gases, steam, or water under various conditions. Gas can be composed of air, oxygen, or a combination of these elements. Fuels with hydrogen content tend to have temperatures at which they vaporize and are more likely to exist in a gaseous state (Higman and Van der Burgt, 2008). Gasification allows for an increase in the hydrogen content through exposure to high pressure hydrogen or indirect exposure to steam under temperature and pressure where hydrogen is introduced. Biomass, which contains around 40% oxygen by weight, can undergo gasification to remove a portion of its
The production of hydrogen via natural gas gasification plays a role in industrial scale ammonia production. By steam reforming natural gas, syngas is produced which can then be used for methanol synthesis. However, these processes heavily rely on renewable natural gas resources and contribute to carbon dioxide emissions. In contrast, biomass has the potential to replace hydrocarbons as a source for fuel and chemicals. The process of converting biomass into carbon monoxide and hydrogen forms the basis for creating fuels (gasoline), synthetic chemicals (methanol), and methane which can be directly used for generating energy (Basu, 2018).

2.3 Hydrothermal liquefaction

Hydrothermal liquefaction (HTL) enables the conversion of biomass into a liquid form and is usually carried out at moderate temperatures ranging from 280-370°C and pressures between 100-250 bar (Zhang, 2016; Behrendt et al., 2008; He et al., 2019). Although these conditions are commonly used, researchers have also explored a range of temperatures and pressures. The main outputs of HTL include a viscous compound with a heating value (biocrude), organic compounds dissolved in water (aqueous), a gas phase rich in carbon dioxide and solid char (hydrochar). The addition of alkaline catalysts can decrease the formation of residue (Toor et al., 2011). The resulting oily component, referred to as biocrude, contains oxygen, nitrogen, carbon, and hydrogen. It has lower oxygen content, compared to biomass, less soluble in water and possesses higher heating value. It has a high carbon content (50-75%) and contains hundreds of organic compounds. The specific composition of biocrude depends on the type and properties of the feedstock material (Kaltschmitt and Neuling 2018; Letcher, 2022). Although the presence of oxygen poses challenges for its utilization, downstream hydrotreatment processes can enhance its quality. The composition and yield of the product are also influenced by temperature along with the specific type of biomass used. Optimal yield is typically achieved around 300°C. However, as temperature increases further, there is an increase in the production of char. Other factors that
influence the process include how quickly it reacts, the amount of pressure applied, how fast it heats up and the size of the particles involved (Akhtar and Amin 2011; Basu and Kaushal 2024). As mentioned above, the overall efficiency of HTL process and the characteristics of its resulting (oily, solid, gaseous and aqueous) products depend on many factors, such as the type of starting feedstock used, the process mode (batch versus continuous-flow) employed, specific process conditions applied during HTL operation, as well as catalysts utilized. The following sections will provide more information.

2.3.1 Impacts of HTL operational parameters

Improving the efficiency of biocrude production can be achieved by reducing the time it takes to process the biomass and minimizing the development cost, while ensuring the process is environmentally sustainable. Previous studies have examined batch and continuous-flow HTL processing of various biomass feedstocks. A continuous-flow HTL process design is necessary for larger-scale and more efficient commercial production, which is superior to batch systems (Singh et al., 2020). Several operational parameters must be optimized to achieve an efficient HTL process, including feed composition, temperature, pressure, reaction time, biomass-to-solvent ratio, and catalyst usage (Rosendahl, 2018). These parameters can significantly impact the quality and yield of the biocrude produced.

2.3.1.1 Feed composition

For optimal biocrude oil production through HTL it is best to use feedstocks that contain fat and nonfibrous carbohydrates (Biller and Ross 2011; Demirbas, 2000). In contrast, feedstocks with protein or fibrous carbohydrate content require higher reaction temperatures and longer retention times. Subcritical water hydrolysis can also be employed to break down amino acids found in feedstocks. In addition to producing biocrude oil, HTL of food processing waste can generate chemicals (Zhong and Wei 2004; Peterson et al., 2008; Kabyemela et al., 1999). The
composition of food processing waste can vary, with fat content tending to yield more biocrude oil (Vardon et al. 2011; Pavlovic et al., 2013). On the other hand, HTL usually leads to char formation when lignin is present. Animal manure contains fat but has higher levels of proteins and carbohydrates requiring a higher temperature for optimal biocrude oil yields. Human feces have also shown potential as an HTL feedstock while offering the added advantage of pathogen destruction. However, the HTL biocrude oil obtained from animal manure and human feces has much higher nitrogen and oxygen content compared to petroleum. If it is intended for use as a transportation fuel, further refining is necessary. Algae and high-lipid containing microalgae show promise as HTL feedstocks due to their photosynthesis ability for growth and reduced need for agricultural land (Toor et al., 2011; Rogalinski et al., 2005; Rosendahl, 2018).

2.3.1.2 HTL temperature and retention time

As mentioned earlier, the important HTL operating conditions include reaction temperature, retention time, pressure, feedstock to water ratio, and the use of catalysts. Out of these factors, the reaction temperature plays a crucial role in determining the quality and yield of biocrude oil obtained from sources like animal manure, algae, and food waste. For producing biocrude oil from microalgae without a catalyst, the ideal reaction temperature ranges between 280-300°C (Rosendahl, 2018). On the other hand, protein containing biomass requires a temperature range of 250-375°C depending on the feedstock used (Li et al., 2012; Brown et al., 2010; Spolaore et al. 2006). When temperatures exceed 320°C during HTL processes, there is an increased production of gaseous products. Notably raising the reaction temperature to 380°C significantly enhances gas product yields. The initial stage of HTL involves hydrolysis and depolymerization reactions followed by repolymerization occurring between temperatures ranging from 220 to 375°C and gasification beyond 375°C (Rosendahl, 2018). If the retention time is too long, it can result in a decrease in the yield of biocrude oil due to the formation of char or gaseous byproducts. However, it is important to have a long enough retention time to achieve the highest
possible yield of biocrude oil (Singh et al., 2020) depending on the feedstock. Retention time ranks as the second (after temperature) most critical parameter in the HTL process (Basar et al., 2021). Different retention times from 0 to 120 minutes were examined, revealing that longer retention times positively impact the yield of bio-crude across all reaction temperatures ranging from 100 to 300°C (Yu et al., 2011). Elevating the severity of the HTL reaction, dictated by higher temperatures and longer retention times, led to reductions in levels of COD, dissolved protein, total carbohydrate, total phenolic compounds, and total short-chain VFAs of HTL aqueous. Conversely, total ammonia, pH, total alkalinity, valeric acid, and iso-valeric acid concentrations increased in HTL aqueous as a result (Basar et al., 2023).

Table 2.2. Different HTL conditions on various types of feedstock.

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>HTL conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Pressure</td>
<td>Retention time</td>
</tr>
<tr>
<td>(°C)</td>
<td>(bar)</td>
<td>(min)</td>
</tr>
<tr>
<td>Rice straw*</td>
<td>220-280</td>
<td>15-60</td>
</tr>
<tr>
<td>Peanut shell*</td>
<td>270-310</td>
<td>30</td>
</tr>
<tr>
<td>Lignin*</td>
<td>250-310</td>
<td>Atmosphere</td>
</tr>
<tr>
<td>Spirulina*</td>
<td>380</td>
<td>Atmosphere</td>
</tr>
<tr>
<td>Wastewater sludge*</td>
<td>325</td>
<td>30</td>
</tr>
<tr>
<td>Cornstalk*</td>
<td>260-300</td>
<td>15-75</td>
</tr>
<tr>
<td>Food waste*</td>
<td>300</td>
<td>Atmosphere</td>
</tr>
<tr>
<td>Wastewater sludge**</td>
<td>300-350</td>
<td>10-100</td>
</tr>
<tr>
<td>Paper sludge*</td>
<td>250-380</td>
<td>15-120</td>
</tr>
<tr>
<td>Pinewood**</td>
<td>300-400</td>
<td>20</td>
</tr>
<tr>
<td>Mixed sludge**</td>
<td>350</td>
<td>15</td>
</tr>
</tbody>
</table>

* Data represents with and without catalyst, respectively.

Table 2.2 shows different HTL conditions with different feedstock. As seen, HTL temperature and pressure are feedstock dependent. Temperature and pressure parameters regulate the state of water, fundamentally determining the reaction mechanisms. On the other hand, where temperature and pressure surpass the critical point, supercritical conditions lead to free-radical reactions (He et al. 2014). The degradation of organic molecules requires substantial
energy, making temperature a crucial factor in HTL. Inadequate energy at low temperatures restricts hydrolysis reactions. Proteins (300°C) and lipids (640°C) necessitate even higher temperatures for complete decomposition. There is a competition between the breakdown of polymers and the formation of new ones. However, when the temperature exceeds 375°C, there is a shift towards the radical mechanism resulting in higher production of gaseous products such as carbon dioxide, carbon monoxide, hydrogen, and alkane gases (Barber, 2016; He et al. 2013; Liu, 2023; Watson et al. 2020).

2.3.1.3 Catalyst

Hydrothermal liquefaction (HTL) is well-suited for employing different catalyst types (alkaline, acid, metal-based, mineral and mixed catalysts). Employing the appropriate type and concentration of catalyst can potentially reduce the necessary HTL temperature and pressure, thereby enhancing bio-crude yield. Moreover, adopting milder process conditions can lead to significant reductions in energy and capital costs (Basar et al., 2021). However, the complexity of phase behavior, intraparticle diffusion limitations, and catalyst deactivation could contribute to the ineffectiveness of catalysts in HTL of protein-containing biomass. Additionally, it is possible that the oil yield without catalysts was already near its maximum limit. HTL biocrude oil yield seems positively impacted by homogeneous catalysts, but catalyst recovery is challenging. Effective and renewable methods for catalyst regeneration in HTL processes are urgently needed (Duan and Savage, 2010; Rosendahl, 2018; Ong, 2013; Biddy et al., 2013).

2.3.2 HTL aqueous

The wastewater that remains after the HTL process, also known as HTL aqueous, accounts for the largest volume among HTL products. HTL aqueous has been considered as the bottleneck for incorporating HTL to treatment plants for sludge treatment since it has a considerable amount of phosphates and ammonia, as well as other harmful organic and inorganic
substances. The composition of HTL aqueous resulting from protein-containing feedstocks differs significantly from that of other feedstocks like crop residue. In particular, it has a higher concentration of nutrients, particularly nitrogen, that can be utilized for biomass production. Conversely, it has numerous nitrogen containing aromatic compounds which may inhibit the growth of algae, microorganisms (bacteria, archaea), algae, plants. Therefore, HTL aqueous requires treatment before being used as a fertilizer or released into water bodies (Rosendahl, 2018).

2.4 HTL aqueous treatment and valorization methods

HTL is a promising technology to convert energy from mixed sludge. Given the high volume and soluble organic compounds in addition to VFAs that accumulate in sludge-derived HTL aqueous, on-site biological treatment represents a low-cost downstream treatment. Details regarding HTL aqueous treatment and valorization methods are discussed in the following sections. A suitable pretreatment option for HTL aqueous treatment depends on characteristics of aqueous phase and efficiency requirements and each pretreatment options has advantages and disadvantages.

2.4.1 Biochemical treatment

Hydrothermal liquefaction (HTL) aqueous contains numerous inhibitors, including organic and inorganic compounds which require significant dilution for a downstream biological treatment. However, dilution is financially impractical. One alternative option is to return it to main wastewater biological treatment processes, such as activated sludge, and treat it with the incoming wastewater. HTL aqueous has also gained recognition as a substrate option for promoting biomass growth, where the resulting biomass can be utilized as a valuable resource for biofuels and other high value chemicals. Microorganisms possess the ability to actively transform components using pathways and numerous strains have been extensively studied in this area.
AD, a cost-effective conversion technology, is well-suited for processing HTL aqueous to produce methane and other valuable byproducts within the same WWTP. While similar wastewater streams have been studied extensively for methane production using AD, the complex composition of HTL aqueous presents unique challenges. Some compounds (aromatics, phenolics) in HTL aqueous could impact AD, and the conversion of certain compounds, such as N-heterocyclic compounds, is relatively slow (Zheng, et al. 2014; Watson, et al. 2020). Azarmina, (2023) studied HTL aqueous effect on thermophilic anaerobic digesters over a year period. HTL aqueous was generated using mixed sludge and 350°C and 15 minutes residence time. It was found that digesters with 12, 23.1 and 43.3% COD contribution from HTL aqueous were inhibited after 42, 29 and 15 days, respectively. Digesters with 2.74% and lower COD contributions showed no inhibition over 180 days, making it a limit for HTL aqueous co-digestion in anaerobic digesters. Basar (2024) studied thermophilic and mesophilic anaerobic digestion, aerobic treatment and dark fermentation of HTL aqueous which is obtained from various temperatures (290-360°C) and residence times (0-30 min.) under batch bioreactor conditions. BOD/COD index showed that HTL reaction temperatures below 340°C was more readily biodegradable under aerobic conditions. Above 360°C, HTL aqueous was not suitable for anaerobic digestion. Among the tested scenarios, aerobic treatment and mesophilic anaerobic digestion emerged as the most appropriate options for the biological treatment of HTL aqueous samples. Thermophilic cultures showed higher sensitivity to HTL aqueous inhibitory compounds.

2.4.2 Chemical and thermo-chemical treatment

Various methods like ozonation, hydrogen peroxide (H₂O₂) treatment, ultraviolet irradiation, chlorine treatment and photo-fenton processes have been employed for HTL aqueous. These approaches could enhance biodegradability and reduce toxicity (Somensi, et al., 2010; Alvares, et al., 2001). Research has shown that ozone can effectively convert inhibitors in HTL
aqueous such as furfural, N-heterocyclic compounds, and aromatic ring structures, into carboxylic acids that can be easily processed biologically (Watson et al., 2020; Andreozzi et al., 1991; Leili et al., 2013; Van Aken et al., 2001; Alvares et al., 2001). Through ozonation, total phenolics and a significant portion of N-heterocyclic compounds in swine manure derived HTL aqueous were transformed, ultimately improving its biodegradability. Similarly, hydrogen peroxide oxidation contributed to reducing concentration of some compounds in HTL aqueous and increasing methane production during fermentation (Yang et al., 2018). It is important to have control over the oxidation process so that toxic compounds are selectively converted without impacting all substances. However, ozone dose optimization is crucial to prevent VFA removal. Additionally, oxidation may require extended contact time and addition of chemicals to stop the process.

The excessive use of hydrogen peroxide had an impact on fermentation, reducing methane yield and prolonging the lag phase. On the other hand, when HTL aqueous was treated with ozone, it also experienced a lag phase but achieved higher rates and yields of methane production while reducing the concentration of some compounds during ozonation (Si et al., 2019; Watson et al., 2020; Jayakody et al., 2018). Another method used as a pretreatment in this context is extraction, which selectively isolates and concentrates chemicals from HTL. Various solvents like petroleum ether, cyclohexane, dichloromethane, and ethyl acetate have been employed for this purpose. Extracting compounds from HTL aqueous has been proven to enhance methane yields by separating furans, ketones (Watson et al., 2020). Total phenolic compounds, known inhibitors for biological conversion, can be effectively extracted using modified resins and two stage nanofiltration (Zhang et al., 2017; Chen et al., 2016). This recovery process has the benefit of improving treatment by preventing any associated inhibitory effects (Shanmugam et al., 2017).

