

Investigating Nutrient Limitation and Microbial Imbalances in Greywater Treatment

DEPARTMENT OF OF PROCESS AND LIFE SCIENCE ENGINEERING | LUND UNIVERSITY

AISWARYA RAPHEL | MASTER THESIS 2025



Investigating Nutrient Limitation and Microbial Imbalances in Greywater Treatment

by

Aiswarya Raphael

Master Thesis number: 2025-05

Water and Environmental Engineering
Division of Chemical Engineering
Lund University

June 2025

Supervisor: **Åsa Davidsson**
External Supervisor: **Ashley Hall**
Examiner: **Michael Cimbritz**

Picture on front page: The ferry terminal, Helsingborg. Photo by Vino Paul Varghese.

Postal address

Box 124
SE-221 00 Lund, Sweden

Web address

<http://www.ple.lth.se>

Visiting address

Kemicentrum
Naturvetarvägen 14
223 62 Lund, Sweden

Telephone

+46 46-222 82 85
+46 46-222 00 00

Postal address

Box 124
SE-221 00 Lund, Sweden

Web address

<http://www.ple.lth.se>

Visiting address

Kemicentrum
Naturvetarvägen 14
223 62 Lund, Sweden

Telephone

+46 46-222 82 85
+46 46-222 00 00

Preface

This master's thesis was carried out as part of the Master's program in Water Resources Engineering at Lund University, in collaboration with RecoLab, Helsingborg. The project investigated nutrient limitation and microbial imbalances in greywater treatment.

I would like to express my sincere gratitude to my supervisor, Åsa Davidsson, for her continuous support, holding regular meetings, insightful feedback, and encouragement throughout the research process. I am also deeply thankful to my external supervisor, Ashley Hall, for providing valuable guidance and facilitating access to RecoLab. I especially appreciate Ashley's help in driving me to and from Lund university to RecoLab for sample transport on several days as well as Åsa's support in providing transportation on one occasion when Ashley was unavailable. Special thanks go to my examiner, Michael Cimbritz, for his constructive suggestions that helped refine the research questions and outcomes.

I also acknowledge the Chemical Engineering Division at Lund University not only for providing the laboratory space, equipment, and assistance needed to carry out the experimental work but also for the vehicle required for transportation during the sampling period, which was essential for conducting this study. I extend my appreciation to the staff at RecoLab and NSVA for their cooperation and support during sample collection and for granting access to the treatment facility.

I am particularly grateful to Gratus for helping me in collecting, analysing samples and capturing pictures. A heartfelt thanks to all my friends for their support throughout this academic journey. Finally, I am deeply grateful to my family for their encouragement and patience during this time.

I also want to express my appreciation for the AI tools that helped get more ideas and enhance the language and overall quality of the text in this thesis.

Lund, June 2025

Aiswarya Raphael

Popular Scientific Summary

The science behind smarter water reuse

Greywater isn't as dirty as toilet water but still needs treatment before it can be reused. Every time when we wash our hands, do the laundry, or take a shower, we create this greywater. For treating greywater separately, an advanced treatment facility called RecoLab has been collecting wastewater from Helsingborg's Oceanhamnen area, for the last two years. In this facility, wastewater is collected through three different pipes and treated individually to recover nutrients, gas, and energy.

A recent master's thesis from Lund University examined how effectively RecoLab is managing this task. The study investigated whether the tiny microbes that break down waste in greywater are receiving the proper nutrients to perform their function. These microbes require a balanced diet, particularly the right combination of carbon, nitrogen, and phosphorus. An imbalance in any of these elements can disrupt the system and lead to issues such as excessive bacterial growth.

Over several weeks, samples were collected from the greywater system and tested in the lab. The study found that while the greywater had enough organic material, and it often contained less nitrogen and phosphorus. Or in another way, the greywater had relatively high levels of organic matter but still contained insignificant amounts of nitrogen and phosphorus. When there isn't enough of nitrogen and phosphorus compared to organic matter, the system can become unbalanced. This may not cause immediate or severe issues, but it could slightly limit microbial activity, especially for high-efficiency treatment systems. In such cases, operators might need to dose nitrogen and phosphorus into the system to maintain microbial health and ensure proper breakdown of organic matter.

This suggests that even the water from washing activities can be managed more intelligently and recycled, preserving drinking water for essential uses. At the same time, it highlights the role of research and development in guiding towards better sustainability and environmental protection. Understanding and managing these nutrient levels can help treatment plants avoid problems, reduce costs, and make better use of recycled water.

Populärvetenskaplig sammanfattning

Vetenskapen bakom smartare vattenåteranvändning

Gråvatten är inte lika smutsigt som toalettavatten men behöver fortfarande behandling innan det kan återanvändas. Varje gång vi tvättar våra händer, tvättar eller duschar, skapar vi detta gråvatten. För att behandla gråvatten separat har en avancerad behandlingsanläggning med namnet RecoLab samlat in avloppsvatten från området Oceanhamnen i Helsingborg de senaste två åren. I denna anläggning samlas avloppsvattnet genom tre olika rör och behandlas individuellt för att återvinna näring, gas och energi.

Ett examensarbete från Lunds universitet undersökte nyligen hur effektivt RecoLab hanterar denna uppgift. Studien undersökte huruvida de små mikrober som bryter ner föroreningar i gråvatten får de rätta näringsämnen för att utföra sin funktion. Dessa mikrober behöver en balanserad kost, särskilt rätt kombination av kol, kväve och fosfor. En obalans i något av dessa element kan störa systemet och leda till problem som överdriven bakterietillväxt.

Under flera veckor samlades prover in från gråvatten-systemet och testades i labbet. Studien fann att även om gråvattnet hade tillräckligt med organiskt material, så innehöll det ofta mindre kväve och fosfor. När det inte finns tillräckligt med kväve och fosfor i förhållande till organiskt material, kan systemet bli obalanserat. Detta kanske inte orsakar omedelbara eller allvarliga problem, men det kan något begränsa den mikrobiella aktiviteten, särskilt för högpresterande behandlingssystem. I sådana fall kan operatörer behöva tillsätta kväve och fosfor i systemet för att upprätthålla mikrobiell hälsa och säkerställa en korrekt nedbrytning av organiskt material.

Resultaten från examensarbetet tyder på att även vattnet från tvättaktiviteter kan hanteras mer intelligent och återvinnas, vilket sparar dricksvatten som då kan användas där det är som viktigast. Att förstå och hantera näringbehovet vid biologisk rening kan hjälpa reningsverk att undvika problem, minska kostnader och bidra till bättre användning av återvunnet vatten.

Summary

Greywater, which comes from household sources such as showers, laundry, and dishwashing, is typically less polluted than toilet wastewater and can be treated and reused. In Helsingborg, Sweden, the RecoLab collects and treats greywater from Oceanhamnen separately as part of a source-separated wastewater system. However, the system has experienced issues with filamentous bacteria overgrowth, raising concerns about potential nutrient imbalances that could disrupt microbial processes.

This thesis investigated nutrient dynamics and potential microbial imbalances in greywater treatment at RecoLab. The study focuses on availability of the concentrations of carbon, nitrogen, and phosphorus in influent greywater and evaluates their balance using Total Organic Carbon (TOC) as a measure of biodegradable carbon.

A total of eight days of influent greywater samples were analyzed, with two sets of samples collected per day: one representing fresh greywater taken directly from Oceanhamnen, and the other taken after the greywater had remained in the collection tank for approximately 12 hours. All samples were analyzed for COD, TN, TP, and other key fractions. TOC is a more accurate indicator of bioavailable carbon. A conversion from COD to TOC was employed for a more accurate representation. However, when using TOC in C:N:P ratio, both nitrogen and phosphorus were found to be inadequate relative to carbon in the influent greywater. It became clear that greywater entering the treatment system may contain more bioavailable carbon in relation to nitrogen and phosphorus. Although this does not necessarily cause the overgrowth of filamentous bacteria, such imbalances can affect microbial stability and treatment efficiency. Implementing effective monitoring alongside the dosing of nitrogen and phosphorus into the system could support microbial health and facilitate the proper decomposition of organic matter.

This study provides a foundation for optimizing nutrient management in greywater treatment systems to improve operational stability and effluent quality. It also indicates that careful monitoring of TOC-based nutrient ratios is essential for effective greywater treatment.

Sammanfattning

Gråvatten, som kommer från hushållskällor som duschar, tvätt och diskning, är vanligtvis mindre förorenat än toalettavlopp och kan behandlas och återanvändas. I Helsingborg, Sverige, samlar och behandlar RecoLab gråvatten från Oceanhamnen separat som en del av ett källseparerat avloppssystem. Dock har systemet haft problem med filamentösa bakterietillväxt, vilket väcker oro för potentiella näringsobalanser som kan störa mikrobiella processer.

Detta examensarbete har undersökt näringsdynamik och potentiella mikrobiella obalanser i gråvattenbehandling vid RecoLab. Studien fokuserar på tillgängligheten av koncentrationerna av kol, kväve och fosfor i inkommande gråvatten och utvärderar deras balans med hjälp av Total Organic Carbon (TOC) som ett mått på biologiskt nedbrytbart kol.

Åtta inkommande gråvattenprover analyserades för COD, TN, TP och andra nyckelfraktioner. TOC är en mer exakt indikator på bioanvändbart kol. En omvandling från COD till TOC användes för en mer exakt representation. När TOC användes i C:N:P-förhållandet, visade det sig att både kväve och fosfor var otillräckliga i förhållande till kol i det inkommande gråvattnet. Det blev tydligt att gråvatten som kommer in i behandlingssystemet kan innehålla mer bioanvändbart kol i förhållande till kväve och fosfor. Även om detta inte nödvändigtvis orsakar överväxt av filamentösa bakterier, kan sådana obalanser påverka mikrobiell stabilitet och behandlingseffektivitet. Att implementera effektiv övervakning tillsammans med dosering av kväve och fosfor i systemet kommer att stödja mikrobiell hälsa och underlätta korrekt nedbrytning av organiskt material.

Denna studie ger en grund för att optimera näringshanteringen i gråvattenreningsystem för att förbättra den operativa stabiliteten och avloppskvaliteten. Den indikerar också att noggrann övervakning av TOC-baserade näringsförhållanden är avgörande för effektiv gråvattenrening.

