Treatment of Dairy Wastewater Using Enzyme Pre-Treatment Coupled with an Expanded Granular Sludge Bed Reactor

BY

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I dedicate this thesis to my mother and my late father.

Conflict of Interest

I, Hugendra Rishay Moodley, thus declare that the work reported in this dissertation was not influenced by any known conflicting financial interests or personal relationships. The funding sources for this study have all been mentioned, and the conclusions in this dissertation are the product of my own investigation and analysis, uninfluenced by other factors.

Furthermore, no organisation or person has provided me with any direct or indirect benefits that might have influenced the results of this study. Every attempt has been taken to guarantee the objectivity and integrity of the work that is provided here.

Signed,

Hugendra Rishay Moodley

02/06/2024

Abstract

Dairy consumption is rising due to the rapid growth of the population, necessitating increased dairy production to meet this demand. Consequently, the wastewater produced by dairy plants is also increasing significantly. The dairy industry, already one of the biggest contributors to pollution, generates large volumes of wastewater contaminated with toxic substances. These substances adversely affect the water sources into which the wastewater is released, whether fully or partially. Contaminants such as high concentrations of fats, oils, and greases (FOGs), nitrogen, phosphate, chemical and biological oxygen demand (COD and BOD) are found in the wastewater.

This study investigates the treatment of dairy wastewater (DWW) using a biological pre-treatment coupled with an anaerobic down-flow expanded granular sludge bed reactor (DEGBR). The DEGBR system, previously utilized in the treatment of PSW, showed satisfactory removal efficiencies with the use of a bioremediation agent, Eco-Flush. However, Eco-Flush was found to contribute to the increase in dissolved oxygen (DO) levels due to aeration, leading to the identification and use of a new enzyme named Momar in this study.

The pre-treatment stage aimed to determine the optimum dosage of Momar, focusing on the removal of FOGs to mitigate clogging in the DEGBR. The results of the pre-treatment stage were satisfactory, with a 42% reduction in DO, and removal efficiencies of 16% for COD, 20% for Total Suspended Solids (TSS), and 70% for FOG. The pre-treatment stage successfully removed a significant amount of FOGs from the wastewater.

The DEGBR was operated with three different hydraulic retention times (HRTs), and the removal efficiencies of COD, TSS, and FOG were compared. At 36 hours, the removal efficiencies were 38% for COD, 55% for TSS and 76% for FOG; at 24 hours, they stood at 36%, 48% and 68%, and at 12 hours, the values were 48%, 43% and 74%. The overall average removal efficiencies of the DEGBR for COD, TSS, and FOG were 39%, 49%, and 73%, respectively. Overall, the combined system produced satisfactory results with removal efficiencies of COD, TSS, and FOG, which are 48%, 56%, and 71%, respectively.

In conclusion, the biological system coupled with a biological pre-treatment effectively treated high-fat wastewater. It is recommended that a thorough analysis be conducted on Momar to identify the impact of the enzymes on wastewater. In the future, the system should be operated again with dairy wastewater for a longer period, addressing system issues, and incorporating regular backwashing to prevent clogging.

Keywords: Dairy wastewater (DWW); Chemical Oxygen Demand (COD); Down flow Expanded Granular Sludge Bed Reactor (DEGBR); Fats, Oil, and Grease (FOG); Total Suspended Solids (TSS).

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Acronyms and Abbreviations

AD – Anaerobic Digestor
BOD - Biochemical Oxygen Demand
COD - Chemical Oxygen Demand
DEGBR- Down-flow expanded granular Bed Reactor
DF – Downflow
DO - Dissolved Oxygen
DWS – Department of Water and Sanitation
EGSB – Expanded Granular Sludge-Bed
HRT – Hydraulic Retention Time
OLR – Organic Loading Rate
FOG – Fats, oil and grease
PSW – Poultry slaughterhouse wastewater
PT – Pre- treatment
SA – South Africa
SGBR – Static Granular Bed Reactor
SS – Suspended solids
TDS – Total dissolved solids
TKN - Total Kjheldahl nitrogen
TN – Total nitrogen
TSS – Total suspended solids
UASB – Up-flow Anaerobic Sludge-Bed
UF – Up-flow

CHAPTER 1: INTRODUCTION

1.1. Background to report

The dairy industry stands as one of the most prominent sectors in South Africa's agricultural landscape (Esterhuizen, Fossey & Lues, 2012). According to Dr Chris van Dijk, CEO of the Milk Producers' Organisation (MPO), the dairy sector contributes approximately R14.5 billion annually to the country's GDP ("*The challenges of …",* 2018). This underscores the industry's substantial role in the country's economic framework. Despite the substantial financial contribution made by the dairy industry, the CEO advocates for its further expansion. The envisaged growth is expected to boost the economy by generating additional revenue. However, this expansion may also lead to an increase in wastewater production, posing potential environmental challenges if the wastewater is discharged untreated or if existing treatment options struggle to manage the escalating discharge load.