Catalytic hydrothermal gasification (CHG) represents a method for producing fuel gas from HTL aqueous. This gas can be utilized for both heating and powering applications. It is also possible to refine it to meet the required standards, for pipeline quality gas. In terms of fuel, it
holds the potential to substitute energy sources in ethanol biorefineries. Furthermore, if converted into gas form, it could serve as an alternative to imported petroleum that is commonly used for transportation purposes. Typical CHG operational parameters are 300-350°C and approximately 160-210 bar for water-based slurries (PNNL, 2009). Sarker et al., (2022) studied different conditions (375-525°C, 15-60 min, 22-35 MPa) in the presence of catalyst and found that the utilization of homogeneous catalysts shows promise. However, overcoming challenges such as corrosion and salt precipitation remains imperative for future advancements.

2.4.3 Adsorption

Adsorption, for treating a solution, occurs when there are imbalanced molecular forces on the surfaces of solids leading to an interaction between the solid surface (adsorbent) and a gas or liquid (adsorbate). Particles or ions of the gas or liquid balance out these forces resulting in a higher concentration of the gas or liquid near the solid surface. Adsorption involves forces such as dipole moments, polarization, dispersive forces range repulsive interactions, as well as chemical forces like valency forces that emerge from electron redistribution between the solid surface and the adsorbed atoms. There are two types of adsorption: physical adsorption and chemisorption.

Physical adsorption occurs through van der Waals forces, while chemisorption involves electron sharing or exchange. The bond formed during chemisorption is stronger than in physical adsorption resulting in a significant change in enthalpy. Physical adsorption is nonspecific in nature, whereas chemisorption is specific and depends on factors like the characteristics of the adsorbate, reactivity of the adsorbents surface area, temperature, pressure during adsorption (Bansal and Goyal, 2005). Furthermore, the thickness of the phase also differs between physical and chemisorption. Intermolecular forces are responsible for physical adsorption, which typically happens at lower temperatures and exhibits a rapid adsorption rate, low adsorption heat and non-selectivity (Pourhakkak et al., 2021). On the other hand, chemical adsorption involves the creation
and breaking of chemical bonds but demands more activation energy and adsorption heat. It is worth noting that both types of adsorption can coexist (Gunay et al., 2021). In the field of wastewater treatment technology various forms of adsorption occur, with certain types being more prominent due to factors such as the adsorbents and adsorbates involved.

2.4.3.1 Adsorption kinetics and isotherms

The rate at which adsorption takes place is known as kinetics. It depends on factors such as the duration of contact, the concentration of solute and the complexity of the surface where adsorption occurs. After considering these factors, optimizing adsorption processes can lead to greater efficiency in removing pollutants from wastewater (Saleh, 2022). Choosing an appropriate model is crucial for analyzing adsorption kinetics, and this depends on the correlation coefficient ($R^2$) calculated from experimental and modelled results.

In adsorption studies, the adsorption capacity of adsorbent at equilibrium ($q_e$, mg/g) is calculated using Eq. (2.1). The removal percentage ($R$, %) is calculated using Eq. (2.2). $C_0$ (mg/L) is the initial concentration of adsorbate, $C_e$ (mg/L) is adsorbate equilibrium concentration, $V$ (L) adsorbate volume, and $m$ (g) is adsorbent mass used. The pseudo-first-order and pseudo-second-order (adsorption capacity at given time) are presented in Eqs. (2.3) and (2.4), respectively (Ho and McKay, 1999; Simonin, 2016).

$$q_e = \frac{C_0 - C_e}{m} \times V \quad (2.1)$$

$$R = \frac{C_0 - C_e}{C_0} \times 100 \% \quad (2.2)$$

$$q_t = q_e (1 - e^{-kt}) \quad (2.3)$$

$$q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t} \quad (2.4)$$
The rate constants for pseudo-first-order \((k_1, \text{min}^{-1})\) and pseudo-second-order \((k_2, \text{g/mg.min})\) are denoted as per the expressions, where \(q_t \text{(mg/g)}\) signifies the COD adsorbed at time \(t\), and \(q_e \text{(mg/g)}\) represents the COD adsorbed at equilibrium.

Isotherms are describing the state of equilibrium in an adsorption system. They offer insights into the adsorbent and the process of adsorption. The method of identifying isotherms helps determine characteristics of the adsorbent material, such as surface area pore volume, size distribution, heat of adsorption and how well it can absorb gases or vapors. There are equations to describe adsorption isotherms such as Langmuir, Freundlich, and Temkin. The Langmuir and Freundlich isotherms play a role in physisorption (Bansal and Goyal, 2005). As a result, the Langmuir equation continues to be significant in theories related to physisorption and chemisorption. While it can be derived using statistical approaches, there are commonly used kinetic methods for its derivation. According to the Langmuir model, entities that undergo adsorption (such as atoms, molecules, or ions) attach themselves specifically to sites, on the surface where each site can accommodate one entity at a time. The Langmuir model, known as the localized model, operates under the assumption of a uniformly distributed surface, with minimal lateral interactions occurring among adsorbed entities.

The Langmuir, Freundlich, and Temkin isotherm equations, provided in Eqs. (2.5), (2.6), and (2.7) by Liu et al. (2021), play a role in modeling the adsorption process. Additionally, the Langmuir separation factor \((R_l, -)\), assessing the favorability of adsorption, is computed through Eq. (2.8) (Adedeji and Jahan, 2023).

\[
q_e = \frac{Q_mC_L C_e}{1+K_L C_e} \tag{2.5}
\]

\[
q_e = K_F C_e^n \tag{2.6}
\]

\[
q_e = B ln(K_T C_e) \tag{2.7}
\]
The parameters involved include $Q_m$ (mg/g) for maximum monolayer adsorption capacity, $K_L$ (L/mg) for the Langmuir constant indicating adsorbent-adsorbate affinity, $K_F$ (mg/g)/(mg/L)$n$ for Freundlich constant, $n$ (−) for Freundlich intensity parameter reflecting adsorption process intensity and heterogeneity, and $B$ (−) and $K_T$ (L/mg) as Temkin constants. For enhanced accuracy, non-linear kinetic and isotherm methods were employed, a method proven more accurate than the linear approach (Tran et al., 2017).

2.4.3.2 Adsorption thermodynamics

Adsorption thermodynamics assess the behaviour of an isolated adsorption system. The assessment and understanding of the adsorption thermodynamics includes the free energy, enthalpy, and entropy of adsorption, through measurement and analysis (Bansal and Goyal, 2005). The free energy change ($\Delta G^\circ$, J/mol) is examined through the equilibrium constant ($K_c$, −) using Eq. (2.9) and (2.10) (Tan et al., 2008). The Gibbs free energy ($\Delta G^\circ$) has a relationship to enthalpy ($\Delta H^\circ$, J/mol) and entropy changes ($\Delta S^\circ$, J/mol K) as seen in Eq. (2.11) (Tran et al., 2017). The values of $\Delta H^\circ$ and $\Delta S^\circ$ can be determined by analyzing the slopes and intercepts of linear relationships between $1/T$ and $K_c$ using Eq. (2.12) where $R$ represents the gas constant (8.314 J/K mol), and $T$ is the absolute temperature ($K$).

\[
\Delta G^\circ = -RT \times \ln K_c \tag{2.9}
\]

\[
K_c = \frac{C_0 - C_e}{C_e} \tag{2.10}
\]

\[
\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{2.11}
\]

\[
\ln K_c = \frac{-\Delta H^\circ}{R} \frac{1}{T} + \frac{\Delta S^\circ}{R} \tag{2.12}
\]
HTL process generates aqueous phase under high temperatures. Thermodynamic investigations become essential when examining the adsorption properties of the HTL aqueous phase.

2.4.3.3 Adsorption materials

Although a wide range of adsorbents are used in practice, this section focuses on materials explored in this thesis. Activated carbon is a material that is widely known for its exceptional ability to adsorb substances. It is created through two steps: carbonization and activation. During carbonization of carbon, elements such as oxygen, hydrogen and nitrogen are removed, leaving behind carbon atoms that come together in irregularly arranged stacks of flat sheets. These sheets create gaps or pores which give activated carbons their adsorption properties (Bansal and Goyal 2005; Saleh, 2022). The activation process further enhances the structure of the material resulting in a high surface area. The final product has a microcrystalline structure distinct from graphite. In activated carbon, the arrangement of sheets is less ordered, and studies have shown that these sheets contain structures with electrons known as free radicals (Bansal and Goyal 2005; Saleh, 2022). The surface groups on activated carbon are predominantly acidic and essential for providing sites for adsorption of substances (Duong, 1998). A typical composition of activated carbon includes 88% carbon and around 7% oxygen along with some elements and inorganic ash constituents making up the rest. The oxygen content within activated carbon can vary depending on the raw material source and activation process conditions employed (Duong, 1998; Hameed et al., 2007).

Biochar is a material produced by processing biomass through methods like pyrolysis and gasification and is commonly used as a soil amendment. Its ability to effectively store carbon over long periods of time has attracted attention as an affordable approach to sequestering atmospheric carbon while simultaneously generating energy with a negative carbon footprint and improving soil quality. Current research is focused on understanding the relationship between
materials used in biochar production processes to its properties, how it affects soil and crop growth responses upon application well as evaluating its economic feasibility (Brewer and Brown 2012). The effectiveness of biochar depends on factors such as soil type and biochar production method. Overall biochar contributes positively to soil health and promotes crop growth (Ramanathan et al., 2022).

Hydrochar is a carbon-based material that contains oxygen containing functional groups. It is produced from biomass through hydrothermal processes (hydrothermal carbonization (HTC), HTL, hydrothermal gasification (HTG)) which utilize water as a solvent during the chemical conversion process. As the severity of treatment goes up from HTC to HTL and HTG, there is a reduced production of solid and liquid phase materials, with an increase in gas yield. The highest yield of biocrude typically occurs during HTL treatment of municipal sludge. This process typically occurs at temperatures ranging between 150-280°C. The presence of acids or bases can further accelerate this breakdown (Zhang et al., 2019). The composition and performance of hydrochar are influenced by the type of feedstock used, with cellulose, hemicellulose and lignin being the components of lignocellulosic biomass. The properties of hydrochar are influenced by the reaction temperature. Higher temperatures tend to result in dehydration and increased condensation of the hydrochar. As the reaction temperature increases, both the energy content and thermostability of hydrochar improve significantly. Additionally, retention time also plays a role in determining the extent of reaction well as the distribution and quality of different product types. Longer retention times may lead to a decrease in yield while promoting levels of oxygenated functional groups with longer residence times for higher biomass concentrations. However excessive exposure can reduce oxygenated group content. It is worth noting that starting materials and lignin content also impact the extent of reaction and formation of oxygenated groups within hydrochar (Zhang et al., 2019).
2.5 Summary

Hydrothermal liquefaction (HTL) has a good potential in converting municipal sludge into energy in the form of biocrude oil. However, achieving high efficiency requires optimization of the process design and operational parameters for dewatered municipal sludge as a feedstock. A significant challenge for adaptation of HTL into conventional WWTPs is the handling of the HTL aqueous byproducts. Furthermore, it is essential to recover carbon and utilize nutrients from the aqueous phase in a downstream biological treatment process to establish a sustainable sludge treatment system. However, HTL aqueous contains a long-list of potential inhibitory compounds to biological processes. Adsorption emerges as a favorable pretreatment method for removal of inhibitory compounds, enabling biogas production from remaining carbon (such as VFAs) in the aqueous phase. However, the optimization of adsorption process for HTL aqueous, derived from municipal sludge, is also necessary. The biggest challenge, regarding the adsorption pretreatment, is the regeneration and reuse of adsorbents due to the high concentration of pollutants in HTL aqueous, posing a constraint on their effectiveness in the process. Finding solutions to these challenges is pivotal for maximizing the potential of HTL in converting waste sludge into valuable energy resources. The future chapters of this thesis will tackle these challenges by lab-scale testing of both adsorption and biological downstream treatment for HTL aqueous, derived from municipal sludge.
Chapter 3: Treatment of aqueous phase from hydrothermal liquefaction of mixed sludge by adsorption: Comparison of biochar, hydrochar, and GAC as adsorbents

3.1 Introduction

Hydrothermal liquefaction (HTL) aqueous, derived from municipal sludge, contains a substantial amount of processed water with high concentrations of COD, organic carbon, ammonium, and potentially harmful substances such as aromatics and nitrogenous organics (Heracleous et al., 2022; Watson et al., 2020). Proper management of this aqueous by-product is crucial in commercializing HTL technology. Various methods have been explored for treating HTL aqueous waste, including anaerobic digestion (AD) (Wang et al., 2021), adsorption (Adedeji and Jahan, 2023; Wang et al., 2022), catalytic hydrothermal gasification (Marrone et al., 2018), etc.

Adsorption stands out as an efficient and economically viable pretreatment for HTL aqueous. Unlike other techniques which often require substantial energy and expensive equipment, adsorption offers a higher efficiency at a lower cost, and easy to implement (Wang et al., 2022). It can effectively remove organic pollutants from aqueous solutions without generating hazardous by-products, and the resulting by-products can be reused (Pham et al., 2013).

Despite several studies on the treatment of HTL aqueous by adsorption (Adedeji and Jahan, 2023; Marrakchi et al., 2023; Marx et al., 2021; Wang et al., 2022), there is a lack of sufficient information on the mechanism and feasibility. This includes the effects of various operating parameters, capacity, adsorption models, and selectivity of different adsorbents, and removal efficiency of specific inhibitors to downstream biological treatment processes. This chapter focused on the adsorption of pollutants in HTL aqueous derived from municipal mixed sludge using hydrochar derived from the HTL process, biochar, and GAC, along with a comprehensive characterization of both adsorbents and the HTL aqueous. Batch adsorption tests were conducted to assess the impact of adsorbent concentration, pH, time, and temperature on
the removal of overall pollution quantified by COD. Additionally, kinetics, isotherms, and thermodynamics were analyzed to evaluate the adsorption mechanism. The study also investigated the desorption performance of GAC and its removal efficiencies on potential biological inhibitors (total phenolics, ammonia, pyrazines, pyridines, and ketones) and VFAs. Finally, the feasibility of different adsorbents for treating HTL aqueous was revealed.