List of Abbreviations

| | |
|------------------------------------|------------------------------|
| BOD = | Biochemical Oxygen Demand |
| C:N:P = | Carbon: Nitrogen: Phosphorus |
| COD = | Chemical Oxygen Demand |
| L/day = | Liters per day |
| MBBR = | Moving bed biofilm reactor |
| NH ₄ ⁺ -N = | Ammonium Nitrogen |
| NO ₂ ⁻ -N = | Nitrite Nitrogen |
| NO ₃ ⁻ -N = | Nitrate Nitrogen |
| PO ₄ ³⁻ -P = | Phosphate Phosphorus |
| TOC = | Total Organic Carbon |
| TN = | Total Nitrogen |
| TP = | Total Phosphorus |
| TS = | Total Solids |
| TSS = | Total Suspended Solids |
| VS = | Volatile Solids |

Table of Contents

| | | |
|-----|--|----|
| 1 | Introduction | 1 |
| 1.1 | Background Information..... | 1 |
| 1.2 | Aim & Objective | 1 |
| 1.3 | Significance, Scope and Limitations of the Study..... | 2 |
| 2 | Literature Review | 3 |
| 2.1 | Greywater characteristics | 3 |
| 2.2 | Greywater Treatment Methods..... | 6 |
| 2.3 | Importance of Nutrient Ratios | 7 |
| 2.4 | Purpose of Measuring Specific Nutrients | 8 |
| 3 | Materials and Methods | 11 |
| 3.1 | Sampling Strategy and Field Collection..... | 11 |
| 3.2 | Sample Preparation and Fractionation..... | 11 |
| 3.3 | Analytical Methods using Hach Lange Cuvette Tests..... | 13 |
| 3.4 | Conversion method from COD to TOC | 14 |
| 3.5 | Data Analysis..... | 14 |
| 4 | Results and Discussions | 17 |
| 4.1 | Overview of Greywater Composition | 17 |
| 4.2 | Characteristics of Unfiltered fresh and stored greywater | 18 |
| 4.3 | Assessment of nutrient balance based on TOC | 19 |
| 4.4 | Nitrogen and Phosphorus Fractionation | 20 |
| 5 | Conclusions | 23 |
| 6 | Future Work..... | 25 |
| 7 | References | 27 |
| | Appendix | 31 |

1 Introduction

Water scarcity is anticipated to be one of the most critical challenges globally in the coming decades, and an effective solution lies in the development of circular water systems, which enables the reuse of treated wastewater to reduce freshwater demand. Among different wastewater streams, greywater is less contaminated than blackwater and is therefore relatively easier to collect and treat, making it a viable option for reuse (Yáñez et al., 2024).

1.1 Background Information

RecoLab is a demonstration site for treating source-separated wastewater that is generated in the city district Oceanhamnen in Helsingborg, Sweden. Wastewater from households in this area is divided into three streams: food waste from kitchen grinders, black water from vacuum toilets, and greywater from washing dishes in the kitchen, bathing and laundry activities. Each stream is treated separately to maximize resource recovery. This includes nutrient extraction for fertilizers, biogas production from carbon in the blackwater and food waste fraction, and heat recovery from greywater (“RecoLab,” 2025).

RecoLab has experienced issues in the greywater treatment process, particularly the overgrowth of filamentous bacteria (Hall et al., 2024). Preliminary studies suggest that this problem may be from the deficiency of biologically available nitrogen and phosphorus. To reduce overgrowth, it may be necessary to adjust the nutrient concentrations within the treatment process. Additionally, if the effluent wastewater exhibits elevated levels of Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), nitrogen (N), and phosphorus (P), it could fail to comply with discharge standards for release into water bodies.

1.2 Aim & Objective

The aim of this master's thesis is to analyse influent greywater and assess the various forms of nitrogen and phosphorus present in the influent wastewater. It also aims to determine whether a nutrient limitation exists and if it affects biological degradation processes by analysing the C:N:P ratios present in influent greywater by laboratory experiments and comparing those values to the theoretical requirements for aerobic respiration.

The following research questions will be addressed:

- What are the concentrations of carbon, nitrogen, and phosphorus in greywater, and how do the measured C:N:P ratios in greywater compare with the theoretical ratios required for effective biological treatment?
- Does storage (hydraulic retention time) improve nutrient bioavailability in greywater?
- What are the dominant forms of nitrogen and phosphorus, and what do they imply?
- Is greywater suitable for biological treatment without nutrient supplementation?

1.3 Significance, Scope and Limitations of the Study

Understanding the nutrient limitations will enable RecoLab to operate the greywater treatment facility more efficiently with less cost. Moreover, it will reduce the environment impact by sustainable reuse of greywater and by reducing eutrophication and environmental pollution.

The study mainly concentrated on the availability of carbon, nitrogen and phosphorus in fresh and stored influent greywater. However, it did not include a detailed analysis of the microbiological community through genetic or sequencing methods.

2 Literature Review

Water scarcity is a growing global concern, driven by population growth and pollution. Treatment is crucial for the sustainability of the environment and human health because prolonged usage of greywater in agricultural lands may decrease its capillarity and permeability (Vuppaladadiyam et al., 2019). It can also alter soil chemistry, increasing salinity which negatively affects plant growth and soil structure. Untreated greywater can contain pathogens, chemicals, and organic matter that pose risks such as waterborne diseases, environmental pollution, and unpleasant odours. If released untreated, it can contaminate freshwater sources, leads to eutrophication, harm aquatic ecosystems, and contribute to soil degradation (Khajvand et al., 2022). Circularity in water management involves reusing and recycling water to reduce dependence on freshwater sources. Greywater treatment enables safe reuse, reducing the strain on freshwater supplies while minimizing environmental impact (Awasthi et al., 2024). This chapter discusses the characteristics of greywater, its treatment methods, and the role of nutrient ratios in the biological treatment process, supported by recent literature and research.

2.1 Greywater characteristics

Characteristics of greywater mainly depends on its source, household activities, peoples' behaviour and geographic location (Oteng-Peprah et al., 2018a). Greywater accounts for 75-90% of total household wastewater (Ghaitidak and Yadav, 2013). The average per capita greywater generation for Sweden is 100 to 150 L/day (Isaksson, 2023). For High-income countries it is 62 to 223 L/day, while for Low-income countries greywater production is 14 to 140 L/day depending on water availability and usage patterns (Shaikh and Ahammed, 2020).

Greywater is divided into two types based on the concentration of contaminants: light greywater and dark greywater. Light greywater includes water from bathrooms and wash basins, whereas dark greywater is from washing machines and dishwashers (Shaikh and Ahammed, 2020). In RecoLab, greywater from various sources is gathered for treatment. The physical and chemical properties of mixed greywater can vary significantly based on its household origins and user practices. It is crucial to understand these properties to assess treatment requirements and the potential for reuse.

2.1.1 Physical characteristics

Physical characteristics of greywater are observable and measurable properties, which influence the treatment process and reuse potential. Most common physical characteristics are temperature, pH, odour, colour, turbidity, total suspended solids and electrical conductivity.

Greywater is normally warmer than potable water, with temperatures ranging between 18 to 38°C depending on the source, due to the use of hot water for different human activities like bathing, washing, laundry (Ghaitidak and Yadav, 2013). High temperatures can promote microbial growth, settling of inorganic salts and odour formation. Greywater has a light grey colour, which tends to darken as time progresses. The pH values generally range between 5.5 and

9.0, with laundry and kitchen greywater tending to be more alkaline due to the presence of soaps and detergents (Shaikh and Ahammed, 2020).

Its odour is mild when fresh, but it can develop an unpleasant smell if stored for an extended period. Turbidity levels and total suspended solids (TSS) levels in kitchen greywater are usually higher and turbidity can range from 12.6 to 444 NTU (Isaksson, 2023), while total TSS vary between 15 to 537 mg/L (Ghaitidak and Yadav, 2013; Shaikh and Ahammed, 2020). The electrical conductivity ranges from 190 to 3000 $\mu\text{S}/\text{cm}$, with higher values found in kitchen greywater. (Isaksson, 2023).

Visually, mixed greywater can appear light to dark grey, with the colour darkening over time due to microbial activity. Fresh greywater may have a mild odour, but if stored without treatment, it rapidly develops a strong, unpleasant smell due to anaerobic decomposition (Shaikh and Ahammed, 2020). Table 2.1 shows a summary of all physical characteristics.

Table 2.1. Physical Characteristics

| Parameter | Range | Notes |
|-------------------------------------|-------------------------------------|---|
| Temperature (°C) | 18–38 | Higher due to use of hot water in showers and laundry (Ghaitidak & Yadav, 2013). |
| pH | 5.5–9.0 | Generally alkaline; influenced by soaps and detergents (Shaikh & Ahammed, 2020; Ghaitidak & Yadav, 2013). |
| Turbidity | 12.6–444 NTU | Higher in kitchen and laundry greywater due to food and fabric particles (Isaksson et al., 2023). |
| Total Suspended Solids (TSS) | 15–537 mg/L | Variable depending on greywater source (Ghaitidak & Yadav, 2013; Shaikh & Ahammed, 2020). |
| Electrical Conductivity (EC) | 14 - 3000 $\mu\text{S}/\text{cm}$ | Kitchen greywater shows higher values (Isaksson, 2023). |
| Color | Light grey to dark | Darkens with kitchen/laundry input or prolonged storage (Isaksson et al., 2023). |
| Odor | Mild when fresh; strong when stored | Anaerobic decomposition causes foul smells (Shaikh & Ahammed, 2020). |

2.1.2 Chemical characteristics

Chemical characteristics are the composition and concentration of various chemicals in greywater, and it includes BOD₅ (Biochemical Oxygen Demand over 5 days) & COD (Chemical Oxygen Demand) for measuring organic matter and nutrients like TN (Total Nitrogen) and TP (Total Phosphorus). BOD₅ reflects the presence of biodegradable organic matter and it ranges from 20 to 1460 mg/L (Ghaitidak and Yadav, 2013; Shaikh and Ahammed, 2020), while higher COD values are likely because of oil and organic food waste from the kitchen and its value lies between 41 to 1815 mg/L (Ghaitidak and Yadav, 2013). To avoid hazardous chemicals used in COD testing, Total Organic Carbon (TOC) is increasingly used as a safer and more precise

alternative. The TOC in mixed greywater can be approximated to range between 14 and 605 mg/L (Dubber and Gray, 2010).