The dairy industry consumes a substantial amount of water for various purposes such as cleaning, sanitation, and processing. This results in wastewater production ranging from 0.2L to 10L per 1L of milk produced (Ates, Ozay & Dizge, 2017). The significant volume of wastewater generated places this industry as one of the most polluting within the food sector (Borbón et al., 2014).

This wastewater is characterized by high levels of COD, BOD, FOG, nitrogen, and phosphorous. These pollutants fall into two categories: conventional pollutants, encompassing those found in domestic, commercial, or industrial wastes (such as BOD, FOG, TSS, and pH), and non-conventional pollutants, which include COD, ammonia, phenols, and others, not falling within the conventional category (Riffat, 2012).

FOGs pose a particular challenge as they hinder the diffusion of oxygen in the water, leading to oxygen depletion due to the presence of oil droplets on the water's surface. This depletion poses a threat to aquatic life and species (Mendes et al., 2010). Addressing the wastewater challenges in the dairy industry becomes crucial for mitigating environmental impacts and preserving aquatic ecosystems.

In Table 1, a summary of the most relevant social, economic, and environmental issues can be seen and how these affect nature as well as society.

Table 1 | Social, Economic and Environmental implications

Implications	Evaluation
Social	- The discharging of partially treated wastewater from these factories into nearby rivers or water sources contaminates the water, and people who use water from rivers will be infected by these contaminants. They could spread it amongst society (Esterhuizen, Fossey & Lues, 2012). - The odour produced from the dairy plant is very potent and not pleasant (Sam, 2022); hence no residential or business areas can be within proximity, which can result in land scarcity with this rise in population and urbanisation.
Environmental	- The process of making dairy products causes immense pollution to the water and biodiversity (Ahmad et al., 2019). - The emissions from the treatment of DWW contaminate the air and can cause problems for bird species. The emissions could rise to 0,19 million tons by 2025, according to Hernández-Sancho and others (2015). - Water pollution can cause eutrophication with an excess of nutrients and put aquatic life at more risk (Khan & Mohammad, 2014). - The excess nutrients can also affect the soil quality and stunt plant growth (Gatiboni, L. 2018).
Economical	- The treatment of DWW is an expensive procedure in order to make sure the wastewater is not harmful when released (Tikariha & Sahu, 2014). - The amount of water it uses for the treatment is massive and contributes heavily to the expenses (Tikariha & Sahu, 2014). - If not treated properly, it can cause more expenses in terms of repairs to specific equipment and pipelines (Kolev Slavov, 2017). - Tourism will go down if the water sources are contaminated, and unpleasant and more water-borne diseases are reported. (Edokpayi, Odiyo & Durowoju, 2017) - The fish industry will decrease as the aquatic life is destroyed (Hernández-Sancho *et al.*, 2015).

1.2. Research Problem statement

With the rapid growth of the population and industrialization, the dairy industry continues to expand, leading to increased production and the release of toxins into the environment. These toxins manifest in the form of solids and liquids, comprising various chemicals (Raghunath et al., 2016). Furthermore, these pollutants pose a significant threat to the environment, impacting aquatic life and the surrounding flora near water sources, while also contributing to eutrophication (Sam, 2022). With this growing issue this research explores a new enzyme to reduce FOGs in DWW and to further treat it in Anaerobic Digester (AD) without the risk of clogging.

1.3. Research hypothesis

It is hypothesised that optimising the biological pre-treatment stage before the DWW enters the DEGBR will result in a reduced concentration of FOGs.

1.4. Research Questions

- What are the general characteristics of DWW?
- How effective is the use of Morma in the removal of FOGs in the pre-treatment stage?
- What dosage of the enzyme is required to pre-treat the DWW effectively?
- What are the optimum conditions of the pre-treatment stage using this new enzyme and can the lab-scale plant treat DWW?

1.5. Aims and Objectives of report

This research aims to assess the performance of the enzyme-based pre-treatment stage coupled with the DEGBR in treating DWW. In order to assess this, the following specific objectives will be looked at in this study:

- The general characterisation of DWW.
- Optimising the parameters of the pre-treatment stage, which include the temperature, HRT and enzyme dosage, of Momar to produce an effluent with a low FOG content.
- To evaluate the robustness of using Mormar as a bioremediation agent in the pre-treatment – DEGBR system.
- To evaluate the robustness of the combined pre-treatment – DEGBR system in treating DWW.

1.6. Scope and Limitations

The scope of this dissertation will be to evaluate the performance of the treatment of DWW using an enzymatic pre-treatment coupled with an DEGBR and to establish if this enzyme is efficient in the removal of FOGs. The duration of the project was from March 2022 to final submission on the 10 February 2024.