3.2 Materials and methods

3.2.1 HTL of dewatered mixed sludge and product recovery

Mixed sludge was obtained from Annacis Island WWTP in British Columbia, Canada. The plant uses primary treatment and a two-stage secondary treatment (trickling filters followed by solids contact tanks with a design hydraulic retention time of 1.3 h and sludge retention time of 1.33 d). Primary sludge (thickened by gravity) and secondary sludge (thickened by dissolved air flotation) were mixed at the WWTP before shipped to Bioreactor Technology Group (BTG) laboratory. At this WWTP, the daily volume ratio of primary to secondary sludge typically changes from 40:60 to 60:40%, with an average ratio of 50:50%, depending on the operation. The mixed sludge was then centrifugated (4,000×g for 8 min then 20,000×g for 8 min) to achieve a total solid (TS) content of 20% wt. before being used as feedstock for HTL to minimize input energy requirement. The HTL process was carried out in a 1-L batch reactor (Figure 3.1) (Parr® 4570) at 350°C and 15 min of residence time, as commonly reported in the literature for maximizing biocrude from mixed sludge (Liu et al., 2023b; Marrone et al., 2018; Ovsyannikova et al., 2019). The resulting hydrochar was extracted, washed with dichloromethane (DCM) and ethanol, and dried at 105°C overnight. The reactor was opened, and a mixture of HTL aqueous solution, biocrude, and hydrochar was obtained. The liquid supernatant was filtered through 0.45 μm membrane (Millipore Sigma). After filtration, HTL aqueous phase was collected for adsorption and biodegradability assessment testing, reported in Chapters 3 and 4, respectively.
3.2.2 Adsorbents used for HTL aqueous treatment

Biochar (Soil-matrix, Canada) and GAC (Calgon, F400) were purchased commercially. Biochar was produced through the pyrolysis of pine, spruce, and cedar woods at temperatures ranging from 450 to 550°C (Basar et al., 2022). GAC was made in the United States using a finely ground mixture of premium, domestically sourced bituminous coals. Hydrochar was obtained from the HTL process itself according to methodology summarized in Figure 3.1. After HTL aqueous was recovered, the remaining mixture of biocrude and hydrochar was washed with approximately 1 liter of DCM and filtered (0.45 μm). This extraction process was repeated until the filtrate became colorless. The solid residue that remained insoluble in DCM was identified as hydrochar (Liu et al., 2022). After drying hydrochar overnight at 105°C, both hydrochar and biochar were grounded and sieved through a mesh of 250 μm size to have uniform particle size distribution. Adsorption capacity of the adsorbent increases with decreasing particle size due to larger surface area and more pore structure (Y. Liu et al., 2021). The reasoning for selection these materials was to utilize...
a HTL aqueous byproduct (hydrochar) and compare it to cheap alternative (biochar) and benchmark (GAC) adsorbent. Activated carbon was chosen in granular form because it is suitable to be utilized in continuous adsorption column rather than powder form.

3.2.3 Characterization of HTL feedstock and aqueous phase

A dual-channel pH meter XL25 (Thermo Fisher Scientific, USA) was used to determine the pH of HTL aqueous and mixed sludge. COD of the HTL aqueous samples and mixed sludge was determined following Standard Methods 5220 (APHA, 2005) using a COD block digester (DRB 200, Hach, Canada). HTL aqueous samples were filtered through 0.45 μm membrane filter before COD test. The colorimetric total ammonium nitrogen test for the samples was conducted based on Standard Methods 4500-NH₃ using UV-VIS (Genesys 10S, Thermo Scientific, Canada). Total alkalinity concentrations were measured using the titrimetric method specified in Standard Methods 2320-B, and the concentration of total phenolic compounds was measured using the Folin-Ciocalteu procedure using a microplate reader (Synergy HT, Biotek, USA) and reported as gallic acid equivalent (GAE) (Rodger and Sanders, 2016).

Volatile fatty acids (VFAs) were analyzed using a 7890A gas chromatography (GC) system (Agilent Technologies, USA) equipped with a flame ionization detector (FID) in both HTL aqueous and mixed sludge. VFA samples were subjected to filtration through a membrane filter with a size of 0.45 μm. Subsequently 0.5 mL of the sample was combined with an amount (0.5 mL) of isobutyric acid which served as an internal standard, based on Ackmans method (1972). The prepared samples were then injected into the Agilent 7890A GC using a column (Agilent 19091F 112) and an autosampler. Detailed experimental setup and calibration curves can be found in Appendix A.

Trace organic compounds were measured in HTL aqueous as well as pretreated HTL aqueous at various GAC dosages using gas chromatography–mass spectrometry (GC-MS). For
sample preparation, HTL aqueous samples were diluted 10 times and adsorbed with various GAC concentrations. 10 mL of the adsorbed sample was extracted using 2 mL of DCM, and the sample was vortexed twice. A 1 mL of the DCM extract was transferred to an autosampler vial that contained internal standard (EPA 8270E). Sample extracts were analyzed using an Agilent 8860 GC with a 5977B mass selective detector (MSD) and a DB 5 Ultra MS column (30 m × 0.25 mm inner diameter, 0.25 μm film). The MSD was run in full scan mode. For the quantitative results, stock solutions were purchased commercially. Mixed calibration standards were made from the stock solutions and included the internal standard. The internal standard technique calibrated the instrument and quantified the compounds extracted from the HTL aqueous adsorption samples. For the semi-quantitative results, the isomers of these compounds in the calibration mix were used to quantitate, and the internal standard technique was used. The list of compounds quantitated and semi-quantitated only included partial compounds found in the extracted HTL aqueous by GC-MS. In addition, detection limit for GC-MS was 1 mg/L, below the lowest organic compound concentration.

### 3.2.4 Characterization of biochar, hydrochar and GAC

An automatic elemental analyzer (Thermo Scientific™ Flash 2000) was used for the elemental analysis (CHNS) at an external laboratory. The oxygen content was determined by subtracting the sum of carbon, hydrogen, nitrogen, and sulfur percentages from 100 (O% = 100 – % CHNS – % ash). To analyze CHNS, the samples were completely combusted at 950°C using pure oxygen (99.95%), converting CHNS into carbon dioxide, water, nitrogen gas, and sulfur dioxide. The resulting gases were separated using a GC column and quantified using a thermal conductivity detector. A Fourier-transform infrared spectrometer (FT-IR) (Perkin Elmer, UATR 2) was employed to identify functional groups. Approximately, 10 mg of materials were measured after drying at 105°C overnight. Nob pressure was adjusted until obtaining suitable wavelength. After each analysis material was removed and compartment was cleaned with ethanol.
Thermogravimetric analysis (TGA) was carried out under a nitrogen flow of 20 mL/min using a PerkinElmer STA 8000 instrument. The analysis was conducted between 30 and 800°C with a heating rate of 10°C/min to determine the optimal conditions for carbonization (Gunay et al., 2021). A Micromeritics TriStar-II Plus apparatus was employed to examine surface and porosity characteristics of adsorbents using nitrogen adsorption at 77K. The Brunauer-Emmett-Teller (BET) surface area was calculated by analyzing the relative pressure range of P/P° = 0.029-0.099, while the total pore volume was determined at P/P° = 0.98. The t-plot method was employed to evaluate the micropore volume, while the mesopore volume was derived by deducting the micropore volume from the total pore volume. Surface morphologies of all materials were examined using a Scanning Electron Microscope (SEM) (Mira 3 XMU, Tescan, Czech Republic). A voltage of 10-kV was aimed at the sample surface, and images were taken under vacuum for SEM. Malvern Zetasizer (ZS, Nano-series) was used for zeta potential and electrical conductivity (EC) with a 50:1 liquid to solid ratio, and Malvern Mastersizer (MAL 3000) was used for particle size distribution analysis. The moisture of samples was measured by weight loss at 105°C overnight and content of total solids (TS) was calculated from the difference (ASTM D3173).

3.2.5 Adsorption tests

The adsorption experiments (Figure 3.2) used COD as a response parameter because COD serves as an indicator for total amount of oxidizable, mostly organic, pollutants (Adedeji and Jahan, 2023). The HTL aqueous solution was diluted 10 times to ensure the effectiveness of adsorbent materials at lower pollutant concentrations. For each test, 50 mL of 10 times diluted HTL aqueous and a certain amount of adsorbent were transferred into a 100 mL bottle and shaken for a certain time at various temperatures in a range of 20 to 50°C. An orbital shaker (Fisher brand, MPS) was used, rotating at 400 rpm. After adsorption, samples were settled, and supernatants were filtered through a membrane filter with 0.45 μm. The filtrates were kept in the dark at 4°C for analysis. All adsorption tests were duplicated, and analyses were triplicated.
3.2.5.1 Effect of pH on adsorption

To determine the optimum pH for adsorption, HTL aqueous was diluted 10 times and adjusted to pH levels of 4, 7, and 10 using 0.1 M NaOH or HNO₃. The tests were conducted at an adsorbent concentration of 200 g/L raw HTL aqueous for 1 h at room temperature (21–22°C).

3.2.5.2 Adsorption kinetics and isotherms

For isotherms tests, an adsorbent concentration of 120–280 g/L raw HTL aqueous was used for a contact time of 4 h at room temperature (22-24°C). For kinetic tests, adsorbents were dosed at 200 g/L raw HTL aqueous and shaken for a time of 10–240 min at room temperature. The kinetic parameters could be estimated through non-linear least square fitting (Ho and McKay, 1999; Simonin, 2016). For enhanced accuracy, non-linear optimization was employed to determine the parameters of kinetic and isotherm models, a method proven more accurate than the linear approach (Tran et al., 2017). Model fitting was accomplished using the least chi-squared
test in OriginPro 2023 (OriginLab Corp.). Adsorption kinetics and isotherms details including equations were provided in Section 2.5.3.1, and not repeated here for brevity.

3.2.5.3 Adsorption thermodynamics

To assess the viability and spontaneity of the adsorption process, the thermodynamics were examined by conducting adsorption at different temperatures. Each time, adsorbent at a concentration of 200 g/L was used for adsorption under different HTL aqueous temperatures (20-50°C). A hot plate with temperature control and magnetic stirring (400 rpm) was employed for the test for 1 h. In preliminary tests, the temperature range extended up to 80°C; however, there was no change in COD removal (%) after reaching 50°C. Furthermore, higher temperatures are not feasible in terms of subsequent biological treatment options. Details of thermodynamics analysis were previously described including equations in Section 2.5.3.2.

3.2.5.4 Adsorbent desorption

Desorption experiments were conducted on GAC only based on the performance from adsorption assays. The desorption efficiency of carbon materials depends on the solubility of compounds and the interactions between compounds and carbon surface (Karanfil and Dastgheib, 2004). Both acid and alkali chemicals are commonly used to dissolve adsorbed substances and increase the sorption capacity of adsorbents. Carbon materials that have adsorbed high concentrations of organic compounds such as total phenolic compounds can be washed with a sodium hydroxide solution (Wang et al., 2022). In this study, GAC saturated with HTL aqueous was washed with 100 mL of 0.1 M NaOH at continuously stirring (400 rpm) for 1 h. Afterward, the adsorbent was separated and rinsed with distilled water until neutral pH was reached and dried at 105°C overnight for further use. The adsorption tests were performed at 200 g/L of GAC for 1 h at room temperature (22°C).
3.3 Results and discussion

3.3.1 Characterization of sludge feedstock and HTL aqueous

Table 3.1 shows the properties of mixed sludge and its HTL aqueous. From literature review regarding HTL aqueous, depending on the feedstock composition and HTL conditions, concentrations of COD can range from 40 g COD/L to 105 g COD/L (Chen et al., 2019; Maddi et al., 2017), while total ammonium nitrogen concentrations can range from 15 g/L to 20 g/L (Chen et al., 2019; Elliott et al., 2012), and VFA concentrations can vary from 3 g/L to 10 g/L (Maddi et al., 2017; Panisko et al., 2015; Tommaso et al., 2015) and reach up to 17 g/L (Basar et al., 2023). The pH can range between 3.7 and 9.5 (Quispe-Arpasi et al., 2018; Tang et al., 2019; Zheng et al., 2017), and total phenolic compounds can vary from 1.5 g/L to 2.3 g/L (Basar et al., 2023; Chen et al., 2017). The results in Table 3.1 were mostly in agreement with ranges reported in the literature. Chemical oxygen demand (COD) measures the concentration of organic molecules and oxidable inorganics (nitrite, sulfide, and ferrous) that remain dissolved in the aqueous phase after HTL. COD level (65 g/L) observed in HTL aqueous samples can be explained by hydrolyzed organic molecules that were not converted into biocrude or hydrochar and molecules that were not gasified into carbon dioxide, CO, or methane (Liu et al., 2022).

Table 3.1. Properties of mixed sludge and the aqueous phase from HTL of mixed sludge.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HTL aqueous</th>
<th>Mixed Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical oxygen demand (COD) (g/L)</td>
<td>65 ± 0.4</td>
<td>8.5 ± 0.2</td>
</tr>
<tr>
<td>pH</td>
<td>6.9 ± 0.1</td>
<td>6.02 ± 0.07</td>
</tr>
<tr>
<td>Total alkalinity (g CaCO₃/L)</td>
<td>7 ± 0.02</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Total ammonium nitrogen (g/L)</td>
<td>2.9 ± 0.01</td>
<td>0.7 ± 0.01</td>
</tr>
<tr>
<td>Total phenolics (g GAE/L)*</td>
<td>2 ± 0.02</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>VFAs** (g/L)</td>
<td>10.3 ± 0.04</td>
<td>3.8 ± 0.01</td>
</tr>
</tbody>
</table>

*GAE = Gallic acid equivalence ** VFAs = Acetic acid, propionic acid, butyric acid, iso-valeric acid, valeric acid, caproic acid, iso-caproic acid, a,b Data represent average ± standard deviation of triplicates.
The dissolved organic molecules mainly consist of organic acids, N-heterocyclics, amides, amines, ketones, total phenolics, esters, and alcohols (Watson et al., 2020). Ammonia is mainly generated from the decomposition of proteins in sludge. The specific conditions of the HTL reaction strongly influenced these concentrations. VFAs, carboxylic acids, represent a significant group of compounds found in HTL aqueous, which are produced during the breakdown of carbohydrates, proteins, and lipids. When hydrothermal conditions lead to carbohydrate decomposition, VFAs are the final products if no amino acids are available for reducing sugars to undergo the Maillard reaction (Fan et al., 2018; Basar et al., 2023). Furthermore, some amino acids can transform into VFAs after the deamination reaction by removing the amine group. While VFAs from lipids are subjected to severe hydrothermal conditions and catalytic effects can cause long-chain lipids to crack into shorter-chain fatty acids (Zhang et al., 2019).

High alkalinity (7 g/L) in HTL aqueous makes it impractical and costly to adjust the pH for subsequent processes. The neutral pH of HTL aqueous is generally well-suited for most downstream processes. Phenolic compounds can contribute to biocrude production or remain in the aqueous phase in various forms, such as phenol and cresol. Phenolic compounds in HTL are typically associated with lignin, can also be associated with carbohydrate, protein, and lipid degradation (Kang et al., 2012; Yang et al., 2018). Carbohydrates degradation is the main source for total phenolics, with a minor contribution from proteins and lipids. Notably, glucose alone has been shown to form a significant amount of phenolic compounds under HTL conditions (Basar et al., 2023; Tang et al., 2019).

### 3.3.2 Characterization of biochar, hydrochar and GAC

#### 3.3.2.1 Elemental analysis

Table 3.2 shows the elemental and ash content of GAC, biochar, and hydrochar. The elevated carbon content in GAC and biochar correlates with higher pyrolysis temperatures.
(Domingues et al., 2017). However, the elemental composition of hydrochar is intricate due to the substantial influence of feedstock parameters, and operational conditions in the HTL process. Despite this complexity, hydrochar exhibits similar content under similar conditions as reported in existing literature (Liu et al., 2021).