In terms of nutrients, Total nitrogen (TN) concentrations in mixed greywater are found to be between 2.7 to 57.7 mg/L and it is from food particles, detergents, and other nitrogen-rich substances (Ghaitidak and Yadav, 2013). Ammonia (NH₄⁺-N) levels typically range from <1 to 17 mg/L, while Nitrate (NO₃⁻-N) is usually low, at <5 mg/L, unless nitrification occurs during storage (Shaikh and Ahammed, 2020). Total Phosphorus (TP) and Phosphate (PO₄³⁻-P) concentrations ranges from 0.06 to 42 mg/L and are due primarily to the use of phosphate-based cleaning agents and detergents (Ghaitidak and Yadav, 2013). Compared to blackwater, greywater contains lower concentrations of nitrogen and phosphorus (Ghaitidak and Yadav, 2013; Shaikh and Ahammed, 2020). The following Table 2.2 shows a summary of all chemical characteristics relevant to this study.

Table 2.2. Chemical characteristics

| Parameter | Range | Notes |
|--|---------------------------------------|---|
| Chemical Oxygen Demand (COD) | 41–1815 mg/L (up to 8071 in kitchens) | Indicator of total organic load; highly variable across sources (Ghaitidak & Yadav, 2013). |
| Biochemical Oxygen Demand (BOD₅) | 20–1460 mg/L (up to 3330 in kitchens) | Reflects biodegradable organic matter (Shaikh & Ahammed, 2020; Ghaitidak & Yadav, 2013). |
| Total Organic Carbon (TOC) | 14 and 605 mg/L | Used as a safer, greener COD alternative (Dubber & Gray, 2010). |
| Total Nitrogen (TN) | 2.75–57.7 mg/L | Includes ammonia and organic nitrogen; higher in laundry and kitchen greywater (Ghaitidak & Yadav, 2013). |
| Ammonia (NH₄⁺-N) | <1 – 17 mg/L | From organic decomposition, urine, and food waste (Shaikh & Ahammed, 2020). |
| Nitrate (NO₃⁻-N) | <5 mg/L | Result of nitrification; usually low unless greywater is stored (Shaikh & Ahammed, 2020). |
| Phosphate (PO₄³⁻-P) | 0.06–42 mg/L | From detergents; important in eutrophication (Ghaitidak & Yadav, 2013). |
| Total Phosphorus (TP) | 0.062–42 mg/L | Includes organic and inorganic P forms (Ghaitidak and Yadav, 2013). |

2.2 Greywater Treatment Methods

Greywater treatment systems involve a variety of techniques designed to remove physical, chemical, and biological contaminants and therefore consists of three steps: physical, chemical and biological treatment technologies for safe reuse (Ghaly et al., 2021). This section mentions already established treatment methods for greywater.

2.2.1 Physical and Chemical Treatment Methods

Physical treatment primarily removes suspended solids and coarse particles from greywater through screening, sedimentation, and filtration using materials such as sand or membranes (Ghaitidak and Yadav, 2013). Chemical treatment processes involve coagulation and flocculation, disinfection methods like ozonation, chlorination and ultraviolet (UV) treatment. These methods are often used when biological treatment alone is insufficient Or when reuse could expose people to potential pathogens still in the water (Shahzeb et al., 2022; Szabolcsik-Izbéki et al., 2024). They serve as 'pathogen barriers' that protect public health.

2.2.2 Biological Treatment Methods

Biological treatment uses living microorganisms to degrade organic matter and nutrients. Microorganisms use organic carbon as a source for their growth hereby facilitating the removal of BOD, COD, and nutrient loads like nitrogen and phosphorus (Oteng-Peprah et al., 2018b; Shaikh and Ahammed, 2020).

Commonly used biological treatment methods are activated sludge processes, constructed wetland treatments, and membrane bioreactors (MBR). The activated sludge system is widely used in municipal and large-scale decentralized systems and involves the aeration of greywater in a tank containing suspended microbial biomass. The microbes consume organic pollutants, forming flocs that settle out in secondary clarifiers. (Ghaitidak and Yadav, 2013; Oteng-Peprah et al., 2018b).

Constructed wetlands are low-energy systems that use vegetation and microbial biofilms to treat greywater as it flows through gravel or sand media. These systems are particularly effective in rural areas due to their natural appearance, low operational cost, and ability to remove organics and nutrients (Ghaitidak and Yadav, 2013; Oteng-Peprah et al., 2018b).

Membrane bioreactors (MBRs) combine biological degradation with membrane filtration, offering high-quality effluent water with minimal space requirements. These systems are effective in removing suspended solids, bacteria, BOD, and nutrients, and are suitable for urban settings with limited land availability (Jadhao and Dawande, 2012).

In addition to these methods, biofilm-based systems such as trickling filters, rotating biological contactors (RBCs), and moving bed biofilm reactors (MBBRs) are also used. These systems support microbial growth on media surfaces, allowing for simultaneous aerobic and anoxic zones within the biofilm, which enhances the removal of nitrogen through nitrification and denitrification (Oteng-Peprah et al., 2018a).

The treatment process adopted in RecoLab is illustrated in Figure 2.1. Influent greywater was obtained from two locations marked by red dots: one from the automatic sampling device positioned immediately before the collection tank, and the other from a grab sample taken after the collection tank.

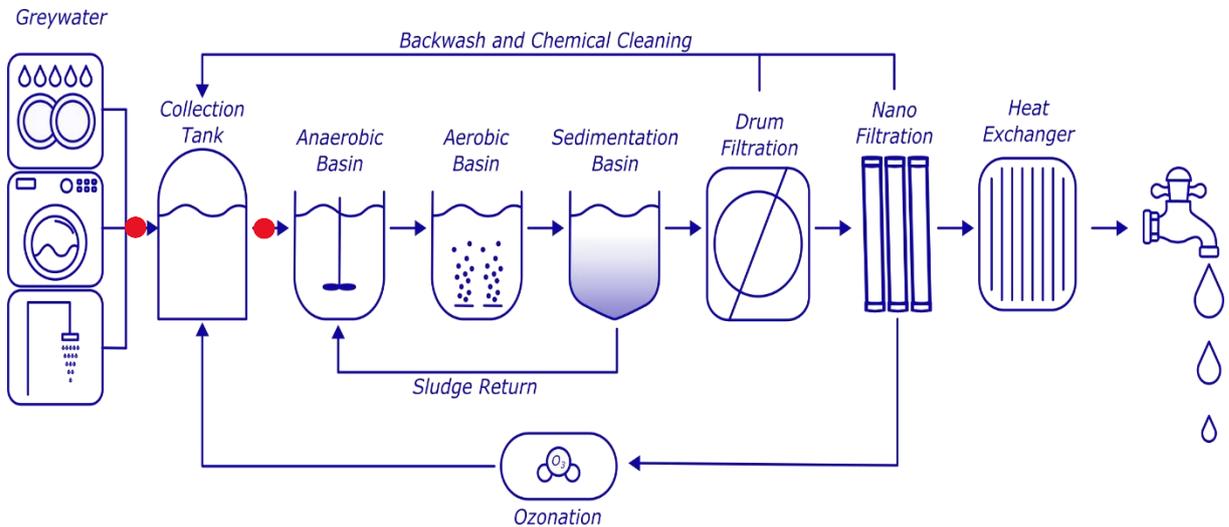


Figure 2.1. Water flow path at RecoLab, Helsingborg, adapted from Hall et al. (2024), under the terms of the CC BY 4.0 license.

2.2.3 Treatment process at RecoLab

Greywater collected in a collection tank is pumped into an anaerobic tank followed by an aerobic tank for biological treatment to remove organic matter, nitrogen and phosphorus. Treated water is then passed through a sedimentation tank to separate solids, then through a 10 μm drum filter and a 400 Da nano-filter for removing micropollutants. This step effectively removes nutrients like nitrogen and phosphorus. The final stage involves ozonation, during which only the reject water retained by the membrane and the backwash water undergo ozonation. Additionally, heat energy is recovered using heat exchangers. (Hall et al., 2024).

2.3 Importance of Nutrient Ratios

In biological treatment systems, the availability of carbon (C), nitrogen (N), and phosphorus (P) is important for sustaining microbial activity. The ideal carbon to nitrogen to phosphorus (C:N:P) ratio for microbial growth is 100:5:1 (Metcalf & Eddy Inc., 2014). COD is more common in practical treatment design and nutrient ratio calculations. According to the study conducted by Hernández Leal et al. (2011), which utilizes Chemical Oxygen Demand (COD) as a measure for carbon, greywater typically has a C:N:P ratio of about 100:3.5:1.6, which suggests a nitrogen shortage for aerobic treatment procedures (Hernández Leal et al., 2011).

In another study by Jefferson et al.(2001), they concluded that greywater supplementing nitrogen and phosphorus with a typical COD:N:P ratio of 100:10:1 improved microbial activity and treatment efficiency (Jefferson et al., 2001). Though ratios are not explicitly mentioned, Li et al.(2009) noted that bathroom and laundry greywater are deficient in both nitrogen and phosphorus, while kitchen greywater has a balanced COD:N:P ratio (Li et al., 2009).

COD is used as a proxy for the carbon content in the C:N:P ratio. However, COD includes both biodegradable and non-biodegradable organics, and can be influenced by oxidizing agents, and is therefore less accurate than TOC for this purpose. Converting COD to TOC provides a more precise estimate of the actual organic carbon available for microbial metabolism, thus allowing for a more accurate assessment and adjustment of the C:N:P ratio (Dubber and Gray, 2010). The COD:TOC ratio typically ranges between 2 and 4 depending on the wastewater characteristics in municipal wastewater and greywater (Dubber and Gray, 2010).

If nitrogen or phosphorus values are less than the required limit, an imbalanced situation will develop. While nutrient deficiencies particularly in nitrogen (N) and phosphorus (P) do not always lead to filamentous bacterial overgrowth, they can create conditions that favour it. Certain filamentous bacteria are better adapted to nutrient-limited environments, and they tend to be filamentous. Therefore, an imbalance in nutrient availability can result in the filamentous species, especially those adapted to low-nutrient conditions (Nielsen et al., 2009).

This filamentous overgrowth disrupts the microbial community structure in activated sludge systems, often resulting in bulking. Bulking is a condition where sludge flocs become poorly compacted and fail to settle properly in the secondary clarifier. This leads to poor sludge settling, increased solids carryover in the effluent, and overall decreased treatment efficiency. Furthermore, some filamentous bacteria produce stable surface foams, which interfere with aeration and sludge management operations (Nielsen et al., 2009).

The TOC:NH₄⁺-N:PO₄³⁻-P ratio is a critical parameter in biological wastewater treatment processes, especially in enhanced biological nutrient removal (EBNR) systems, as it reflects the biologically available fractions of nitrogen and phosphorus. Maintaining an optimal TOC:NH₄⁺-N:PO₄³⁻-P ratio of approximately 100:5:1 is essential for effective biological nutrient removal in wastewater treatment, as it ensures sufficient carbon availability for microbial growth, enhances nitrogen and phosphorus removal, and improves overall treatment efficiency (Metcalf & Eddy Inc., 2014).

2.4 Purpose of Measuring Specific Nutrients

Measuring the different forms of carbon, nitrogen and phosphorus present in greywater is important in evaluating the performance of treatment and helps to understand the microbial growth, treatment efficiency, and effluent quality of the system. Nutrients exist in various chemical forms (e.g., organic vs inorganic, dissolved vs particulate) and may undergo biological or chemical transformations like nitrification, denitrification, or phosphate precipitation. Understanding the chemistry or the potential for these to occur based on what forms of N and P are present helps to optimize conditions for microbial degradation of pollutants and maintain treatment stability (Henze et al., 2008).

2.4.1 Measuring Carbon: COD, TOC, and bCOD

Since carbon acts as the primary energy source for microorganisms, COD is widely used to estimate the organic load and indirectly indicates the amount of carbon available for microbial metabolism (Ghaitidak and Yadav, 2013). COD is a measure of the oxygen required to

chemically oxidize organic and inorganic substances in water. But COD is not a precise indicator of bioavailable carbon. It does not distinguish between biodegradable and non-biodegradable organics. This means that not all carbon measured by COD is available to bacteria (Dubber and Gray, 2010). For greater accuracy in assessing bioavailable carbon, TOC or biodegradable COD (bCOD) should be measured or estimated (Wojnárovits et al., 2024). The COD:TOC ratio typically ranges between 2 and 4 depending on the wastewater characteristics in municipal wastewater and greywater (Dubber and Gray, 2010).

2.4.2 Measuring Nitrogen Forms

Total nitrogen (TN) represents the sum of all nitrogenous species, including organic nitrogen and inorganic forms such as ammonia ($\text{NH}_4^+\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), and nitrate ($\text{NO}_3^-\text{-N}$). Measuring TN helps determine if additional nitrogen supplementation is required for biological processes (Shaikh and Ahammed, 2020).

Ammonia is the most accessible form of nitrogen for microbial uptake and helps to initiate the nitrification in aerobic conditions. Its concentration gives insight into the nitrogen available for microbial growth (Metcalf & Eddy Inc., 2014). Nitrate is the end product of nitrification and indicates the successful conversion of ammonia through the nitrification pathway. Elevated levels of nitrate signify complete nitrification, while the presence of nitrite, an intermediate product, may indicate incomplete or imbalanced nitrification processes (Nielsen et al., 2009).

However, nitrate is still environmentally harmful, as it contributes to eutrophication and can contaminate drinking water. Therefore, it is essential to remove nitrate through denitrification, a biological process that reduces nitrate to harmless nitrogen gas (N_2) under anoxic conditions. Ideally, ammonia levels should be reduced without corresponding to major increases in nitrate, indicating more complete nitrogen removal and less environmental risk.

2.4.3 Measuring Phosphorus Forms

Total phosphorus (TP) quantifies all phosphorus species, both particulate and dissolved, and is important for evaluating whether phosphorus levels meet microbial nutrient demands. Phosphate ($\text{PO}_4^{3-}\text{-P}$) refers to the readily bioavailable portion of phosphorus (Isaksson, 2023).

The following Table 2.3. shows the purpose and importance of different parameters in greywater (Metcalf & Eddy Inc., 2014; Tchobanoglous, G., Burton, F. L., & Stensel, H. D., 2003; U.S. Environmental Protection Agency; U.S. Agency for International Development, 2012).

Table 2.3. Core nutrient components and their importance

| Parameter | Measured Form | Key Purpose | Importance |
|----------------------------------|----------------------|-----------------|---|
| Phosphate (PO_4^{3-}) | Inorganic phosphorus | Bioavailability | Prevents eutrophication, helps phosphorus removal |

| | | | |
|--|-----------------------|-------------------------|---|
| Total Phosphorus (TP) | All phosphorus forms | Overall P load | Determines phosphorus pollution & treatment needs, Key factor for C:N:P ratio |
| Nitrite (NO₂⁻) | Intermediate nitrogen | Nitrification indicator | High levels = incomplete nitrification, toxic |
| Nitrate (NO₃⁻) | Final nitrogen form | Pollution indicator | High levels = risk for drinking water, eutrophication |
| Total Nitrogen (TN) | All nitrogen forms | Overall N load | Key factor for C:N:P ratio, wastewater regulations |
| Ammonia (NH₃/NH₄⁺) | Free & ionized | Toxicity & treatment | Important for nitrification, grey-water reuse |

3 Materials and Methods

This chapter explains how the research was conducted, including what materials were used, sample collection methods, the procedure of the experiments conducted at the lab and methods adopted for analysis of sample.

3.1 Sampling Strategy and Field Collection

In RecoLab, greywater from the residents in Oceanhamnen flows into an 80 m³ storage tank prior to treatment. The storage tank is completely mixed and has a retention time of roughly 12 hours. An automated sample collects flow proportional samples once an hour throughout the day and stores the sample in a refrigerated cabinet at 4°C, before the influent greywater enters the storage tank.

For analysis, greywater samples were collected from two separate points in the plant: one is 24-hour flow proportional samples of fresh influent greywater from the automatic sampling device and the other is a grab samples of greywater from the storage tank. These points were marked by red dots in figure 2.1. The first sample was taken on 11 February 2025, and samples were collected every Tuesday until 8 April 2025, for a total of eight samples. However, a sample was not collected on 1 April due to technical problems that affected RecoLab's operations for the previous three days. This may also influence the quality of the sample collected on the 8th.

The routine began by collecting two 1-liter greywater samples: a 24-hour composite from an automatic sampler and a grab sample from a pipeline tap. Figure 3.1 shows the arrangement in RecoLab to collect daily samples at specified time intervals. Figure 3.2 shows collecting a grab sample from the greywater pipeline into a one-liter plastic container. Both samples were promptly transported on ice back to Lund University.

3.2 Sample Preparation and Fractionation

The influent greywater samples in this report were analysed using the procedure established in "Description of methods for characterization of municipal wastewater," developed by Christoffer Wärrff at the Research Institutes of Sweden for wastewater fraction analysis (Wärrff, 2022).

Upon reaching the university lab, the bottles were shaken and approximately 40 mL of unfiltered water from each sample was poured into labelled Falcon tubes. Eight Falcon tubes were required for a single day's analysis. Unfiltered samples were analysed for the total fraction without any prior treatment.

Another 100 mL of unfiltered water from each sample was placed in a 500 mL glass jar with a magnetic stirrer. While stirring at 600 rpm, 0.2 mL of undiluted PAX XL 60 solution was added, and the pH was adjusted to 10.5 using around 0.5 to 0.6 mL of 2M NaOH. The solution was stirred again for 30 seconds, then left to settle for 15 minutes. The PAX XL 60 solution helped to destabilize the suspended particles by neutralizing their charges and forming flocs that can

be removed by filtration or sedimentation. From the clear supernatant, 30 mL was filtered through a 0.45 μm syringe filter into a labelled Falcon tube for later analysis. These flocculated and filtered samples were processed to obtain the non-reactive and dissolved fraction, which can be removed using a membrane.



Figure 3.1. Automatic Sampler



Figure 3.2. Collecting grab sample from the pipeline

Two 1.2 μm filter papers were labelled, weighed, and used to filter 25 mL from each sample via vacuum filtration, using a separate filter apparatus for each. The filter papers were folded, placed in ceramic dishes, and dried at 105°C for at least one hour. The filtered water was saved in labelled Falcon tubes. After drying, the filter papers were cooled to room temperature in a desiccator, weighed again, and stored in the desiccator for later ignition at 550°C and re-weighing. Vacuum-filtered fractions eliminate coarse particles, thereby revealing only the dissolved fraction.

Again, 30 to 40 mL from each original 1 L bottle were filtered using an ultra-fine 0.45 μm syringe filter into separate labelled Falcon tubes. Syringe filtration was employed to separate the dissolved ionic fraction which can be removed chemically.

This process results in four Falcon tubes per sample: one with unfiltered water, one with vacuum-filtered water, one with syringe-filtered water and one with soluble nonreactive water. The unfiltered, vacuum-filtered and soluble nonreactive samples were tested for COD, TN and TP; syringe-filtered sample was analysed for $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{PO}_4^{3-}\text{-P}$ using Hach Lange Cuvettes.

The following Figure 3.3 is a visual representation of all four fractions of the samples used to analyse different aspects of greywater quality and tests required for the project. The materials used for collecting the samples and conducting the experiments are in Appendix 1.