<table>
<thead>
<tr>
<th>Total Elements (wt. %)</th>
<th>GAC</th>
<th>Biochar</th>
<th>Hydrochar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen</td>
<td>0.3</td>
<td>0.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Total Carbon</td>
<td>90.5</td>
<td>97.9</td>
<td>31.5</td>
</tr>
<tr>
<td>Total Hydrogen</td>
<td>1</td>
<td>1.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Total Sulfur</td>
<td>0.3</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Total Ash</td>
<td>7.9</td>
<td>0.1</td>
<td>63.5</td>
</tr>
</tbody>
</table>

### 3.3.2.2. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of hydrochar, biochar, and GAC are shown in Figure 3.3. In hydrochar's FT-IR spectrum, peaks observed at 3200-3400 cm\(^{-1}\) indicated the presence of carboxyl and hydroxyl groups. The main peak at 2900 cm\(^{-1}\) was likely the asymmetric stretching of aliphatic C-H bonds. On the other hand, both biochar and GAC exhibited relatively flat peaks around 3200-3400 cm\(^{-1}\) (Liu et al., 2008), suggesting lower moisture content. Additionally, a peak around 2500 cm\(^{-1}\) was observed in the FT-IR spectra of biochar and GAC, which was likely to be the symmetric vibration of aliphatic C-H bonds (Behazin et al., 2016). The peak at approximately 2090 cm\(^{-1}\) indicated the presence of -C=N vibration. Furthermore, the peak at around 1620 cm\(^{-1}\) was stretching vibration of C-N bonds (Li et al., 2019). The appearance of a peak at 1440 cm\(^{-1}\) was likely to be the stretching of C-C bonds. The strong and broad peak around 1000 cm\(^{-1}\) indicated the presence of C-O vibration (Yu et al., 2019). In hydrochar, peaks around 800 cm\(^{-1}\) and 500 cm\(^{-1}\) were likely associated with the stretching vibrations of plane aromatic C-H bonds and asymmetric Fe-O bonds, respectively (Liu et al., 2023a, 2021). The presence of these functional groups effects surface heterogeneity which is directly related to surface energy. Surface energy
refers to the energy associated with the adsorption of a molecule to adsorbent surface (Ali et al., 2020).

**Figure 3.3.** FT-IR analysis of hydrochar, biochar, and GAC.

### 3.3.2.3 Thermogravimetric analysis

The total weight losses for GAC, hydrochar, and biochar were 11, 33, and 51%, respectively (Figure 3.4). The initial increase in weight in TGA was due to nitrogen adsorption onto porous materials. A weight loss of approximately 5% up to 200°C indicated water removal through desorption from GAC and hydrochar. In comparison, a slightly higher weight loss of more than 5% was due to the presence of organic substances within the pores of biochar. Between 200°C and 450°C, most volatile organic matter was eliminated. Beyond 750°C, only the ash content remained. These findings showed that commercially obtained GAC was thermally stabilized, whereas biochar and HTL-generated hydrochar were less thermally stable than GAC (Dittmann et al., 2022; Mitchell et al., 2013). The moisture level in the adsorbent impacts its weight, and reducing this moisture content enhances adsorption efficiency. This is because water vapor
competes during the adsorption process, occupying the adsorption sites within the pores and consequently diminishing adsorption effectiveness (Zhou et al., 2001).

Figure 3.4. TGA results of hydrochar, biochar, and GAC.

3.3.2.4 Brunauer-Emmett-Teller (BET) analysis

The Brunauer–Emmett–Teller (BET) theory explains gas molecules adsorption to a solid surface, forming an analytical method used to determine the specific surface area of materials. The Barrett, Joyner, and Halenda (BJH) theory, offers a technique for determining pore size distributions based on experimental isotherms. BJH pore volumes for GAC, biochar, and hydrochar were 0.17, 0.0075, and 0.0012 cm$^3$/g, respectively. As anticipated during BET analysis, GAC exhibited the highest surface area, 791 m$^2$/g, which significantly surpassed surface areas of biochar (29 m$^2$/g) and hydrochar (11 m$^2$/g). The surface area of hydrochar was the lowest due to the accumulation and condensation of hydrocarbons on its surface (Masoumi and Dalai, 2020), which obstructed pores and diminished its adsorption capacity (Marrakchi et al., 2023).
Adsorbents with a limited specific surface area require a longer duration for the adsorption process to reach equilibrium (Adedeji and Jahan, 2023).

### 3.3.2.5 Scanning electron microscope (SEM) images

Figure 3.5 shows SEM images of biochar (a-1, a-2, a-3), hydrochar (b-1, b-2, b-3), and GAC (c-1, c-2, c-3). Biochar had abundant macropore structures (a-1) such as uniform long channels with some pore structures (a-2, a-3), whereas hydrochar (b-1, b-2, b-3) had very limited pore structures. A-1, b-1, and c-1 were magnified 200-fold, while a-2, b-2, and c-2 were enhanced by a factor of 1000, and a-3, b-3, and c-3 were magnified 2000 times.

**Figure 3.5.** SEM images of biochar (a-1, a-2, a-3), hydrochar (b-1, b-2, b-3), and GAC (c-1, c-2, c-3).
Even though hydrochar was smaller than biochar, its pore structure had limited capacity, as seen with particle size distribution, and BET analysis, discussed in the next section. The clogged pore structure of hydrochar could be a result of biocrude oil in its pores. Although repeated DCM washing had a considerable effect on reducing biocrude oil content, it was insufficient to eliminate it from pores. GAC had the highest surface area due to its various pore and inter-connected pore structures (c-2, c-3).

Also, GAC displayed a coarse external surface with ridges, consistent with the literature (Abulikemu et al., 2023). Upon closer examination at higher magnifications, the images revealed detailed views of the surface ridges and crevasses, along with some discernible submicron macropores ranging from nanometers to micrometers in size.

3.3.2.6 Physicochemical properties of materials

The properties of adsorbents were analyzed and summarized in Table 3.3, including moisture content, zeta potential, pH, BET surface area, particle size distribution, and electrical conductivity (EC). The findings showed that hydrochar had the highest moisture content while GAC had the lowest, which aligned with FT-IR results. In terms of EC, hydrochar demonstrated a higher value (0.021 mS/cm) compared to GAC (0.011 mS/cm) and biochar (0.005 mS/cm). The moisture level in the adsorbent impacts its weight, and reducing this moisture content enhances adsorption efficiency. This is because water vapor competes during the adsorption process, occupying the adsorption sites within the pores and consequently diminishing adsorption effectiveness (Zhou et al., 2001).

Generally, the conductivity of carbonaceous materials increases with particle sizes (Shanmugam et al., 2018; Wang et al., 2022). Other properties, such as volume and density changes resulting from compression, may also influence conductivity (Adinaveen et al., 2016). All materials had a pH close to neutral: hydrochar (7.8), biochar (8.1), and GAC (8.9).
Table 3.3. Characteristics of hydrochar, biochar, and GAC.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hydrochar&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Biochar&lt;sup&gt;b&lt;/sup&gt;</th>
<th>GAC&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (wt. %)</td>
<td>7.4 ± 0.3</td>
<td>6.7 ± 0.2</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>Electrical conductivity (μS/cm)</td>
<td>0.021</td>
<td>0.005</td>
<td>0.011</td>
</tr>
<tr>
<td>pH (1:10 v/v)</td>
<td>7.8 ± 0.1</td>
<td>8.1 ± 0.1</td>
<td>8.9 ± 0.1</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>-25 ± 4</td>
<td>-29 ± 5</td>
<td>-22 ± 4</td>
</tr>
<tr>
<td>BET Specific surface area (m&lt;sup&gt;2&lt;/sup&gt;/g)</td>
<td>11 ± 0.1</td>
<td>29 ± 0.1</td>
<td>791 ± 21</td>
</tr>
<tr>
<td>BJH Pore volume (cm&lt;sup&gt;3&lt;/sup&gt;/g)</td>
<td>0.0012</td>
<td>0.0075</td>
<td>0.17</td>
</tr>
<tr>
<td>BJH Average pore diameter (nm)</td>
<td>0.8</td>
<td>1.1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

| Particle size distribution                  |                        |                     |                  |
| D10 (μm)                                   | 1.2 ± 0.1              | 7.2 ± 0.1           | 550-750<sup>d</sup> |
| D50 (μm)                                   | 4.1 ± 0.1              | 30 ± 2              | -<sup>e</sup>    |
| D90 (μm)                                   | 93 ± 4                 | 216 ± 15            | -<sup>e</sup>    |

<sup>a, b, c</sup> Data represent average ± standard deviation of triplicates. <sup>d</sup> Provided by the manufacturer. <sup>e</sup> Could not be detected.

Particle size analysis of hydrochar found that 90% of particles were < 93 μm and 50% were < 4.1 μm. The particle size analysis of biochar showed that 90% of particles were < 216 μm and 50% were < 30 μm. This made GAC (0.55-0.75 mm) the biggest and hydrochar the smallest in particle size. The zeta potential of hydrochar, biochar, and GAC were found to be -25, -29, and -22 mV, respectively and zeta potential of HTL aqueous was found to be -43 mV. When the zeta potential falls below -30 mV, it indicates a significant increase in the adsorption capacity due to enhanced electrostatic attraction between the cationic adsorbate and the carbon surface (Dai, 1994). However, when zeta potential ranges between -30 mV and 60 mV, the electrostatic force is weakened, and the adsorption capacity of carbonaceous materials remains relatively unchanged (Adedeji and Jahan, 2023). On the other hand, conductivity of carbonaceous materials impacts the adsorption of polar molecules. However, mechanisms behind this phenomenon remain unclear, and it is typically overlooked in adsorption modeling (El Oufir et al., 2020). Additionally, reducing particle size leads to a notable increase in the maximum adsorption capacity of the adsorbent.
3.3.3 Adsorption results

3.3.3.1 Effects of pH on COD removal

During adsorption, COD removal percentages at various initial pH of HTL aqueous by biochar, hydrochar, and GAC are shown in Figure 3.6. When a particle moves in an electric field, a decreased potential is observed at the Stern-diffuse layer boundary, known as zeta potential. It significantly depends on pH; the point where zeta potential equals zero is called the point of zero charge ($pH_{pzc}$).

The surface charges of hydrochar (Liu et al., 2023a), biochar (Tran et al., 2016), and GAC (Abulikemu et al., 2023) were positive at pH 4 ($pH < pH_{pzc}$). In contrast, it became negative at pH 11 ($pH > pH_{pzc}$). At a low pH of 4, positive-charge sites were generated on the surface of carbon materials, reducing electrostatic attraction between the positively charged surface of carbon materials and the organic compounds that contribute to COD (Nayl et al., 2017). Under neutral pH conditions, there was a slight improvement in the final removal efficiency compared to pH 4, indicating favorable conditions for removing positively charged organic compounds with carbon materials. This may be primarily due to an induced electrostatic attraction mechanism resulting from a more negatively charged surface (hydrochar > GAC > biochar) at neutral pH. In addition, it may be caused by the deprotonation reaction that increases the number of anionic binding sites available for interaction with positively charged compounds, especially for biochar (Eljamal et al., 2022).

Overall, electrostatic attraction and deprotonation reactions could work together in the removal of COD from HTL aqueous using GAC. The electrostatic attraction could allow charged contaminants to adhere to the surface of GAC particles, while deprotonation reactions may facilitate the removal of acidic compounds by generating negatively charged species that are also attracted to GAC surface. This combined mechanism could help to effectively remove COD. At a
high pH of 10, the negatively charged surface of carbon materials hindered the adsorption of negatively charged inorganic compounds and organic compounds like total phenolics. Furthermore, the solubility of certain organic compounds and NH$_4^+$ in water decreased as pH increased.

**Figure 3.6.** COD removal at various initial pH of HTL aqueous by biochar, hydrochar, and GAC.

Adsorption conditions: 200 g/L, 400 rpm, room temperature (22°C) for 1 h (n = 2).

Adsorption of HTL aqueous with carbon materials was impeded at lower pH due to the positively charged surface. In comparison, it diminished at a higher pH due to increased hindrance to the diffusion of organic compounds caused by an abundance of OH$^-$ ions and reduced solubility of organic and inorganic compounds (Eljamal et al., 2022; Nayl et al., 2017; Sapillado et al., 2022). For ionic compounds containing basic anions, solubility decreases as the solution pH increases, whereas the solubility of ionic compounds with anions remains unaffected by pH variations. Overall, a neutral pH of HTL aqueous is favorable for adsorption.
3.3.3.2 Adsorption kinetics

Pseudo-first-order and pseudo-second-order kinetic models results can be found in Table 3.4. Figure 3.7 shows the graphs for all materials' pseudo-first and second-order models. The pseudo-second-order kinetic model provided the best fit for the adsorption of HTL aqueous onto all adsorbents, exhibiting the highest correlation coefficient ($R^2$). This agrees with a previous finding about the adsorption of nitrogenous compounds in bio-oil to activated carbon (Li et al., 2019).

Table 3.4. Adsorption kinetic parameters for COD removal from HTL aqueous by biochar, hydrochar, and GAC.

<table>
<thead>
<tr>
<th>Kinetics</th>
<th>Biochar</th>
<th>Hydrochar</th>
<th>GAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudo-first-order</td>
<td>$q_e$ (mg/g)</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>$k_1$ (min$^{-1}$)</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.82</td>
<td>0.93</td>
</tr>
<tr>
<td>Pseudo-second-order</td>
<td>$q_e$ (mg/g)</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>$k_2$ (min$^{-1}$)</td>
<td>0.0016</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.94</td>
<td>0.98</td>
</tr>
</tbody>
</table>

The chemisorption process, which operates through valence forces involving the exchange of electrons, was considered the rate-controlling step (Albadarin et al., 2017). GAC exhibited the greatest adsorption capacity at 184 mg/g, surpassing biochar (44 mg/g) and hydrochar (42 mg/g). As a result, GAC could remove 53% COD from HTL aqueous at 200 g/L dose while biochar and hydrochar removed 13% and 9%, respectively. However, Marrakchi et al. reported that hydrochar after thermal activation could achieve 93% COD removal at 30°C with 20 g/L dose, which might also be attributed to their pretreatment with coagulation prior to adsorption (Marrakchi et al., 2023).
Figure 3.7. Adsorption kinetics of COD removal from HTL aqueous by (top) biochar, (middle) hydrochar, and (bottom) GAC. Adsorption conditions: 200 g/L, 400 rpm, room temperature (22°C) (n = 2).
The adsorption mechanism of HTL aqueous to hydrochar, biochar, and GAC is intricate due to the numerous compounds present in HTL aqueous. However, plausible explanations expand on chemical and physical sorption through the adsorption of nitrogen-containing compounds (Li et al., 2019; Mondol et al., 2020) and other organic compounds (Dai et al., 2019; Marrakchi et al., 2023). These descriptions may be broadly categorized as follows: electrostatic attraction (Rosales et al., 2017), pore-filling (Nguyen and Pignatello, 2013), π-π electron-donor acceptor interaction (Wathukarage et al., 2019), H-bonding (Ahmed et al., 2016), complex adsorption (Chaukur et al., 2017), and hydrophobic interaction (Zhang et al., 2018). Various hydroxyl and amine groups within carbon materials may serve as π electron-donor sites, as discussed by Ahmed et al. in 2018. The surface of carbon materials typically carries a negative charge, contingent on factors like pH, pH_{pzc}, and solubility, resulting in electrostatic attraction between the surface and positively charged organic compounds. The strength of this electrostatic attraction is determined by the magnitude of each atomic charge and the distance between two atoms, as highlighted by Rosales et al. in 2017. Additionally, carbon materials with low surface oxidation may exhibit hydrophobic properties (Dai et al., 2019). Adsorption capacities ($q_e$) were found to be 42 mg/g, 44 mg/g, and 184 mg/g for hydrochar, biochar, and GAC, respectively. These findings indicated that GAC displayed the most advantageous adsorption conditions and had the greatest adsorption capacity compared to the other adsorbents that were examined.

### 3.3.3.3 Adsorption isotherms

Sorption isotherms play a crucial role in understanding the interaction between a sorbate and sorbent, and they allow the establishment of a connection between adsorption equilibrium and various mathematical models (Fil, 2016). Three commonly used sorption isotherms for this purpose are the Langmuir, Freundlich, and Temkin models (Figure 3.8). In order to identify the most suitable model for all materials, values of $R^2$ were calculated in Table 3.5.
Figure 3.8. Adsorption isotherms of COD removal from HTL aqueous by (top) biochar, (middle) hydrochar, and (bottom) GAC. Adsorption conditions: 400 rpm, temperature (22°C) for 1 h (n=2).
The Langmuir isotherm, which examines the adsorption of molecules onto a solid surface, is based on several assumptions: certain number of accessible sites, reversible, one-time adsorption, and no interaction between adsorbates (Tran et al., 2017). The Freundlich isotherm is an empirical formula used for nonideal sorption involving heterogeneous sorption (Hameed et al., 2007). The Temkin model introduces a factor that helps discern the interaction between the adsorbate and the adsorbent (Ma et al., 2019).

**Table 3.5.** Adsorption isotherm parameters for COD removal from HTL aqueous by biochar, hydrochar, and GAC.

<table>
<thead>
<tr>
<th>Isotherms</th>
<th>Biochar</th>
<th>Hydrochar</th>
<th>GAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Langmuir</td>
<td>$Q_m$ (mg/g)</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>$K_L$ (L/mg)</td>
<td>0.006</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>$R_L$ (–)</td>
<td>0.21</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.85</td>
<td>0.87</td>
</tr>
<tr>
<td>Freundlich</td>
<td>$K_F$ (mg/g)/(mg/L)$^n$</td>
<td>2.0</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>$n$ (–)</td>
<td>0.33</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.87</td>
<td>0.89</td>
</tr>
<tr>
<td>Temkin</td>
<td>$K_T$ (L/mg)</td>
<td>0.06</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>$B$ (mg/g)</td>
<td>4.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.86</td>
<td>0.88</td>
</tr>
</tbody>
</table>

The correlation coefficients of the Freundlich isotherm adsorption model were slightly higher than those of the Langmuir and Temkin isotherm adsorption models for all adsorbents. This is consistent with a previous study about adsorption of phenolic compounds to mixed solid waste-derived hydrochar (Marx et al., 2021). This indicated that the adsorption of HTL aqueous compounds on hydrochar, biochar, and GAC primarily occurred through a multilayer adsorption process under these conditions. However, $R^2$ values were closer indicating the complexity of HTL aqueous adsorption. The Freundlich heterogeneity factor ($n$) and Langmuir $R_L$ values, falling within the range of 0 to 1, further confirmed that the adsorption reaction was favorable and demonstrated a strong affinity for compounds in HTL aqueous with carbon surfaces (Tran et al.,
The maximum adsorption capacities \( (q_m) \) were found to be 9 mg/g, 20 mg/g, and 481 mg/g for hydrochar, biochar, and GAC, respectively. These results suggested that GAC exhibited the most favorable adsorption conditions and possessed the highest adsorption capacity among the tested adsorbents.

### 3.3.3.4 Adsorption thermodynamics

For all adsorbents, COD removal slightly increased at higher adsorption temperatures. This suggests that the adsorption point should be close to the effluent of HTL reactors where aqueous phase is still hot to favor the adsorption process. Various thermodynamic parameters were determined. The positive values of \( \Delta H^\circ \) shown in Table 3.6 indicated that the adsorption of HTL aqueous by all adsorbent was endothermic. The positive values of \( \Delta S^\circ \) suggested an increase in randomness and disorderliness at the interface between adsorbents and HTL aqueous during adsorption. The negative values of \( \Delta G^\circ \) validated the spontaneous and feasible nature of the adsorption process. Furthermore, as the temperature went up, the magnitude of \( \Delta G^\circ \) also increased, implying that the spontaneity of the system increased with higher temperatures (Nayl et al., 2017). Figure 3.9a shows linear relationships between \( 1/T \) and \( K_c \) and \( \Delta H^\circ \) and \( \Delta S^\circ \) could be determined by analyzing the slopes and intercepts using Eq. (2.12). Figure 3.9b illustrates that COD removal increases slowly over increasing HTL aqueous temperatures until 50°C. This could be useful given the elevated HTL aqueous temperature coming out of HTL process.

### Table 3.6. Thermodynamic parameters for COD adsorption from HTL aqueous by biochar, hydrochar, and GAC.

<table>
<thead>
<tr>
<th>Parameters ( \Delta H^\circ ) (kJ/mol)</th>
<th>Biochar</th>
<th>Hydrochar</th>
<th>GAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta H^\circ ) (kJ/mol)</td>
<td>16.0</td>
<td>10.8</td>
<td>5.3</td>
</tr>
<tr>
<td>( \Delta S^\circ ) (kJ/mol K)</td>
<td>36.5</td>
<td>19.6</td>
<td>19.5</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.93</td>
<td>0.99</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Figure 3.9. (a) Thermodynamic analysis results of biochar, hydrochar and GAC, (b) COD removal change in response to HTL aqueous temperature using biochar, hydrochar and GAC (n=2).
3.3.3.5 GAC desorption

Figure 3.10 illustrates the COD removal from HTL aqueous by desorbed GAC in each cycle. The adsorption capacity of GAC declined sharply (15%) after first use and kept decreasing around 5% after each cycle. As the amount of desorption increased, the COD removal declined from 53% to 23%, reaching 43% of the initial adsorption capacity. This sharp decline also coincided with adsorption kinetics results which suggest chemisorption as a primary adsorption mechanism. Following four desorption cycles, the adsorption capacity of all materials declined. This decline could be attributed to pore blockage and a reduction in the specific surface area, which was not fully recovered after each desorption. Pore blockage limited the adsorption by decreasing accessible surface area, activated site, and connectivity of existing pore space. It can be said that pore blockage was mostly irreversible due to a nearly 50% decrease in adsorption capacity after four desorption cycles (Brennan et al., 2002).

Figure 3.10. COD removal from HTL aqueous vs. desorption cycles of GAC. Adsorption conditions: 200 g/L, 400 rpm, room temperature (22°C) for 1 h (n=2).
Additionally, phenolic compounds that adsorbed from HTL aqueous react with NaOH, which is easily eliminated during the desorption phase. Phenol undergoes a reaction with NaOH, resulting in the formation of a soluble phenolate salt. This salt can be easily eliminated during the desorption process, aided by the electrostatic repulsion between the negatively charged carbon surface and the phenolate anion (Larasati et al., 2020). Chemical regeneration may present a more suitable and economically viable choice for industrial use when compared with thermal regeneration. However, thermal regeneration could have more potential to regenerate exhausted adsorption capacity.

3.3.3.6 Removal of various pollutants in HTL aqueous by GAC

Figure 3.11 illustrates the removal of various pollutants in HTL aqueous at different doses of GAC. It was selected due to its superior adsorption capacity for HTL aqueous compounds. The removal efficiency of COD, total ammonium nitrogen, total phenolics, and VFAs was collectively evaluated. In Figure 3.10a, it was evident that total phenolics and COD exhibited substantial removal which further increased with higher GAC doses (120–280 g/L), while VFAs and total ammonium nitrogen had limited removal (< 10%). At a GAC concentration of 280 g/L HTL aqueous, the highest removal efficiency was achieved for total phenolics (94%), COD (66%), VFAs (9%), and total ammonium nitrogen (8%). Fig. 3.10b presents the contributions of various compounds to COD in HTL aqueous calculated based on chemical equations (Appendix B). Total phenolics account for a very small fraction (3%) of COD compared to VFAs (21%) and others (50%) in raw HTL aqueous. With the increase of GAC dose, most total phenolics and other COD compounds can be removed while VFAs remained in the HTL aqueous as a major portion (up to 50% of COD) after adsorption. Small organic acids, such as VFAs, can be valorized and have the potential to produce methane through AD. However, abundant phenolic and other compounds (e.g., nitrogenates) can inhibit biological activities involved in AD (Si et al., 2019).
Figure 3.11. (a) Removal of various pollutants in HTL aqueous at different GAC doses (b) COD equivalence of each pollutant in raw and adsorbed HTL aqueous at different GAC doses.

Adsorption conditions: 400 rpm, room temperature (22°C) for 1 h (n=2).
Other unidentified organic compounds make up the majority of COD in HTL aqueous. Previous quantitative studies suggested that HTL aqueous from various biomass were mainly composed of organic acids, oxygenated aromatics (mostly total phenolics), cyclic oxygenates (e.g., cyclic ketones), and nitrogenates (mainly amides, pyrazines, pyridines, and their derivates) (Madsen et al., 2017, 2016). Among these, aromatic and nitrogenous compounds are considered as the primary inhibitors. Aromatic compounds are particularly resistant to biodegradation due to their stable structure (Watson et al., 2020; Li et al., 2001; Maddi et al., 2017). Fermenting certain compounds like pyrazine, pyridine, and pyrrolidinone anaerobically is possible, but it requires significant dilution and prolonged treatment time.

Moreover, these inhibitors can also impede acetogenesis, which is a crucial step in the AD process (Shanmugam et al., 2017; Watson et al., 2020; Zheng et al., 2017). Therefore, it is important to reveal the removal efficiencies of those potential inhibitors by GAC. Table 3.7 illustrates some other compounds in HTL aqueous quantified by GC-MS and their removal efficiencies by GAC. These compounds were selected for their frequent occurrence in HTL aqueous derived from sludge feedstock. Among tested compounds, pyrazines were more abundant than pyridines and cyclic ketones, which could be one magnitude higher. This is due to more formation pathways to pyrazines from carbohydrates and protein (Madsen et al., 2017).

Notably, GAC was quite effective in removing all compounds, while it seemed to be more effective in adsorbing pyrazines and pyridines than cyclic ketones. A GAC concentration of 280 g/L eliminated most (> 90%) of those potentially inhibitory compounds. GAC exhibited a high removal rate in adsorbing undesired compounds while keeping VFAs in HTL aqueous. The findings could suggest that adsorption could be a possible option for pretreating HTL aqueous and removing potential inhibitors for valorization through AD for simultaneous production of biocrude and biogas from mixed sludge at treatment plants.
Table 3.7. The removal efficiency of organic compounds identified by GC-MS in HTL aqueous derived from mixed sludge using different doses of GAC.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in raw HTL aqueous (mg/L)a</th>
<th>Removal by GAC dose (%)</th>
<th>120 g/Lb</th>
<th>200 g/Lc</th>
<th>280 g/Ld</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylpyrazine*</td>
<td>830.4 ± 38.3</td>
<td>68 ± 2</td>
<td>89 ± 1</td>
<td>94 ± 4</td>
<td></td>
</tr>
<tr>
<td>Ethylpyrazine*</td>
<td>144.7 ± 14.5</td>
<td>55 ± 1</td>
<td>88 ± 0</td>
<td>95 ± 3</td>
<td></td>
</tr>
<tr>
<td>2,3-Dimethylpyrazine*</td>
<td>76.7 ± 12</td>
<td>74 ± 1</td>
<td>94 ± 0</td>
<td>98 ± 2</td>
<td></td>
</tr>
<tr>
<td>2,3,5-Trimethylpyrazine*</td>
<td>70.1 ± 0.6</td>
<td>79 ± 0</td>
<td>96 ± 0</td>
<td>99 ± 1</td>
<td></td>
</tr>
<tr>
<td>2,5-Dimethylpyrazine**</td>
<td>164.3 ± 11.1</td>
<td>73 ± 1</td>
<td>94 ± 0</td>
<td>99 ± 0</td>
<td></td>
</tr>
<tr>
<td>2-Ethyl-3-methylpyrazine**</td>
<td>19.7 ± 1.1</td>
<td>67 ± 1</td>
<td>95 ± 1</td>
<td>98 ± 0</td>
<td></td>
</tr>
<tr>
<td>2-Ethyl-5-methylpyrazine**</td>
<td>34.9 ± 2</td>
<td>79 ± 1</td>
<td>95 ± 0</td>
<td>99 ± 0</td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methyl-pyridine*</td>
<td>51.5 ± 0.6</td>
<td>79 ± 1</td>
<td>95 ± 0</td>
<td>98 ± 1</td>
<td></td>
</tr>
<tr>
<td>2-Ethyl-pyridine*</td>
<td>4.9 ± 0.1</td>
<td>83 ± 1</td>
<td>98 ± 3</td>
<td>100 ± 0</td>
<td></td>
</tr>
<tr>
<td>5-ethyl-2-methyl-pyridine*</td>
<td>5.8 ± 0.1</td>
<td>87 ± 1</td>
<td>99 ± 0</td>
<td>100 ± 0</td>
<td></td>
</tr>
<tr>
<td>2,3-Dimethyl-pyridine**</td>
<td>8 ± 0.2</td>
<td>73 ± 2</td>
<td>92 ± 1</td>
<td>100 ± 0</td>
<td></td>
</tr>
<tr>
<td>3,4-Dimethyl-pyridine**</td>
<td>14.3 ± 0.4</td>
<td>74 ± 2</td>
<td>92 ± 0</td>
<td>97 ± 0</td>
<td></td>
</tr>
<tr>
<td>Cyclic ketone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylcyclopentanone*</td>
<td>24.5 ± 0.9</td>
<td>65 ± 1</td>
<td>85 ± 0</td>
<td>91 ± 5</td>
<td></td>
</tr>
<tr>
<td>2-methylcyclopentanone**</td>
<td>33.3 ± 0.3</td>
<td>63 ± 2</td>
<td>83 ± 0</td>
<td>95 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

*Quantitated using standards by GC-MS, **Semi-quantitated using standards of isotherms by GC-MS, a, b, c, d Data represent average ± standard deviation of triplicates.

3.4 Summary

As one of the major waste streams from HTL, the treatment and valorization of HTL aqueous are crucial for the incorporation of HTL into treatment plants for sludge treatment. This study evaluated the performance of adsorption for HTL aqueous treatment and compared the effects of biochar, hydrochar, and GAC on removing pollutants from HTL aqueous. The main findings of the study are as follows:

- Neutral pH conditions resulted in better removal efficiency than acidic and alkaline environments for all adsorbents. GAC was the most susceptible to higher pH.
• The adsorption of COD in HTL aqueous by all adsorbents was best described by the pseudo-second-order kinetic model. The rate-controlling step was likely the chemisorption process, which involves valence forces and electron exchange. GAC exhibited the highest adsorption capacity at 184 mg/g, surpassing biochar (44 mg/g) and hydrochar (42 mg/g) which had limited surface area and mesopores.

• The Freundlich isotherm adsorption model showed slightly higher correlation coefficients compared to the Langmuir and Temkin isotherm models. This suggests that the adsorption of COD in HTL aqueous compounds to hydrochar, biochar, and GAC may occur through a multilayer adsorption process.

• The adsorption of HTL aqueous COD to hydrochar, biochar, and GAC was found to be endothermic, which can be favored by increasing temperatures. The positive $\Delta S^0$ values suggest an increase in randomness and favor the adsorption between adsorbents and HTL aqueous during adsorption.

• The adsorption capacity of desorbed GAC declined sharply (15%) after initial use and kept decreasing around 5% after each reuse cycle. This sharp decline also coincides with adsorption kinetics results which implied chemisorption as a primary adsorption mechanism.

• GAC was efficient in removing total phenolics (up to 94%) and COD (up to 66%) but not VFAs ($\leq 9\%$) and total ammonium nitrogen ($\leq 8\%$) from HTL aqueous. GAC could highly selectively eliminate inhibitory organic compounds and retain VFAs in HTL aqueous, allowing subsequent valorization through AD, which warrants further testing.