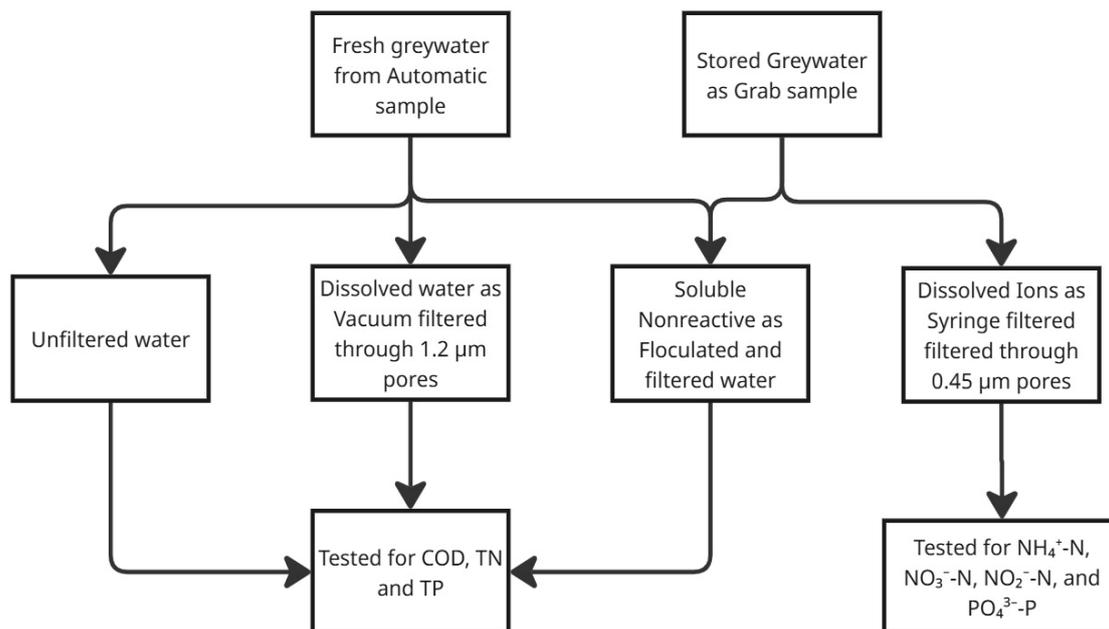


Figure 3.3. Flow chart of different types of water used for analysis and details of analytical methods conducted.

3.3 Analytical Methods using Hach Lange Cuvette Tests

Analyses were conducted to determine the concentrations of key physical and chemical parameters. Hach Lange cuvette tests are factory-prepared test kits that contain precise amounts of reagents. These are well-established, reliable colorimetric methods that provide rapid and accurate measurements, especially suitable for environmental and wastewater analysis.

Hach Lange cuvettes of different ranges were used to find COD, TN, TP, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N and PO₄³⁻-P. The measurement range for each parameter varies depending on the specific Hach Lange cuvette used. COD in influent unfiltered and vacuum filtered greywater samples were tested using Hach Lange LCK114 cuvettes for COD levels ranging from 150 to 1000 mg/L and COD on flocculated and filtered greywater was determined using Hach Lange LCK 314 cuvettes, which are designed for COD values from 15 to 150 mg/L. In cases where the COD of vacuum-filtered and flocculated greywater samples exceeded the LCK 314 limit, LCK 114 cuvettes were employed for measurement.

LCK 138 cuvettes were used to test TN of all samples, with a detection range of 1 to 16 mg Hach Lange /L. For NH₄⁺-N, Hach Lange LCK 303 was typically employed, covering a range of 2 to 47 mg/L, with Hach Lange LCK 304 being used less frequently for values between 0.015 and 2 mg/L. For all samples NO₃⁻-N was tested using Hach Lange LCK 339 cuvettes, which have a range of 0.23 to 13.5 mg/L and NO₂⁻-N was measured with LCK 341 cuvettes, ranging from 0.015 to 0.6 mg/L.

Additionally, Hach Lange LCK 348 cuvettes were utilized for TP testing, applicable for unfiltered and some vacuum-filtered samples within the range of 0.5 to 5 mg/L, while Hach Lange LCK 349 was used to test TP for remaining samples and $\text{PO}_4^{3-}\text{-P}$ of all samples with a range of 0.05 to 1.5 mg/L. Figure 3.4 shows measuring COD using Hach Lange LCK 314 and phosphate using Hach Lange LCK 348.



Figure 3.4. Measurements using Hach Lange Cuvettes

3.4 Conversion method from COD to TOC

To standardize carbon quantification and improve the accuracy of nutrient dosing calculations, a conversion method from COD to TOC was adopted. Laboratory analyses from RecoLab's internal laboratory determined the actual COD/TOC ratio to be 3.34 ± 0.18 . This ratio derived from site-specific water matrices, offers a reliable basis for carbon estimation. It was used in the report to ensure consistency in conversion and ease of calculation. These values were used for reference but were not generated as part of this study and are attached as Appendix 2.

COD measurements were used instead of TOC due to their relative ease and available materials at the University laboratory. So, conversion is necessary to facilitate the calculation. TOC was thus used in all C:N:P ratio calculations presented in the results and discussion chapters.

3.5 Data Analysis

Collected data were analysed to evaluate trends and variations in water quality parameters over the monitoring period. Results were analysed for mean, standard deviation and range of each parameter across the given span of time. These statistical measures provided insight into the central tendency, variability, and spread of the data, helping to assess the stability and performance of the treatment process.

To support visual interpretation and comparative analysis, graphical representations were developed using both clustered and stacked bar charts. Clustered bar charts were used to visualise the changes in COD, TN and TP. Stacked bar charts were used to break down the individual fractions of nitrogen and phosphorus. For nitrogen, this included species such as $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{NO}_2^-\text{-N}$. For phosphorus, breakdowns typically included $\text{PO}_4^{3-}\text{-P}$ and particulate phosphorus. These charts helped to visualize the relative contributions of each form to the total nutrient load and track their transformation during the treatment process.

Data were interpreted based on optimal microbial growth requirements and previously documented studies.

4 Results and Discussions

This chapter presents and analyses the findings obtained from laboratory work with characterization of influent greywater using fractionation methods. Rather than testing treatment technologies, this study aimed to analyse the distribution of organic carbon, nitrogen, and phosphorus in particulate, dissolved, and ionic forms to assess greywater suitability for biological treatment. To ensure accurate data representation, measurements were averaged across the samples, and bar graphs were constructed, incorporating standard deviation as error bars to illustrate variability.

4.1 Overview of Greywater Composition

The following Table 4.1 below consolidates all measured parameters across eight experimental samples from fresh greywater (24-hour automatic sampler) and stored greywater (grab sample with an approximate hydraulic retention time of 12 hours). The data summarizes total (unfiltered), dissolved (filtered through 1.2 μm), ionic (filtered through 0.45 μm), and nonreactive fractions (floculated and filtered), offering a comprehensive representation of the dataset. The detailed observations for each experimental day are in Appendix 3.

Table 4.1. Average values for different parameters by sample type and fraction

| Parameter (mg/L) | Fresh Greywater | Stored Greywater (HRT\approx12 h) |
|--|------------------------|---|
| Total (unfiltered) | | |
| COD | 413 | 534 |
| TN | 10.3 | 14.1 |
| TP | 1.45 | 2.14 |
| TSS | 76 | 79 |
| VSS | 70 | 75 |
| Dissolved (filtered through 1.2 μm pores) | | |
| COD | 228 | 283 |
| TN | 6.7 | 9.2 |
| TP | 0.55 | 1.27 |
| Dissolved Ions (filtered through 0.45 μm pores) | | |
| NH₄⁺-N | 2.6 | 5.8 |

| | | |
|---|------|------|
| NO₃⁻-N | 0.12 | 0.12 |
| NO₂⁻-N | 0.01 | 0.02 |
| PO₄³⁻-P | 0.05 | 0.48 |
| Soluble Nonreactive (flocculated and filtered) | | |
| COD | 130 | 179 |
| TN | 5.6 | 7.7 |
| TP | 0.04 | 0.08 |

Since unfiltered greywater is the one practically used in the treatment plant, its nutrient values were compared against standard benchmarks from scientific literature to assess its suitability for biological treatment processes. This forms the basis of the following discussions.

4.2 Characteristics of Unfiltered fresh and stored greywater

Stored greywater samples always exhibited higher COD values than fresh greywater samples. Unfiltered stored greywater samples recorded the highest COD levels of 534 mg/L, compared to 413 mg/L in fresh greywater samples, reflecting particulate and dissolved organic matter were potentially accumulating in the storage tank. As per Ghaitidak & Yadav (2013), the COD levels observed in this study are positioned in the moderate to high range of 41-1815 mg/L (Ghaitidak and Yadav, 2013).

Stored greywater samples consistently showed higher TN values than fresh greywater samples, indicating that nitrogen compounds accumulate during storage. Unfiltered stored greywater samples had TN levels of 14.1 mg/L, compared to 10.3 mg/L in fresh greywater samples, emphasizing suspended nitrogen presence. Unfiltered stored greywater samples had TP levels of 2.14 mg/L while TP was only 1.45 mg/L in fresh greywater samples.

In fresh greywater, the overall average of all measured concentrations was 2.6 mg/L for NH₄⁺-N and 0.05 mg/L for PO₄³⁻-P. The results also demonstrate higher concentrations in stored greywater, especially for ammonium and phosphate. This may be due to microbial activity and particle settlement during the retention period. Microbial activity during storage can lead to the breakdown of organic nitrogen and phosphorus containing compounds, releasing inorganic, bioavailable forms such as ammonium and phosphate. Hydrolysis likely contributes to this release, as water reacts with complex organic compounds breaking them down into simpler ionic forms. Additionally, the settlement of particles during storage can lead to further decomposition of trapped organic matter at the bottom. The increase in dissolved inorganic nutrient fractions may benefit biological treatment by supporting microbial growth.

Stored greywater sample mirrors the real influent wastewater entering biological treatment and includes suspended solids, colloids, and soluble fractions. However, COD includes both biodegradable and non-biodegradable fractions. For this reason, this report focuses primarily on TOC as a better indicator of bioavailable carbon (Dubber and Gray, 2010). For assessment based on COD:TN:TP, average values of TN and TP corresponding to COD 100 mg/L is also attached as Appendix 4

4.3 Assessment of nutrient balance based on TOC

Based on laboratory analyses from RecoLab’s internal laboratory, the actual COD/TOC ratio of 3.34 ± 0.18 was used in the report to calculate TOC values. 124 mg/L and 160 mg/L are the values of TOC corresponding to COD level of 413 mg/L and 534 mg/L for unfiltered fresh and stored greywater. Calculated TOC values corresponding to average COD values are in Appendix 5.

To standardize comparisons, TN and TP values are normalized to a TOC equivalent of 100 mg/L. This allows for a clearer assessment of nutrient dynamics relative to organic carbon content. The average TOC:TN:TP ratio for fresh greywater is 124:10.3:1.45 which is equivalent to 100:8.3:1.17 and that for stored greywater is 160: 14.1:2.14 which is equivalent to 100: 8.8: 1.33.

The following Table 4.2 shows TOC:TN:TP and TOC:NH₄⁺-N:PO₄³⁻-P (readily biologically available forms) ratios for unfiltered fresh greywater samples and stored greywater samples.

Table 4.2. C:N:P ratios for unfiltered fresh and stored greywater samples

| | | |
|-------------------------|--|----------------|
| Fresh greywater | TOC:TN:TP | 100:8.3:1.17 |
| | TOC:NH ₄ ⁺ -N:PO ₄ ³⁻ -P | 100:2.1:0.04 |
| Stored greywater | TOC:TN:TP | 100: 8.8: 1.33 |
| | TOC:NH ₄ ⁺ -N:PO ₄ ³⁻ -P | 100: 3.6: 0.3 |

Stored greywater shows improved nutrient ratios due to natural conversion processes, moving closer to ideal microbial growth conditions. As per the study conducted by Hall et al., (2024), the TOC:TN:TP ratio in influent greywater in RecoLab was 80:8:1, which is equal to 100:10:1.25 (Hall et al., 2024). From the current analysis, fresh greywater showed a lower ratio of 100:8.3:1.17, and unfiltered stored greywater samples analysed here showed a TOC:TN:TP ratio of 100: 8.8: 1.33.

Low TOC: NH₄⁺-N ratios of 100:2.1 in the fresh sample and 100:3.6 in the stored sample indicates that a large portion of nitrogen is not immediately bioavailable and requires ammonification to become biologically available.

Even though, TP is relatively high in both fresh and stored greywater, $\text{PO}_4^{3-}\text{-P}$ is very low, especially in fresh greywater ($\text{TOC}:\text{PO}_4^{3-}\text{-P} = 100:0.04$), indicating severe phosphorus limitation. $\text{PO}_4^{3-}\text{-P}$ is the only immediately available form of phosphorus for microbial uptake. A minimum of 1 mg/L must be available as orthophosphate for 100mg/L of TOC to support biomass growth (Henze et al., 2008). Storing greywater significantly increases $\text{PO}_4^{3-}\text{-P}$, to 0.27mg/L, but this is still far away from the optimal range and require supplementation to reach the optimal $\sim 1\text{--}2$ mg/L per 100 mg/L TOC (Henze et al., 2008). A $\text{TOC}:\text{NH}_4^+\text{-N}:\text{PO}_4^{3-}\text{-P}$ ratio of 264:7:1 which is same as 100:2.7:0.37 was found in influent greywater and this shows a deficiency of biologically available phosphorus (Hall et al., 2024).

4.4 Nitrogen and Phosphorus Fractionation

Figure 4.1 presents the nitrogen and phosphorus fractionation. First diagram shows TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and Organic N across fresh greywater and stored greywater sampling methods, with standard deviation included as error bars.

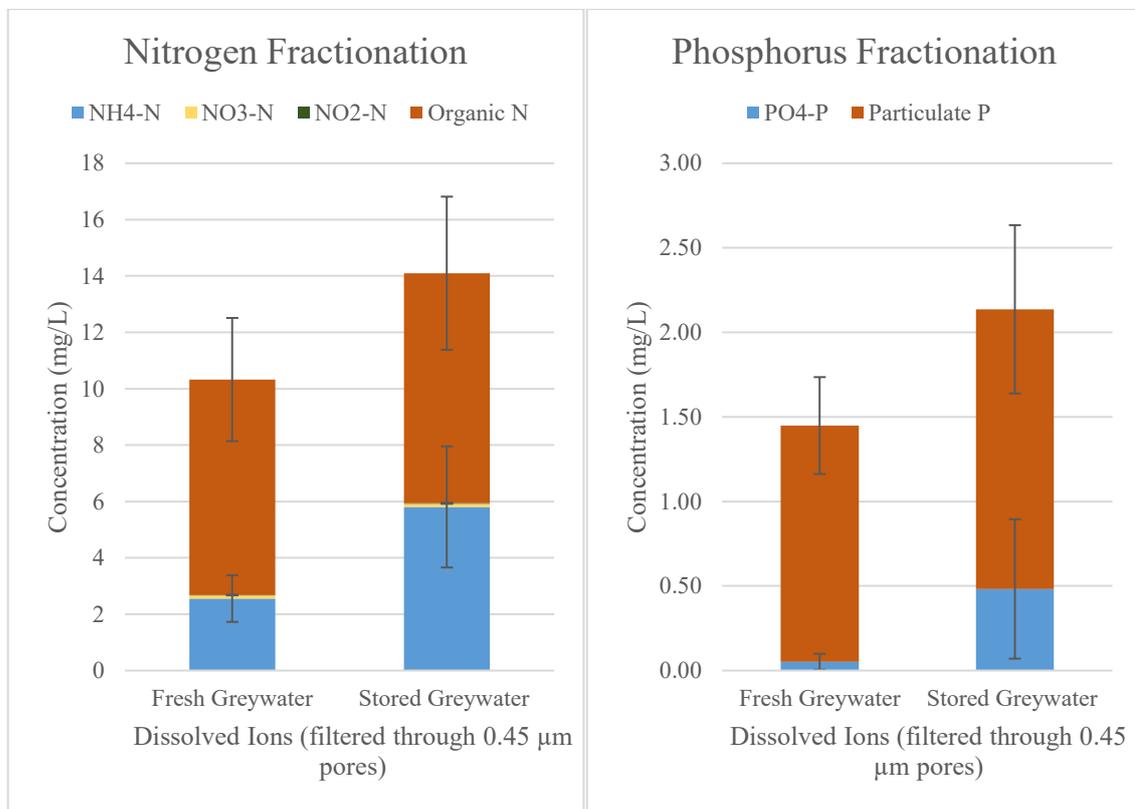


Figure 4.1. Nitrogen and phosphorus fractions breakdown

$\text{NH}_4^+\text{-N}$ concentration were 5.8 mg/L in stored greywater samples accounting 41% of the 14.1 mg/L of TN versus fresh greywater samples where the $\text{NH}_4^+\text{-N}$ concentration was lower at 2.6 mg/L or 25% of 10.3 mg/L TN. $\text{NO}_3^-\text{-N}$ levels were always below the limit of detection, which was 0.23 mg/L. For calculation purposes, half the value of the limit of detection, or 0.12 mg/L

(less than 1% of TN) was considered and therefore, it is identical in both sampling methods, indicating minimal variation in nitrate composition.

In the case of NO_2^- -N concentrations, some sample had very low concentrations, below the limit of detection, 0.015mg/L. For calculation purposes, it was treated as half of the limit of detection, 0.0075mg/L. Nitrite concentration showed slight differences, with stored greywater samples at 0.02 mg/L, potentially reflecting microbial nitro-gen transformations. NO_3^- -N and NO_2^- -N were both negligible (<1% of TN), indicating minimal nitrification activity.

Organic N was calculated using the following equation 1.

$$\text{Organic N} = \text{TN} - (\text{NH}_4^+ \text{-N} + \text{NO}_3^- \text{-N} + \text{NO}_2^- \text{-N}) \quad (\text{Equation 1})$$

Organic N levels were higher in stored greywater samples compared to fresh greywater samples. On average 74% of TN in fresh greywater is Organic N with a concentration of 7.64 mg/L. Organic N had a concentration of 8.15 mg/L, nearly 58% of TN, in stored greywater. This higher value in the stored greywater sample indicated retention effects influenced organic nitrogen distribution. The majority of nitrogen in influent greywater is organic, meaning it needs to undergo ammonification to be biologically available.

The second diagram presents TP, PO_4^{3-} -P, and particulate P concentrations measured from stored greywater, with error bars representing standard deviation. Particulate phosphorus was calculated using the following equation 2.

$$\text{Particulate phosphorus} = \text{TP} - \text{PO}_4^{3-} \text{-P} \quad (\text{Equation 2})$$

PO_4^{3-} -P concentrations were also greater in stored greywater samples versus fresh greywater samples. PO_4^{3-} -P concentration of 0.48 mg/L was almost 23% of TP in stored greywater samples whereas, fresh greywater sample had just 0.05 mg/L PO_4^{3-} -P, which was 4% of TP, indicating that soluble phosphorus might increase during retention. The particulate phosphorus concentration of 1.65 mg/L was 78% of TP in stored greywater samples, suggesting that storage may allow for gradual sedimentation of phosphorus-containing particles. In comparison, 96% of TP in fresh greywater was particulate P.

5 Conclusions

This study investigated the nutrient characteristics of influent greywater in both fresh and stored conditions. The goal was to assess whether the nitrogen and phosphorus content could support effective biological treatment. The results were interpreted through total, dissolved, and ionic nutrient fractions and compared with theoretical stoichiometric requirements.

Although stored greywater shows improved nutrient availability compared to fresh greywater, its TOC:NH₄⁺-N:PO₄³⁻-P ratio (100:3.63:0.3) still deviates significantly from the theoretical microbial requirement of 100:5:1 for optimal biological treatment (Metcalf & Eddy, 2014). This suggests that bioavailable phosphorus (PO₄³⁻-P) remains critically limiting, as it is only 30% of the required value. Ammonium (NH₄⁺-N) is moderately deficient, at about 73% of the target level. The total nutrient ratio (TOC:TN:TP) in stored greywater is 100:8.8:1.33, but this overstates the actual nutrient availability because a large share of nitrogen and phosphorus exists in organic or particulate forms that microbes cannot readily use. Therefore, even after 12 hours of storage, greywater still lacks sufficient readily bioavailable nitrogen and phosphorus, and supplementation would be required to achieve stable microbial activity and treatment performance.

When greywater is freshly generated, nitrogen and phosphorus are locked in organic or particulate forms, which are not immediately usable by microbes. Most nitrogen is present as organic nitrogen rather than ammonium (NH₄⁺) and most phosphorus exists as particulate phosphorus, not as phosphate (PO₄³⁻). Storage increased NH₄⁺-N and PO₄³⁻-P concentrations likely due to microbial activity and particle breakdown, but not enough to fully meet microbial requirements.

Organic nitrogen (58%) and particulate phosphorus (78%) still dominate in stored greywater. These require conversion into biologically available forms before microbial uptake, implying delays in nutrient availability and potential treatment inefficiency without sufficient retention or pre-treatment.

Greywater, especially in its fresh form, lacks sufficient bioavailable nitrogen and phosphorus for effective biological treatment. Even after storage, nutrient levels improve but do not meet the ideal balance needed for microbial health. Even after 12 hours of storage, PO₄³⁻-P remains critically low (<0.5 mg/L), suggesting there is a need for phosphorus supplementation to achieve stable biological treatment and maintain microbial activity.