• From the engineering perspective, using a large amount of GAC ($> 200\ \text{g/L}$) for treating HTL aqueous is neither practical nor economic despite that it can remove most undesirable compounds. Future studies should focus on minimizing the required GAC dose for detoxification of full-strength HTL aqueous pretreatment and demonstrating AD performance of pretreated HTL aqueous.
Chapter 4: Enhanced biological treatment of aqueous phase from hydrothermal liquefaction of mixed sludge by GAC adsorption

4.1 Introduction

In the previous chapter several adsorbents and adsorption conditions were assessed to optimize adsorption parameters for diluted hydrothermal liquefaction (HTL) aqueous, derived from municipal sludge. This chapter focuses on the biological treatment of HTL aqueous after using the optimized adsorption conditions as a pretreatment method. Additionally, the effects of GAC dosage on the removal of contaminants and potential inhibitors from full-strength (non-diluted) HTL aqueous through adsorption were shown. Biochemical methane potential (BMP) and biochemical oxygen demand (BOD) tests were used to evaluate the biodegradability of pretreated HTL aqueous under anaerobic and aerobic conditions, respectively. Both mesophilic and thermophilic BMP assays were performed for comparison. Furthermore, N-heterocyclic compounds, as the main potential inhibitors of anaerobic digestion, were monitored throughout adsorption and BMP assays to understand their fate and removal.

4.2 Materials and methods

4.2.1 Characterization of sludge feedstock, HTL aqueous and GAC

HTL aqueous samples used in Chapters 3 and 4 were derived from the same HTL operation of mixed sludge. Details of HTL of mixed sludge and product recovery were previously described in Section 3.2.1. Characterization steps of mixed sludge feedstock and HTL aqueous produced were also described in Section 3.2.3. Characterizations included pH, COD, total ammonium-N, total alkalinity, total phenolics, organic compounds using GC-MS, and VFAs using GC-FID. Characterization of GAC (adsorbent) was described in Section 3.2.4. Characterizations included elemental analysis, physiochemical properties, FT-IR, TGA, BET and SEM analysis.
Detailed analysis of physiochemical properties of GAC was previously described in Section 3.3.2.6.

4.2.2 Adsorption tests

The effect of HTL aqueous pH on adsorption, thermodynamics, kinetics and isotherms, adsorbent dose and regeneration were the main objectives of Chapter 3. In this chapter, non-diluted HTL aqueous was used to find the optimum adsorption conditions for pretreatment before biological treatment and valorization of non-diluted HTL aqueous. In each experiment, 50 mL of HTL aqueous and a specific quantity of GAC were placed in a 100 mL bottle and mixed for 1 hour at 400 rpm by an orbital shaker (Fisher Scientific, USA). The amounts of adsorbent used (5, 10, 20, 50, and 100 g/L) were determined based on the results obtained in Chapter 3 and preliminary work with non-diluted HTL aqueous. Preliminary studies were focused on the COD removal performance of different dose of GAC in non-diluted HTL aqueous. The experiments were conducted at room temperature (22-24°C) with a neutral pH (6.8-7). Only GAC was employed in this study because biochar and hydrochar had low adsorption capacity in Chapter 3. After adsorption, samples were settled, and supernatants were filtered through a membrane filter with 0.45 μm pore size. The filtrates were kept in the dark at 4°C for analysis. All adsorption tests were duplicated, and analyses were triplicated.

4.2.3 Biodegradability assessment under anaerobic conditions

The BMP assays were performed under both mesophilic and thermophilic conditions following the method described by Holliger et al. (2016). Each bottle received 50 mL of inoculum and a certain amount of HTL aqueous to maintain a food/microorganism (F/M) ratio of 0.5 mg COD substrate/mg volatile solids (VS) inoculum. The F/M ratio was selected at a low value to decrease the toxic effect of HTL aqueous on the inoculum. No alkalinities addition was applied due to the high total alkalinity (7 g CaCO₃/L) of HTL aqueous. These assays were carried out in 160
mL serum bottles (Figure 4.1). The inoculum and total liquid volume were consistent across all assays within the same batch, and the substrate quantity was determined according to the COD.

Figure 4.1. Serum bottles used for batch biodegradability assessment of HTL aqueous under anaerobic conditions.

Deionized water was added to achieve a maximum total liquid volume of 70 mL. Before sealing with a butyl rubber and aluminum cap, each bottle was purged with N₂ gas to remove dissolved oxygen. The bottles were then incubated at 35°C (mesophilic) and 55°C (thermophilic), with a mixing speed of 95 rpm in the rotary shaking incubators (Figure 4.2).

Figure 4.2. Temperature controlled incubator shaker used for anaerobic biodegradability assays.
The BMP assays were set-up in triplicates, including positive controls (glucose replacing HTL aqueous as substrate) and blanks (no substrate, inoculum only). To analyze the composition of biogas, a syringe was introduced into the headspace of the BMP bottle, and a 0.5 mL gas sample was extracted. The sample was manually injected into an Agilent 7820A GC-TCD for the quantification of methane, carbon dioxide, oxygen, and nitrogen content. Calibration of the GC involved using a gas mixture comprising 7% nitrogen, 20% carbon dioxide, and 73% methane, with injections made using a packed column called Agilent G3591 and helium as the carrier gas.

The gas pressure in the BMP bottles were measured with a compact LEO2 digital manometer (KELLER Pressure, Winterthur, Switzerland). The ideal gas law was used to standardize the cumulative biogas volume at the standard temperature and pressure (0°C, 1 atm). The specific methane potential of the substrate (HTL aqueous before and after adsorption) was determined by deducting the methane production from the blank (inoculum only) from the other assays (inoculum and HTL aqueous) and reported as mL CH₄/mg COD of HTL aqueous. Additional information on BMP assay set-up can be found in Appendix C.

Mesophilic and thermophilic inoculum were taken from lab-scale digesters operating under semi-continuous flow conditions, utilizing mixed sludge with a sludge retention time of 20 days for a minimum of 60 days. Mixed sludge was taken from Annacis Island WWTP, and details of these inoculum can be found in Table 4.1. The cultures were starved of substrate for three days before the BMP tests to reduce methane production originating from the inoculum. Additionally, details regarding analytical for inocula characterization tests were given in Section 3.2.3 not repeated here for brevity. The exception to this is BOD analysis which is described in Section 4.2.4.
Table 4.1. Characterization data of aerobic and anaerobic inoculum used in the biochemical assays (n=3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aerobic inoculum</th>
<th>Mesophilic anaerobic inoculum</th>
<th>Thermophilic anaerobic inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.4</td>
<td>7.73</td>
<td>8.07</td>
</tr>
<tr>
<td>Total solids (wt. %)</td>
<td>-</td>
<td>1.69 ± 0.02</td>
<td>1.82 ± 0.01</td>
</tr>
<tr>
<td>Volatile solids (wt. %)</td>
<td>-</td>
<td>1.15 ± 0.08</td>
<td>1.27 ± 0.01</td>
</tr>
<tr>
<td>Total suspended solids (mg/L)</td>
<td>1339 ± 35.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile suspended solids (mg/L)</td>
<td>1112.2 ± 50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total COD (mg/L)</td>
<td>1977 ± 46</td>
<td>20021 ± 518</td>
<td>13646 ± 855</td>
</tr>
<tr>
<td>Soluble COD (mg/L)</td>
<td>190 ± 22</td>
<td>608 ± 97</td>
<td>1628 ± 67</td>
</tr>
<tr>
<td>BOD₅ (mg/L)</td>
<td>940 ± 35.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total ammonium-N (mg/L)</td>
<td>39.5 ± 0.4</td>
<td>1625 ± 24</td>
<td>1493 ± 14</td>
</tr>
<tr>
<td>Acetic acid (mg/L)</td>
<td>56.4 ± 3.6</td>
<td>24.4 ± 4</td>
<td>202 ± 4.4</td>
</tr>
<tr>
<td>Propionic acid (mg/L)</td>
<td>16.9 ± 3.3</td>
<td>12.9 ± 5.4</td>
<td>10.2 ± 0.1</td>
</tr>
<tr>
<td>Butyric acid (mg/L)</td>
<td>389 ± 30</td>
<td>24 ± 3.7</td>
<td>0</td>
</tr>
<tr>
<td>Total VFA (mg/L)</td>
<td>463 ± 69.8</td>
<td>61.3 ± 13</td>
<td>212 ± 4.6</td>
</tr>
<tr>
<td>Total alkalinity (mg CaCO₃/L)</td>
<td>188.7</td>
<td>5925 ± 35</td>
<td>5150 ± 71</td>
</tr>
<tr>
<td>Total phenolics (mg GAE/L)</td>
<td>3.7 ± 0.2</td>
<td>15.7 ± 0.8</td>
<td>42.8 ± 3</td>
</tr>
</tbody>
</table>

COD: Chemical oxygen demand, BOD₅: Five-day biochemical oxygen demand, VFA: Volatile fatty acid, GAE: Gallic acid equivalent. Soluble COD samples were filtered through a 0.45 μm filter.

4.2.4 Biodegradability assessment under aerobic conditions

The PF-8000 Pulse Flow Respirometer (Respirometer Systems and Applications, USA) was employed to conduct BOD tests (Figure 4.3), according to the Standard Methods 5210D (APHA, 2005). The aerobic inoculum was obtained from the solids contact tanks at Annacis Island WWTP. The inoculum characterization can be found in Table 4.1. The inoculum was pre-aerated and acclimatized to HTL aqueous (substrate) before the tests. Acclimatization was achieved by supplying 0.3% of the reactor volume with HTL aqueous over the course of 2 weeks before conducting tests. Aerobic inoculum was added to the BOD bottles at a F/M ratio of 10 g COD substrate/g volatile suspended solids (VSS) inoculum. The borosilicate bottle reactors were filled up to approximately 500 mL by adding deionized water, mineral base, nutrient base, nitrification inhibitor (10 mg/L of allylthiourea), inoculum, and sample (5 mL of HTL aqueous). The details of
mineral and nutrient base are provided in Appendix D. In each reactor bottle a tube, 5 mL of 30% potassium hydroxide solution was introduced to absorb carbon dioxide generation. The bottles were then sealed, submerged in a water bath (20°C), and connected to a gas counter fed with pure oxygen, constantly mixing at 600 rpm with magnetic stirrers for 18 days. The oxygen uptake in sample bottles (inoculum and HTL aqueous) and blank sets (inoculum only) was determined by the respirometer and recorded. After subtracting the oxygen uptake rate of the blank from the sample bottles, BOD of HTL aqueous samples, in units of mg cumulative oxygen uptake per L of sample, could be calculated. Further details regarding BOD assays can be found in Appendix D.

![Image](image_url)

**Figure 4.3.** Biodegradability assessment of HTL aqueous under aerobic conditions.

### 4.2.5 Biodegradability data analysis

The modified Gompertz equation (Eq. 4.1) was used to fit the cumulative oxygen uptake (BOD$_t$) curve or cumulative methane production (BMP$_t$) curves for kinetics analysis. Where $P$ is the maximum specific methane production (mL CH$_4$/g COD) or oxygen consumption (mg O$_2$/L), $R_m$ is the maximum rate (mL CH$_4$/g COD/h or mg O$_2$/L/h), and $\lambda$ is the lag phase duration (h).
\[ \text{BMP}_t \text{ or BOD}_t = P \times \exp \left\{ - \exp \left[ \frac{R_m \times e}{P} \times (\lambda - t) + 1 \right] \right\} \] (4.1)

4.3 Results and discussion

4.3.1 Characterization of HTL feedstock, aqueous phase and GAC

Characterization results of mixed sludge feedstock and HTL aqueous were described in Section 3.3.1. Characterization results of GAC, in terms of elemental analysis, physiochemical properties, FT-IR, TGA, BET and SEM analysis, was also previously described in Section 3.3.2 and are not repeated here for brevity.

4.3.2 Adsorption tests

In the adsorption test, removal efficiencies of COD, total ammonium nitrogen, total phenolics, protein, and VFAs in raw (non-diluted) HTL aqueous were evaluated. Figure 4.4a demonstrates the removal of different pollutants at varying concentration of GAC, which was chosen for its exceptional ability to adsorb HTL aqueous compounds effectively in Chapter 3. It was evident that total phenolics, protein, and COD showed significant removal, which further improved with higher GAC doses (50-100 g/L). However, total ammonium nitrogen exhibited limited removal (< 10%). When the GAC concentration reached 100 g/L in HTL aqueous, the highest removal rates were achieved for total phenolics (87%), COD (61%), protein (91%), and total ammonium nitrogen (6%). Figure 4.4b presents the contributions of different compounds to COD in HTL aqueous, calculated using molar stoichiometry in oxidation reactions (Appendix B). Total phenolics constituted a small fraction (3%) of COD compared to VFAs (20%) and other substances (47%) in raw HTL aqueous. Additional information on organic removal results for specific group of compounds can be found in Appendix E. Small organic acids, like VFAs, which remained in the HTL aqueous have the potential for valorization and can produce methane under anaerobic conditions.
Figure 4.4. a) Removal of various pollutants in HTL aqueous at different GAC doses, and (b) COD equivalence of each pollutant in raw and adsorbed HTL aqueous at different GAC doses. Adsorption conditions: 400 rpm, room temperature (22°C) for 1 h (n=2).
It is suspected that the reason for the preferential adsorption of phenolic compounds and N-heterocyclic compounds over VFAs could be their higher molecular weight. VFAs predominantly consist of acetic acid (60.05 g/mol) and propionic acid (74.08 g/mol), which are smaller compared to highly adsorbed compounds. While molecular weight alone may not determine adsorption behavior, it can indirectly influence the strength and nature of interactions between the adsorbate and adsorbent (Ren et al., 2022). Even though, chemisorption (chemical bond forces and covalent bonds) is the driving force, higher molecular weight compounds may exhibit stronger van der Waals forces (physical adsorption) (Pourhakkak et al., 2021) or hydrophobic interactions with the bigger carbon surface (Ania et al., 2007), thereby enhancing adsorption. Talebi et al. (2020) conducted a VFAs adsorption study in another concentrated medium, landfill leachate. Longer adsorption time (up to 24 hours), higher GAC concentration, (up to 20 wt.%), and lower pH (3) showed improvement regarding VFAs adsorption onto GAC.

4.3.3 Biodegradability assessment under anaerobic conditions

Figures 4.5a and b show cumulative specific methane yield (mL CH₄/mg COD) from HTL aqueous over time under mesophilic and thermophilic temperatures, respectively. Under both temperatures, BMP assays with glucose were also set up as positive controls. Glucose samples yielded cumulative specific methane yield of 330 and 340 mL CH₄/g COD which were 94% and 97% of theoretical yield under mesophilic and thermophilic conditions. The utilization of HTL aqueous as a substrate resulted in a methane production of 160 mL CH₄/g COD over a 28-day period, significantly lower than the yield of 350 mL CH₄/g COD achieved with glucose at standard temperature and pressure (STP). For the HTL aqueous samples pretreated with various doses of GAC (for 1 h), mesophilic anaerobic degradation was enhanced.
Figure 4.5. (a) Mesophilic (35°C), (b) thermophilic (55°C) cumulative specific methane yield from pretreated HTL aqueous at different GAC doses with time (n=3).
The specific methane yields and reaction rates increased with higher GAC doses. Specifically, GAC doses of 5, 10, 20, 50, and 100 g/L achieved specific methane yield increases of 8, 13, 28, 39, and 97%, respectively, for pretreated HTL aqueous, compared to raw HTL aqueous samples. Pretreatment for HTL aqueous before biological treatment under anaerobic conditions. Notably, the 100 g/L GAC dosage exhibited high enhancement in specific methane yield of HTL aqueous, reaching 320 mL CH₄/g COD, which approached the theoretical yield (350 mL CH₄/g COD at STP). In thermophilic BMPs, GAC doses of 5, 10, 20, 50, and 100 g/L led to specific methane yield increases of 10, 15, 32, 32, and 83%, respectively. These results suggest that adsorption could be a pretreatment option for HTL aqueous. The results in Figure 4.5 also indicate that thermophilic microorganisms may be more susceptible to the inhibitors of HTL aqueous as mesophilic microorganisms could produce more ultimate methane from the same pretreated HTL aqueous samples, especially at higher doses. These findings align with the results given in Fig. 4.4. The removal of well-known recalcitrant and inhibitory compounds, such as pyrazines and phenolics, by GAC enabled an observed increase in the specific methane potential of HTL aqueous, even at the lowest GAC dose (Figure 4.5).

During the HTL operations, dewatered mixed sludge had already undergone significant thermal hydrolysis and decomposition at a temperature of 350°C for 15 min. The hydrolysis and decomposition processes involve breaking down chemical bonds in complex organic molecules into simpler soluble forms like VFAs and protein fragments (such as amino acids, amino acid esters, N-heterocyclic compounds, amines, and amides), and monosaccharides (Basar et al., 2023; 2024). This could be the reason for VFA increase in HTL aqueous. VFAs removal results also suggested that thermophilic archaean took longer to convert VFAs into methane (Appendix F). Due to high alkalinity of HTL aqueous samples, the pH levels ranged from 7.7 to 8.02 in mesophilic and from 7.61 to 7.83 in thermophilic conditions during BMP tests (Appendix F), even without the addition of a buffer. The lowest pH value was 7.61 in both BMP reactors using HTL.
aqueous. This suggests that HTL aqueous possesses sufficient alkalinity to effectively buffer pH fluctuations under both anaerobic conditions. In terms of rate of anaerobic treatment, specific cumulative methane curves were used to identify kinetic parameters using the Modified Gompertz equation (Table 4.2). The trend observed in the kinetic parameters indicated that an increase in adsorbent concentration increased the kinetic reaction rate ($R_m$), while the lag phase ($\lambda$) parameter suggested the absence of a lag phase. Even the lower dose of GAC increased the kinetic rate in both mesophilic and thermophilic BMPs.

Table 4.2. Mesophilic and thermophilic BMP kinetic analysis results.

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Mesophilic biochemical methane potential test</th>
<th>Thermophilic biochemical methane potential test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated HTL aqueous</td>
<td>5 g/L GAC treated HTL aqueous</td>
</tr>
<tr>
<td>$P$</td>
<td>144</td>
<td>156</td>
</tr>
<tr>
<td>$R_m$</td>
<td>40.0</td>
<td>44.9</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9899</td>
<td>0.9910</td>
</tr>
<tr>
<td>Adj-$R^2$</td>
<td>0.9859</td>
<td>0.9874</td>
</tr>
<tr>
<td>Glucose</td>
<td>159</td>
<td>176</td>
</tr>
<tr>
<td>$R_m$</td>
<td>11.1</td>
<td>12.3</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9865</td>
<td>0.9893</td>
</tr>
<tr>
<td>Adj-$R^2$</td>
<td>0.9821</td>
<td>0.9857</td>
</tr>
</tbody>
</table>

$P$: Specific maximum production (mL CH₄/g COD), $R_m$: Maximum reaction rate (mL CH₄/g COD.d⁻¹), $\lambda$: Lag phase (d), Adj-$R^2$: Adjusted $R^2$, HTL: Hydrothermal liquefaction, GAC: Granular activated carbon.

In this study, the kinetic rate in mesophilic BMPs is higher than thermophilic BMPs. This may suggest that the mesophilic microorganisms are more robust, making it easier to adapt to HTL aqueous substrate. Wang et al. (2022) reported 34% kinetic rate increase (from 11.7 to 15.7 mL CH₄/g COD.d⁻¹) in anaerobic conditions (37°C) using GAC for adsorption. It was suggested that the overall pore volume and adsorption capacity of materials (biochar and GAC) emerged as
pivotal factors, driving enhancements in both the structure and activity of the microbial community. Previous studies on HTL aqueous also pointed out advantages of adsorption as a pretreatment, such as: adsorbing inhibitory compounds (Adedeji et al., 2023; Adedeji and Jahan, 2023; Erkelens et al., 2015; Zhou et al., 2015), minimizing ammonia inhibition (Ran et al., 2015), improved methane yield (R. Li et al., 2019; Shanmugam et al., 2017b; Zheng et al., 2017) reduced lag phase (R. Li et al., 2019), and removing recalcitrant compounds (Pham et al., 2013; Zheng et al., 2017).

Other studies assessed the performance of mesophilic and thermophilic digesters utilizing the same substrate. For example, Bicakci et al. (2020) reported a comparative analysis of bacterial and archaeal community comparing thermophilic and mesophilic anaerobic digesters utilizing mixed sludge. It was indicated that organic overload increased VFAs and decreased methane production in thermophilic digesters. Hydrogenotrophic methanogenesis, primarily dominated by the genus *Methanothermobacter*, emerged as a significant methane production pathway in thermophilic digesters. However, in mesophilic digesters, aceticlastic methanogenesis, predominantly associated with the genus *Methanosaeta*, was identified as the primary methane generation route. Overall, with the robust and diversity of mesophilic condition, it can be said that mesophilic conditions are more favorable and reliable than thermophilic for HTL aqueous treatment under anaerobic conditions.

Basar et al. (2024) reported that thermophilic AD process showed higher susceptibility to inhibition compared to mesophilic AD for substrate of mixed sludge. Thermophilic AD methane production was observed to be 32% lower than that of mesophilic AD. N-heterocyclic compounds, such as pyrazines, pyridines, pyrrolidinones, and N-heterocyclic compounds, are known to have extended biological treatment time requirements (Shanmugam et al., 2017a; Watson et al., 2020; Zheng et al., 2017). When protein-rich feedstocks like sludge are used, the resulting HTL aqueous solution contains a high concentration of N-heterocyclic compounds. However, the conversion
efficiency of these N-heterocyclic compounds during AD is relatively slow (Li et al., 2001; Maddi et al., 2017). Aromatic compounds and nitrogen organics were identified as inhibitors due to their aromatic ring structure and the resistance of their C-C and C-H bonds against biodegradation (Maddi et al., 2017, 2016; Watson et al., 2020). Detailed analysis and insight into the organic pollutants and their removal by adsorption was described in Section 3.3.3.6 and not repeated here for brevity. Figure 4.6 shows that in this study, mesophilic organisms can utilize more pyrazines than pyridines during anaerobic treatment. For example, green color indicates initial 100% of organic compounds present in HTL aqueous. Yellow color shows mass % of compounds remaining in digestate, while orange and purple colors represent mass % of compounds digested in BMPs before and after adsorption, respectively. During 28 days of mesophilic incubation, BMPs degraded 75% of mass of the pyrazines in HTL aqueous, meanwhile only 28% of mass of pyridines were utilized.

**Figure 4.6.** The percent mass distribution of pyrazines and pyridines of HTL aqueous between adsorption pretreatment (50 and 100 g/L GAC) and mesophilic anaerobic treatment processes (n=3).
These findings agree with literature and show that pyridines may not easily be biodegradable under mesophilic anaerobic conditions. It also can be postulated that pyridines could be more inhibitory than pyrazines. However, further investigation on HTL aqueous inhibitory compounds under anaerobic conditions is required. Table 4.3 provides a list of organic compounds in (full-strength) HTL aqueous quantified by GC-MS and their removal efficiencies by GAC at various doses. These compounds were selected for their frequent occurrence in HTL aqueous derived from mixed sludge feedstock.

**Table 4.3.** The removal efficiency of organic compounds identified by GC-MS in HTL aqueous derived from mixed sludge using different doses of GAC.

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Concentration in raw HTL aqueous (mg/L)</th>
<th>Removal by GAC dose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyrazine</strong></td>
<td></td>
<td>20 g/L</td>
</tr>
<tr>
<td>2-Methylpyrazine*</td>
<td>830.4 ± 4.6</td>
<td>3.7 ± 2</td>
</tr>
<tr>
<td>Ethylpyrazine*</td>
<td>144.7 ± 10</td>
<td>8.9 ± 2</td>
</tr>
<tr>
<td>2,3-Dimethylpyrazine*</td>
<td>76.7 ± 15.6</td>
<td>22.3 ± 1</td>
</tr>
<tr>
<td>2,3,5-Trimethylpyrazine*</td>
<td>70.1 ± 0.8</td>
<td>34 ± 6</td>
</tr>
<tr>
<td>Pyrazine, 2,5-dimethyl**</td>
<td>164.3 ± 6.8</td>
<td>8.4 ± 1</td>
</tr>
<tr>
<td>Pyrazine, 2-ethyl-5-methyl**</td>
<td>34.9 ± 3.9</td>
<td>32.3 ± 4</td>
</tr>
<tr>
<td>Pyrazine, 2-ethyl-3-methyl**</td>
<td>19.7 ± 11.7</td>
<td>25.7 ± 2</td>
</tr>
<tr>
<td><strong>Pyridine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylpyridine*</td>
<td>51.5 ± 1.1</td>
<td>37.5 ± 10</td>
</tr>
<tr>
<td>2-Ethylpyridine*</td>
<td>4.9 ± 2.4</td>
<td>47.8 ± 10</td>
</tr>
<tr>
<td>2,6-Dimethylpyridine*</td>
<td>11.4 ± 2.6</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>5-Ethyl-2-methylpyridine*</td>
<td>5.8 ± 1.9</td>
<td>54.5 ± 4</td>
</tr>
<tr>
<td>Pyridine, 3,4-dimethyl**</td>
<td>14.3 ± 2.8</td>
<td>45.7 ± 4</td>
</tr>
<tr>
<td>Pyridine, 2,3-dimethyl**</td>
<td>8.0 ± 2.5</td>
<td>44.8 ± 4</td>
</tr>
<tr>
<td>Pyridine, 3-ethyl**</td>
<td>1.3 ± 1.9</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>Pyridine, 3-methyl**</td>
<td>30.3 ± 4.3</td>
<td>38.7 ± 5</td>
</tr>
<tr>
<td><strong>Cyclic compound</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Methyl-cyclopentanone*</td>
<td>24.5 ± 3.8</td>
<td>27.2 ± 4</td>
</tr>
<tr>
<td>Cyclopentanone, 2-methyl**</td>
<td>33.3 ± 0.9</td>
<td>31.8 ± 5</td>
</tr>
<tr>
<td>Cyclohexanone*</td>
<td>8.7 ± 7.4</td>
<td>28.9 ± 4</td>
</tr>
</tbody>
</table>

*Quantitated using standards by GC-MS, **Semi-quantitated using standards of isotherms by GC-MS, a, b, c, d, Data represent average ± standard deviation of triplicates.
Notably, GAC was effective in removing all compounds, while it seemed to be more efficient in adsorbing pyridines than pyrazines and cyclic ketones. GAC concentration of 100 g/L eliminated most (> 90%) of the identified potentially inhibitory compounds while keeping VFAs in HTL aqueous. The findings suggest that adsorption could be a possible option for pretreating HTL aqueous and removing potential inhibitors for valorization through AD for simultaneous production of biocrude and biogas from mixed sludge at treatment plants.

4.3.4 Biodegradability assessment under aerobic conditions

Different concentrations of GAC were used to pretreat HTL aqueous to test its ability to enhance aerobic biodegradability. BOD progression curves for raw and adsorption pretreated HTL aqueous samples at various GAC doses were displayed in Figure 4.7. Appendix G includes oxygen uptake rate curves for all the samples with respect to incubation time.

![Figure 4.7](image)

**Figure 4.7.** Respirometric biochemical oxygen demand (BOD) concentrations of untreated (raw) and GAC-treated HTL aqueous samples at different GAC doses. Shaded regions indicate error bands with standard deviations (n=3).
Furthermore Table 4.4 shows aerobic biodegradability assessment of HTL aqueous samples, quantified by various parameters, such as BOD$_5$, ultimate BOD (or BOD$_{18}$), biodegradability index (ultimate BOD/COD), and average BOD removal (%). Removal of ultimate BOD from HTL aqueous by adsorption followed a similar trend as COD removal. As GAC concentration increased from 5 to 100 g/L GAC, ultimate BOD removal from HTL aqueous increased from 11 ± 0.2 to 50 ± 1.4 %, respectively. The BOD/COD index represents the impact of pretreatment on biodegradability, as BOD detects the oxygen demands required by aerobic organisms to break down organic matter, while COD represents the total oxygen demand to oxidize most of the organic compounds.

Table 4.4. Biodegradability assessment of raw and pretreated HTL aqueous under aerobic conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average$^a$ COD (g/L)</th>
<th>Average$^b$ BOD$_5$ (g/L)</th>
<th>Average$^c$ Ultimate BOD (g/L)</th>
<th>Average biodegradability index (Ultimate BOD/COD)</th>
<th>Average BOD removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTL aqueous</td>
<td>67 ± 0.8</td>
<td>42 ± 0.3</td>
<td>54 ± 0.6</td>
<td>0.81 ± 0.01</td>
<td>11 ± 0.2</td>
</tr>
<tr>
<td>5 g/L GAC</td>
<td>62 ± 0.9</td>
<td>38 ± 0.1</td>
<td>48 ± 0.1</td>
<td>0.77 ± 0.01</td>
<td>13 ± 3.5</td>
</tr>
<tr>
<td>10 g/L GAC</td>
<td>59 ± 0.6</td>
<td>37 ± 0.3</td>
<td>47 ± 1.9</td>
<td>0.80 ± 0.03</td>
<td>21 ± 0.6</td>
</tr>
<tr>
<td>20 g/L GAC</td>
<td>51 ± 0.4</td>
<td>35 ± 0.6</td>
<td>43 ± 0.3</td>
<td>0.84 ± 0.01</td>
<td>35 ± 0.4</td>
</tr>
<tr>
<td>50 g/L GAC</td>
<td>46 ± 1</td>
<td>29 ± 0.7</td>
<td>35 ± 0.2</td>
<td>0.76 ± 0.01</td>
<td>50 ± 1.4</td>
</tr>
<tr>
<td>100 g/L GAC</td>
<td>29 ± 0.6</td>
<td>23 ± 0.5</td>
<td>27 ± 0.7</td>
<td>0.93 ± 0.03</td>
<td>50 ± 1.4</td>
</tr>
</tbody>
</table>

a, b, c, Data represent average ± standard deviation of triplicates. Ultimate BOD=BOD$_{18}$

In this study, the rate of aerobic degradation of HTL aqueous was also assessed. The trend observed in the kinetic parameters indicated that an increase in adsorbent concentration decreased the kinetic reaction rate ($R_m$), while the lag phase ($\lambda$) parameter suggested the absence of a lag phase (Table 4.5). This suggested that GAC adsorption also removes readily biodegradable substrates with BOD potential as well as inhibitory compounds. High correlation coefficient values are due to an extensive set of data collected at 10 minute intervals during 18 days of monitoring.
Table 4.5. Kinetic parameters for ultimate BOD test.