6 Future Work

This study highlighted key nutrient limitations in greywater and suggested that this could influence microbial growth. While nutrient analysis offered important insights, several new questions and areas for further exploration emerged.

The next step should involve tests to confirm how nutrient ratios influence microbial behaviour. This could include microscopic analysis and molecular techniques to track changes in microbial communities such as filamentous bacteria, floc-forming microbes, and phosphorus-accumulating organisms (PAOs) under varying nutrient conditions.

A key unknown is how much of the carbon in greywater is actually biodegradable. Total Organic Carbon (TOC) and COD may overestimate what microbes can use. Conducting biodegradability assays, such as BOD₅/COD ratios or respirometry tests, would help determine if greywater supports healthy microbial metabolism or if carbon is present in more resistant forms.

This study showed that storing greywater increases ammonium and orthophosphate levels. Samples were stored in ice packs and transported to the university for experimental analysis. So, the effects of temperature were not determined. Future work could investigate how nutrient forms change over longer storage periods (e.g., 24–48 hours), under different conditions (temperature, aeration), and how this affects treatment performance.

Installing real-time nutrient sensors (for NH₄⁺, PO₄³⁻, TOC) could support automated nutrient dosing, improving reliability without excessive chemical use. This is especially important for decentralized or low-maintenance greywater systems.

Depending on the incoming greywater's quality (e.g., low P, variable COD), treatment steps may need to adapt dynamically. Future studies could explore adaptive control strategies that react to feed composition.

7 References

- Awasthi, A., Gandhi, K., Rayalu, S., 2024. Greywater treatment technologies: a comprehensive review. *Int. J. Environ. Sci. Technol.* 21, 1053–1082. <https://doi.org/10.1007/s13762-023-04940-7>
- Droste, R.L., 1997. *Theory and practice of water and wastewater treatment*. J. Wiley, New York.
- Dubber, D., Gray, N.F., 2010. Replacement of chemical oxygen demand (COD) with total organic carbon (TOC) for monitoring wastewater treatment performance to minimize disposal of toxic analytical waste. *J. Environ. Sci. Health Part A* 45, 1595–1600. <https://doi.org/10.1080/10934529.2010.506116>
- Ghaitidak, D.M., Yadav, K.D., 2013. Characteristics and treatment of greywater—a review. *Environ. Sci. Pollut. Res.* 20, 2795–2809. <https://doi.org/10.1007/s11356-013-1533-0>
- Ghaly, A.E., Emam, R.H., Abdelrahman, E.N., Sayed, M.S.E., Abdellatif, H.R., Ibrahim, M.M., Mostafa, E.A., Kassem, A., Hatem, M.H., 2021. Overview of Physical and Chemical Greywater Treatment Technologies for Effective Recycling. *Int. J. Bioprocess Biotechnol. Adv.* 7, 426–452.
- Hall, A., Widén, A., Edefell, E., Davidsson, Å., Kjerstadius, H., 2024. Treatment of greywater with nanofiltration for nutrient removal – 2-year experience from Helsingborg. *Water Pract. Technol.* 19, 900–910. <https://doi.org/10.2166/wpt.2024.050>
- Henze, M., Van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D., 2008. *Biological Wastewater Treatment: Principles, Modelling and Design*. IWA Publishing. <https://doi.org/10.2166/9781780401867>
- Hernández Leal, L., Temmink, H., Zeeman, G., Buisman, C.J.N., 2011. Characterization and anaerobic biodegradability of grey water. *Desalination* 270, 111–115. <https://doi.org/10.1016/j.desal.2010.11.029>
- Isaksson, F., 2023. *Greywater reuse for different purposes in Sweden: A literature review (Technical Report)*. Luleå University of Technology, Luleå.
- Jadhao, R.K., Dawande, S.D., 2012. A Review on Application of Membrane Bioreactor for Wastewater Treatment. *Int. J. Biotechnol.*
- Jefferson, B., Burgess, J.E., Pichon, A., Harkness, J., Judd, S.J., 2001. Nutrient addition to enhance biological treatment of greywater. *Water Res.* 35, 2702–2710. [https://doi.org/10.1016/S0043-1354\(00\)00553-4](https://doi.org/10.1016/S0043-1354(00)00553-4)
- Khajvand, M., Mostafazadeh, A.K., Drogui, P., Tyagi, R.D., 2022. Management of greywater: environmental impact, treatment, resource recovery, water recycling, and decentralization. *Water Sci. Technol.* 86, 909–937. <https://doi.org/10.2166/wst.2022.226>

- Li, F., Wichmann, K., Otterpohl, R., 2009. Review of the technological approaches for grey water treatment and reuses. *Sci. Total Environ.* 407, 3439–3449. <https://doi.org/10.1016/j.scitotenv.2009.02.004>
- Metcalf & Eddy Inc., 2014. *Wastewater Engineering: Treatment and Resource Recovery* (5th Edition), 5th ed. McGraw-Hill Education, Newyork.
- Nielsen, P.H., Kragelund, C., Seviour, R.J., Nielsen, J.L., 2009. Identity and ecophysiology of filamentous bacteria in activated sludge. *FEMS Microbiol. Rev.* 33, 969–998. <https://doi.org/10.1111/j.1574-6976.2009.00186.x>
- Oteng-Pepurah, M., Acheampong, M.A., deVries, N.K., 2018a. Greywater Characteristics, Treatment Systems, Reuse Strategies and User Perception—a Review. *Water. Air. Soil Pollut.* 229, 255. <https://doi.org/10.1007/s11270-018-3909-8>
- Oteng-Pepurah, M., Acheampong, M.A., deVries, N.K., 2018b. Greywater Characteristics, Treatment Systems, Reuse Strategies and User Perception—a Review. *Water. Air. Soil Pollut.* 229, 255. <https://doi.org/10.1007/s11270-018-3909-8>
- RecoLab, 2025.RecoLab. URL <https://www.recolab.se/utvecklingsanlaggning/> (accessed 20.3.25).
- Shahzeb, Khan, Z.M., Kanwar, R.M.A., Ameen, A., Khalid, A., Rasool, S., Raza, H., 2022. Greywater Characterization and Treatment Using Chemical Coagulation. *J. Qual. Assur. Agric. Sci.* 2, 46–52. <https://doi.org/10.38211/jqaas.2022.2.1.7>
- Shaikh, I.N., Ahammed, M.M., 2020. Quantity and quality characteristics of greywater: A review. *J. Environ. Manage.* 261, 110266. <https://doi.org/10.1016/j.jenvman.2020.110266>
- Szabolcsik-Izbéki, A., Bodnár, I., Fábrián, I., 2024. The removal of pollutants from synthetic bathroom greywater by coagulation-flocculation and filtration as a fit-for-purpose method. *J. Environ. Chem. Eng.* 12, 114250. <https://doi.org/10.1016/j.jece.2024.114250>
- Tchobanoglous, G., Burton, F. L., & Stensel, H. D., 2003. *Wastewater Engineering: Treatment and Reuse*, 4th ed. McGraw-Hill, Newyork.
- U.S. Environmental Protection Agency; U.S. Agency for International Development, 2012. *Guidelines for Water Reuse* (No. EPA/600/R-12/618).
- Vuppaladadiyam, A.K., Merayo, N., Prinsen, P., Luque, R., Blanco, A., Zhao, M., 2019. A review on greywater reuse: quality, risks, barriers and global scenarios. *Rev. Environ. Sci. Biotechnol.* 18, 77–99. <https://doi.org/10.1007/s11157-018-9487-9>
- Wärff, C., 2022. RISE Fractionation Method: Metodbeskrivning karakterisering (Internal Report No. Utkast 8). RISE Research Institutes of Sweden.
- Water Quality and Health - Review of Turbidity: Information for Regulators and Water Suppliers, n.d.

Wojnárovits, L., Homlok, R., Kovács, K., Tegze, A., Takács, E., 2024. Wastewater Characterization: Chemical Oxygen Demand or Total Organic Carbon Content Measurement? *Molecules* 29, 405. <https://doi.org/10.3390/molecules29020405>

Yáñez, D., Espinoza, L.C., Vargas, I., Romero, J., Aguirre, M.J., Arce, R., Quijada-Maldonado, E., Abejon, R., 2024. Treated greywater as a novel water resource: The perspective of greywater treatment for reuse from a bibliometric analysis. *Water Sci. Technol.* 90, 3076–3110. <https://doi.org/10.2166/wst.2024.384>

Appendix

Appendix -1 Materials required

For collecting greywater two plastic 1-liter bottles, ice packs and an insulated bag were required, and safety glasses and gloves were also essential to collect samples from the treatment plant and for conducting experiments. Falcon tubes, Hach Lange cuvettes, TSS filters of 1.2 μm pore size, Syringe Filters of 0.45 μm , pH meter, magnetic stirrer, oven and vacuum apparatus were used for analysing the samples. Chemicals such as PAX XL 60 and 2M NaOH were utilized during the experiments.