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Untreated HTL aqueous</th>
<th>5 g/L GAC treated HTL aqueous</th>
<th>10 g/L GAC treated HTL aqueous</th>
<th>20 g/L GAC treated HTL aqueous</th>
<th>50 g/L GAC treated HTL aqueous</th>
<th>100 g/L GAC treated HTL aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P$</td>
<td>51708</td>
<td>46037</td>
<td>44764</td>
<td>40690</td>
<td>33615</td>
<td>26020</td>
</tr>
<tr>
<td>$R_m$</td>
<td>10517</td>
<td>9955</td>
<td>9589</td>
<td>9633</td>
<td>9067</td>
<td>8144</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9708</td>
<td>0.9743</td>
<td>0.9677</td>
<td>0.9741</td>
<td>0.9592</td>
<td>0.9182</td>
</tr>
<tr>
<td>Adj-$R^2$</td>
<td>0.9707</td>
<td>0.9743</td>
<td>0.9677</td>
<td>0.9741</td>
<td>0.9592</td>
<td>0.9181</td>
</tr>
</tbody>
</table>

$P$: Ultimate biochemical oxygen demand (mg O$_2$/L), $R_m$: Maximum oxygen consumption rate (mg O$_2$/L.d$^{-1}$), $\lambda$: Lag phase (d), Adj-$R^2$: Adjusted $R^2$, HTL: Hydrothermal liquefaction, GAC: Granular activated carbon.

Figure 4.8 shows biodegradability percentage of raw and GAC-treated HTL aqueous samples under aerobic, mesophilic anaerobic, and thermophilic anaerobic treatment conditions. All adsorption dose conditions, except 50 g/L GAC, had an increasing trend. The removal of well-known recalcitrant and inhibitory compounds, such as pyrazines and phenolics, by GAC enabled an observed increase in the biodegradability of HTL aqueous under different conditions. The biggest improvement was seen in mesophilic BMPs, followed by thermophilic BMPs and aerobic treatment conditions.

Furthermore, mesophilic BMPs performed better than thermophilic BMPs. This could be due to, the type and accessibility of the substrate significantly affect the thermophilic microbial population which might need an extended period of acclimatization to establish a stability, making them more vulnerable to alterations and less likely to adjust to operational changes (Gagliano et al., 2015). Aerobic conditions were the most robust against concentrated pollutants and inhibitory compounds in HTL aqueous. This could be due to more diverse and resilient aerobic microbial composition (Basar et al., 2024). In terms of robustness and diversity, it can be concluded that aerobic microorganisms showed the highest potential whereas thermophilic microorganisms showed the lowest potential.
Figure 4.8. Biodegradability percentages of raw and GAC-treated HTL aqueous samples under aerobic, mesophilic anaerobic, and thermophilic anaerobic biological treatment conditions.

4.4 Summary

This Chapter assessed the efficacy of adsorption as a method for treating full-strength HTL aqueous and examined the impact of GAC in removing various contaminants from HTL aqueous. Subsequently, the study investigated the influence of GAC adsorption on downstream aerobic and anaerobic biological treatment processes. The main findings of the study are as follows:

- GAC was efficient in removing total phenolics (up to 87%) and COD (up to 61%) but not VFAs and total ammonium nitrogen (< 6%) from full-strength HTL aqueous. GAC could
highly selectively eliminate inhibitory organic compounds and retain VFAs in HTL aqueous, allowing subsequent valorization through methane production.

- In mesophilic BMP, GAC at concentrations of 5, 10, 20, 50, and 100 g/L resulted in enhancements in specific methane yield by 8, 13, 28, 39, and 97%, respectively. The dosage of 100 g/L of GAC demonstrated a high increase in specific methane yield (320 mL CH₄/g COD), approaching the theoretical yield at STP.

- In thermophilic BMP, GAC at concentrations of 5, 10, 20, 50, and 100 g/L resulted in specific methane yield enhancements of 10, 15, 32, 32, and 83%, respectively.

- Results also indicate that thermophilic microorganisms could exhibit susceptibility to inhibitory compounds in HTL aqueous, while mesophilic microorganisms may generate more methane with adsorption pretreatment, particularly at higher doses.

- Removal of BOD from HTL aqueous by adsorption followed a similar trend as COD removal. As GAC concentration increased from 5 to 100 g/L GAC, ultimate BOD removal increased from 11 ± 0.2 to 50 ± 1.4%, respectively, corresponding to a biodegradability index (ultimate BOD/COD) increase from 0.81 ± 0.01 to 0.93 ± 0.03.

- Aerobic treatment exhibits the least susceptibility to inhibition while attaining the highest biodegradability and followed by biological treatment under mesophilic anaerobic conditions.
Chapter 5: Conclusion

5.1 Overall summary and conclusions

This thesis addresses some of the HTL of municipal sludge challenges by proposing adsorption as a pretreatment to remove potential inhibitory compounds in HTL aqueous. Furthermore, it assesses hydrochar from HTL as an adsorbent. Through optimizing adsorption and biodegradability assessment of HTL aqueous, the thesis contributes to literature regarding recovering valuable resources from HTL process. The following conclusions can be drawn from this research:

- All adsorbents in HTL aqueous medium favor neutral pH adsorption conditions.
- The adsorption HTL aqueous followed the pseudo-second-order kinetic model. Among GAC, biochar, hydrochar tested, GAC was the suitable adsorbent. GAC demonstrated the highest adsorption capacity at 184 mg/g, surpassing biochar (44 mg/g) and hydrochar (42 mg/g), which had limited surface area and mesopores.
- The Freundlich isotherm adsorption model slightly exhibited higher correlation coefficients compared to the Langmuir and Temkin isotherm models.
- The adsorption of HTL aqueous onto hydrochar, biochar, and GAC was found to be endothermic, favoring higher temperatures.
- GAC proved effective in removing total phenolics and COD but less so for VFAs and total ammonium nitrogen from HTL aqueous and lacked robust desorption capacity.
- GAC (100 g/L) could achieve 97% and 83% specific methane yield enhancement in mesophilic and thermophilic BMP, respectively, compared to raw HTL aqueous.
- Removal of BOD followed a similar trend to COD removal. As GAC concentration went up from 5 g/L GAC to 100 g/L BOD removal increased from 11 ± 0.2 to 50 ± 1.4%, respectively while increasing BOD/COD from 0.81 ± 0.01 to 0.93 ± 0.03.
• Mesophilic cultures were more tolerant to inhibitors in HTL aqueous compared to
thermophilic cultures.
• Aerobic treatment exhibits a higher biodegradability but no opportunity for methane
recovery.

5.2 Limitations and recommendations

One limitation of this study is that only one batch of mixed sludge and one HTL conditions
(350°C, 15 min) were used to generate HTL aqueous, potentially limiting its ability to simulate full-
scale HTL operations at WWTP. However, the properties of HTL products (aqueous and
hydrochar) are consistent with literature (Liu et al., 2021; Liu et al., 2022). Another limitation is
that the study only investigated batch adsorption tests and batch BMP tests. Therefore, the results
of this study require verification under continuous flow operation conditions. Additionally, the study
focused on COD removal for optimization, given the complexity of HTL aqueous. Based on these
limitations, some recommendations for future research are:

• The results of the kinetic and isotherm study suggest the use of an adsorbent with high
adsorption capacity. However, highly favorable adsorption of HTL aqueous would limit the
regeneration of an adsorbent. Also, from a thermodynamic point of view, 50°C is more
suitable than room temperature for HTL aqueous adsorption.
• Different sludge compositions and HTL conditions should be investigated. This is
necessary to understand the impact of feedstock on the composition of HTL aqueous.
Especially in a continuous-flow HTL process, operational and seasonal changes will
impact the sludge composition thereby changing HTL aqueous composition.
• Fixed bed adsorption columns are the most common industrial adsorption applications.
Therefore, testing GAC in a continuous-flow fixed bed adsorption column is necessary for
wastewater treatment plants. Additionally, thermochemical regeneration option for
industrial application is also crucial. The favorable adsorption conditions could exhaust GAC capacity after several cycles and chemical regeneration may not be an option due to limited regeneration efficiency.

- Further investigation on activation and utilization of hydrochar is necessary to increase its adsorption capacity. However, thermochemical activation may be the only suitable option given the persistent biocrude oil presence in the pore structure. Also, hydrochar should undergo re-agglomeration process to be used in a fixed column bed.

- Continuous flow reactors for biological treatment are crucial to see long term effect of feeding HTL aqueous on beneficial cultures. Long term effects of recalcitrant and inhibitory compounds in anaerobic cultures should be investigated.

- Life cycle assessment and life cycle costing could provide more about the cost and the feasibility of the processes from a cost/benefit point of view.
Bibliography


Appendices

Appendix A Detailed explanation of laboratory experiments

The total solids (TS) and volatile solids (VS) content of samples were determined, following standard methods 2540B and 2540E. Samples of 5 to 10 grams were placed in ceramic crucibles and subjected to controlled drying. After measurements were taken, the crucibles were cleaned by soaking them overnight in a solution containing 20% sulfuric acid, thoroughly rinsed, and then incinerated at 550°C for 30 minutes using a muffle furnace. Total suspended solids (TSS), and volatile suspended solids (VSS) were measured following Standard Methods 2540. Samples of 5 to 10 mL were filtered through a glass fiber filter (1.6 μm). The filter is dried in an oven at 105 °C for 24 hours for TSS, and then placed in a 550 °C furnace oven for 2 hours for VSS.

For COD determination, a GENESYS™10S UV VIS spectrophotometer was set at an optical wavelength of 600 nm. Sample preparation included diluting the sample with distilled water and mixing it with digestion and catalyst solutions. Total alkalinity content was measured following standard method 2320B, involving titration using sulfuric acid until reaching a pH level of approximately 4.6. Total phenolics in the samples were measured using the Folin Ciocalteu method. This method converts compounds into a colored mixture with the Folin Ciocalteu reagent. After spinning the samples at 15,000 rpm for 20 minutes, the supernatant was further filtered using a nylon filter with a size of 0.45 μm. Subsequently, 0.1 mL of the filtered sample was mixed with 0.5 mL of a 10 times diluted solution of the Folin Ciocalteu reagent and 0.4 mL of Na₂CO₃. The resulting mixture was transferred to centrifuge tubes with a volume capacity of 3 mL, allowed to react in the Synergy HT microplate detection reader, and finally, the absorbance was measured at a wavelength of 735 nm. To establish a calibration curve, concentrations were prepared by
diluting a solution containing gallic acid, and the results were expressed as GAE per liter (Azarmina, 2023).

**Figure A-1.** Experiments for certain colorimetric analyses (COD, phenol, etc.).

**Figure A-2.** Total phenolics compound measurement.
**Figure A-3.** Calibration curve for COD experiments.

![COD Calibration Curve](image1)

**Figure A-4.** Calibration curve for total phenolics experiments.

![Total Phenolics Calibration Curve](image2)
Figure A-5. Calibration curve for total ammonium-N experiments.

Figure A-6. Calibration curve for soluble protein experiments.
Appendix B COD equivalence of phenol and VFAs

Table B-1. Conversion of organic compounds into chemical oxygen demand (COD) equivalence.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Equation</th>
<th>Molar weight (g/mol)</th>
<th>COD eq. (mol O₂/mol)</th>
<th>COD eq. (g O₂/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>C₇H₆O₅+6O₂=7CO₂+3H₂O</td>
<td>170.12</td>
<td>6</td>
<td>1.13</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>C₂H₄O₂+2O₂=2CO₂+2H₂O</td>
<td>60.05</td>
<td>2</td>
<td>1.07</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>2CH₃CH₂COOH+7O₂=6CO₂+6H₂O</td>
<td>74.08</td>
<td>3.5</td>
<td>1.51</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>C₄H₈O₂+5O₂=4CO₂+4H₂O</td>
<td>88.11</td>
<td>5</td>
<td>1.82</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>2C₅H₁₀O₂+13O₂=10CO₂+10H₂O</td>
<td>102.13</td>
<td>6.5</td>
<td>2.04</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>2C₅H₁₀O₂+13O₂=10CO₂+10H₂O</td>
<td>102.13</td>
<td>6.5</td>
<td>2.04</td>
</tr>
<tr>
<td>Caproic acid</td>
<td>C₆H₁₂O₂+8O₂=6CO₂+6H₂O</td>
<td>116.1583</td>
<td>8</td>
<td>2.20</td>
</tr>
<tr>
<td>Isocaproic acid</td>
<td>C₆H₁₂O₂+8O₂=6CO₂+6H₂O</td>
<td>116.1583</td>
<td>8</td>
<td>2.20</td>
</tr>
</tbody>
</table>
Appendix C BMP setup conditions and change of pH in assays overtime

**Table C-1.** Mesophilic BMP setup parameters.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Substrate COD mg</th>
<th>Inoculum VS (mg)</th>
<th>Volume Inoculum (mL)</th>
<th>Calculated F/M mg COD/mg VS</th>
<th>Total Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>251.20</td>
<td>502.39</td>
<td>40.00</td>
<td>0.50</td>
<td>70.00</td>
</tr>
</tbody>
</table>

**Table C-2.** Thermophilic BMP setup parameters.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Substrate COD mg</th>
<th>Inoculum VS (mg)</th>
<th>Volume Inoculum (mL)</th>
<th>Calculated F/M mg COD/mg VS</th>
<th>Total Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>240.73</td>
<td>481.46</td>
<td>40.00</td>
<td>0.50</td>
<td>70.00</td>
</tr>
</tbody>
</table>
Appendix D Respirometric BOD assay details

Mineral Base I

Add the following to 800 mL of reagent quality water. Dilute to 1 L.

- **CoCl$_2$.6H$_2$O** 0.25 g  **Na$_2$MoO$_4$.2H$_2$O** 0.005 g
- **FeCl$_2$.4H$_2$O** 4 g  **NiCl$_2$.6H$_2$O** 0.025 g
- **MnCl$_2$.4H$_2$O** 0.05 g  **Na$_2$SeO$_4$** 0.025 g
- **H$_3$BO$_3$** 0.025 g  **CuCl$_2$.2H$_2$O** 0.007 g
- **ZnCl$_2$** 0.025 g

Mineral Base II

Dissolve the following in 800 mL distilled water. Dilute to 1 L.

- **CaCl$_2$** 15 g
- **MgCl$_2$.6H$_2$O** 20 g

Nutrient Base

Dissolve the following in 800 mL distilled water. Neutralize to pH using 50% NaOH. Dilute to 1 L.

- **NH$_4$Cl** 53 g
- **KH$_2$PO$_4$** 12 g
- **Na$_2$SO$_4$** 15 g
Appendix E Removal of organic compounds

E-1. Comparison of organic compounds in pyridines and cyclic ketones.

E-2. Removal (%) of organic compounds in HTL aqueous by group during pretreatment at different GAC doses.
Appendix F pH and VFAs change over time in BMPs

Figure F-1. Change of pH during thermophilic BMP assay.

Figure F-2. Change of pH during mesophilic BMP assay.
Figure F-3. Change of VFAs during thermophilic BMP assay.

Figure F-4. Change of VFAs during mesophilic BMP assay.
Appendix G BOD test results of HTL aqueous

G-1. Oxygen uptake rate of non-pretreated and pretreated HTL aqueous at different GAC doses with time.