Appendix - 2: COD/TOC Ratio

Table A1. COD/TOC ratio from the analyses from RecoLab's internal laboratory

| Date | TOC | COD | COD/TOC |
|-------------------|------------|------------|----------------|
| 07-04-2025 | 137 | 461.5 | 3.37 |
| 09-04-2025 | 154 | 460 | 2.99 |
| 11-04-2025 | 170 | 572 | 3.36 |
| 14-04-2025 | 179 | 592 | 3.31 |
| 16-04-2025 | 198 | 697 | 3.52 |
| 25-04-2025 | 217 | 763 | 3.52 |
| 28-04-2025 | 151 | 500 | 3.31 |
| Average | 172 | 586 | 3.34 |
| Std. Dev | 28 | 111 | 0.18 |

Appendix -3: Daily results

Cuvette Analyses: Sample 1, collected on 11/02/2025

Date of Analysis: 11/02/2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 447 | 272 | | 94.2 * |
| TN | 10.4 | 6.52 | | 6.00 * |
| NH ₄ ⁺ -N | | | 2.18 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | <0.015 | |
| TP | 1.55 | 0.515 | | 0.061 * |
| PO ₄ ³⁻ -P | | | <0.05 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 541 | 325 | | 98.5 * |
| TN | 15.9 | 9.94 | | 6.59 * |
| NH ₄ ⁺ -N | | | 6.04 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.045 | |
| TP | 2.03 | 1.11 | | 0.076 * |
| PO ₄ ³⁻ -P | | | <0.05 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited filter weight (g) | [E]=[C]- [A]/([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|--------------------------------|-----------------------------------|---|---|--|--|----------------------------------|
| Fresh greywater sampler | 0.1317 | 25 | 0.1355 | 0.1305 | 0.152 | 0.0032 | 0.1488 |
| Stored greywater sample | 0.1301 | 25 | 0.1341 | 0.1286 | 0.16 | -0.0088 | 0.16 |

* Analysis conducted on 25-02-2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 343 | 163 | | 88.1 |
| TN | 5.52 | 6.18 | | 5.53 |
| NH ₄ ⁺ -N | | | 2.6 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | <0.015 | |
| TP | 1.46 | 0.448 | | <0.15 |
| PO ₄ ³⁻ -P | | | <0.05 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 610 | 296 | | 203 |
| TN | 7.47 | 7.90 * | | 8.26 * |
| NH ₄ ⁺ -N | | | 4.24 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.021 | |
| TP | 2.54 | 1.25 | | 0.093 |
| PO ₄ ³⁻ -P | | | <0.05 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited filter weight (g) | [E]=[C]- [A]/ ([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/ ([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|--------------------------------|-----------------------------------|---|---|---|--|----------------------------------|
| Fresh greywater sampler | 0.1297 | 25 | 0.1314 | 0.1279 | 0.068 | -0.0208 | 0.068 |
| Stored greywater sample | 0.1263 | 25 | 0.1283 | 0.125 | 0.08 | -0.0008 | 0.08 |

* Analysis conducted on 20-02-2025

Cuvette Analyses: Sample 3, collected on 25/02/2025

Date of Analysis: 25/02/2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 452 | 243 | | 158 |
| TN | 12.7 | 8.77 | | 7.74 |
| NH ₄ ⁺ -N | | | 3.85 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.015 | |
| TP | 1.65 | 0.688 | | 0.087 |
| PO ₄ ³⁻ -P | | | 0.054 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 621 | 368 | | 236 |
| TN | 15.6 | 9.48 | | 8.34 |
| NH ₄ ⁺ -N | | | 4.56 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.025 | |
| TP | 2.46 | 1.95 | | 0.111 |
| PO ₄ ³⁻ -P | | | 0.659 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited fil- ter weight (g) | [E]=[C]- [A])/ ([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/ ([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|--------------------------------|-----------------------------------|---|---|---|--|----------------------------------|
| Fresh greywater sampler | 0.1312 | 25 | 0.1329 | 0.1302 | 0.068 | 0.0112 | 0.0568 |
| Stored greywater sample | 0.1322 | 25 | 0.1346 | 0.1312 | 0.096 | 0.0112 | 0.0848 |

Cuvette Analyses: Sample 4, collected on 04/03/2025

Date of Analysis: 04/03/2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 386 | 201 | | 107 |
| TN | 9.79 | 5.52 | | 4.02 |
| NH ₄ ⁺ -N | | | 1.90 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | <0.015 | |
| TP | 1.13 | 0.382 | | <0.05 |
| PO ₄ ³⁻ -P | | | <0.05 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 632 | 280 | | 176 |
| TN | 14.0 | 7.73 | | 6.54 |
| NH ₄ ⁺ -N | | | 5.06 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.020 | |
| TP | 1.71 | 0.829 | | <0.05 |
| PO ₄ ³⁻ -P | | | 0.335 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited fil- ter weight (g) | [E]=[C]- [A]/ ([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/ ([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|--------------------------------|-----------------------------------|---|---|--|--|----------------------------------|
| Fresh greywater sampler | 0.1326 | 25 | 0.1342 | 0.1314 | 0.064 | 0.0032 | 0.0608 |
| Stored greywater sample | 0.1335 | 25 | 0.1339 | 0.1325 | 0.016 | 0.0112 | 0.0048 |

Cuvette Analyses: Sample 5, collected on 11/03/2025

Date of Analysis: 11/03/2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 473 | 294 | | 202 |
| TN | 12.1 | 7.56 | | 5.64 |
| NH ₄ ⁺ -N | | | 2.89 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.020 | |
| TP | 1.43 | 0.602 | | <0.05 |
| PO ₄ ³⁻ -P | | | <0.05 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 558 | 285 | | 209 |
| TN | 14.8 | 8.28 | | 7.06 |
| NH ₄ ⁺ -N | | | 4.70 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.020 | |
| TP | 2.05 | 0.996 | | 0.070 |
| PO ₄ ³⁻ -P | | | 0.385 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited fil- ter weight (g) | [E]=[C]- [A])/ ([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/ ([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|--------------------------------|-----------------------------------|---|---|---|--|----------------------------------|
| Fresh greywater sampler | 0.1338 | 25 | 0.1355 | 0.1331 | 0.068 | 0.0232 | 0.0448 |
| Stored greywater sample | 0.1322 | 25 | 0.1342 | 0.1304 | 0.08 | -0.0208 | 0.08 |

Cuvette Analyses: Sample 6, collected on 18/03/2025

Date of Analysis: 18/03/2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 453 | 217 | | 112 |
| TN | 10.3 | 4.57 | | 3.49 |
| NH ₄ ⁺ -N | | | 1.12 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.019 | |
| TP | 1.45 | 0.428 | | <0.05 |
| PO ₄ ³⁻ -P | | | <0.05 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 577 | 298 | | 212 |
| TN | 14.9 | 8.29 | | 6.78 |
| NH ₄ ⁺ -N | | | 5.06 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.022 | |
| TP | 2.12 | 1.03 | | 0.062 |
| PO ₄ ³⁻ -P | | | 0.451 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited fil- ter weight (g) | [E]=[C]- [A])/ ([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/ ([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|--------------------------------|-----------------------------------|---|---|---|--|----------------------------------|
| Fresh greywater sampler | 0.1315 | 25 | 0.1339 | 0.1301 | 0.096 | -0.0048 | 0.096 |
| Stored greywater sample | 0.1310 | 25 | 0.1337 | 0.1299 | 0.108 | 0.0072 | 0.1008 |

Cuvette Analyses: Sample 7, collected on 25/03/2025

Date of Analysis: 25/03/2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 431 | 231 | | 156 |
| TN | 12.2 | 7.87 | | 6.67 |
| NH ₄ ⁺ -N | | | 2.76 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | <0.015 | |
| TP | 1.88 | 0.743 | | <0.05 |
| PO ₄ ³⁻ -P | | | 0.077 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 474 | 277 | | 195 |
| TN | 14.1 | 9.10 | | 7.41 |
| NH ₄ ⁺ -N | | | 5.89 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | 0.019 | |
| TP | 2.06 | 1.26 | | 0.064 |
| PO ₄ ³⁻ -P | | | 0.680 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited fil- ter weight (g) | [E]=[C]- [A]/ ([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/ ([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|-----------------------------|--------------------------------|-----------------------------------|--|--|--|----------------------------------|
| Fresh greywater sampler | 0.1288 | 25 | 0.1305 | 0.1274 | 0.068 | -0.0048 | 0.068 |
| Stored greywater sample | 0.1317 | 25 | 0.1335 | 0.1301 | 0.072 | -0.0128 | 0.072 |

Cuvette Analyses: Sample 8, collected on 08/04/2025

Date of Analysis: 08/04/2025

Sample Point: Fresh greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 319 | 200 | | 122 |
| TN | 9.58 | 6.88 | | 5.30 |
| NH ₄ ⁺ -N | | | 23.14 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | <0.015 | |
| TP | 1.04 | 0.597 | | 0.060 |
| PO ₄ ³⁻ -P | | | 0.159 | |

Sample Point: Stored greywater sample

| Parameter (mg/L) | Unfiltered | Dissolved | Syringe Filtered | Soluble nonreactive |
|----------------------------------|------------|-----------|------------------|---------------------|
| COD | 255 | 132 | | 105 |
| TN | 16.0 | 12.7 | | 10.2 |
| NH ₄ ⁺ -N | | | 10.9 | |
| NO ₃ ⁻ -N | | | <0.23 | |
| NO ₂ ⁻ -N | | | <0.015 | |
| TP | 2.12 | 1.77 | | 0.167 |
| PO ₄ ³⁻ -P | | | 1.30 | |

Solids Analyses:

| Sample | [A] Filter Weight (g) | [B] Filtered Volume (mL) | [C] Dried Filter Weight (g) | [D] Ig- nited fil- ter weight (g) | [E]=[C]- [A])/ ([B]/1000) TSS (mg/L) | [F]= ([D]- [C])/ ([B]/1000) Non-VSS (mg/L) | [G]=[E]- [F] VSS (mg/L) |
|-------------------------------|--------------------------------|-----------------------------------|---|---|---|--|----------------------------------|
| Fresh greywater sampler | 0.1319 | 25 | 0.1324 | 0.1301 | 0.02 | -0.0208 | 0.02 |
| Stored greywater sample | 0.1326 | 25 | 0.1331 | 0.1309 | 0.02 | -0.0168 | 0.02 |

Appendix - 4: Average values of TN and TP

Table A2. Average values of TN and TP corresponding to COD 100 mg/L

| Sampling Point | Type of Sample | Avg. TN (mg/L) | Avg. TP (mg/L) |
|-------------------------|---------------------|----------------|----------------|
| Fresh greywater sample | Unfiltered | 2.49 | 0.35 |
| | Dissolved | 3.01 | 0.24 |
| | Soluble nonreactive | 4.48 | 0.03 |
| Stored greywater Sample | Unfiltered | 2.92 | 0.43 |
| | Dissolved | 3.71 | 0.51 |
| | Soluble nonreactive | 4.76 | 0.05 |

Appendix - 5: COD to TOC conversion

Table A3. COD to TOC conversion

| Sampling Point | Type of Sample | Avg. COD (mg/L) | Avg. TOC (mg/L) |
|-------------------------|-----------------------|------------------------|------------------------|
| Fresh greywater sample | Unfiltered | 413 | 124 |
| | Dissolved | 228 | 68 |
| | Soluble nonreactive | 130 | 39 |
| Stored greywater Sample | Unfiltered | 534 | 160 |
| | Dissolved | 283 | 85 |
| | Soluble nonreactive | 179 | 54 |