The limitations in this project are as follows:

- The project was limited to just the first stage of the lab scale plant as this was a 120 credit (equivalent to a required 1200 hours of work) dissertation required for the partial fulfilment of the MSc programme.
- Initially during batch testing, Eco-Flush was used as well to see if it produced a higher removal rate of FOGs than Momar but due to the excessive foaming it was removed from the study.
- Initially TKN was being tested as well but due to lab equipment producing inconsistent results countless of times it was removed from the project.
- This study did not collect the biogas produced.
- There was a few cases of restarting the system due unexpected breakages within the system.

1.7. Plan of development (P.O.D.)

This dissertation is divided into five chapters. The first being the introduction which showcases the need for this research including the background on the topic and the objectives of this dissertation. Chapter 2 is a review paper on enzymatic pre-treatment coupled with an AD. Chapter 3 describes the methodology used in this study from collection of wastewater to testing of each parameter. Chapter 4 is a paper that analyses the data and showcases the results with a discussion of the data and evaluating the performance of the reactor with DWW as well the performance of the enzymatic pre-treatment on the removal of FOGs. Chapter 5 includes the future recommendations and limitations with the use of this system and enzyme as well as concluding the findings that were found. The diagram below is a visual representation of the P.O.D.

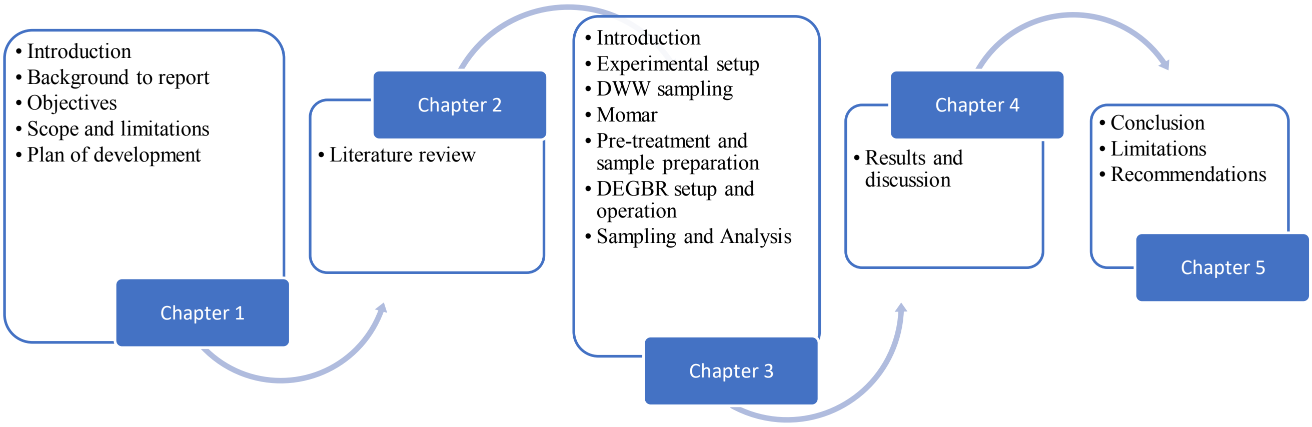


Figure 1.1 | Plan of development of thesis

CHAPTER 2: LITERATURE REVIEW

Moodley, H.R., Gutu, L., Ayinde, W.B., Ikumi, D. and Basitere, M., 2024. Enhancing anaerobic digestion efficiency in dairy waste water treatment: a comprehensive review of enzyme-based pre-treatment by microorganisms in South Africa. Water Practice & Technology, p.wpt2024095.

2.1. Introduction

Clean water refers to water devoid of any physical, chemical, or biological contaminants and can be consumed, used for domestic, and employed for cleaning purposes (Pandit & Kumar, 2015). Access to clean water is considered a fundamental human right necessary for survival. Conversely, untreated water poses a heightened risk of illnesses like diarrhoea, malaria, and other waterborne diseases. It is crucial to adhere to established potable discharge standards to ensure that all individuals have access to clean and safe drinking water (Hove et al., 2019).

With the rapid population growth and industrialization, the dairy industry continues to grow due to the increasing population and urbanization, resulting in more production of wastewater and the release of toxins into the environment. The dairy industry is a significant contributor to the agricultural sector on a global scale, and South Africa (SA) is certainly no exception. Addressing the pollution associated with this industry is critical (Esterhuizen, Fossey & Lues, 2012).

The substantial freshwater consumption in the dairy industry’ s cleaning, sanitation, and processing activities results in large volumes of wastewater, ranging from 0.2 to 10 L per litre of milk produced (Ates et al. 2017). The cleaning process involves several stages, such as cleansing the boilers, softening, and backwashing of filters. Additionally, detergents are employed in cleaning milk cans, tankers, and flooring, giving rise to a different kind of sewage called sanitary wastewater. Lastly, the water used in the cleaning equipment (CIP) process also contributes to industrial wastewater pro- duction (Preeti et al. 2017).

The toxins released in the wastewater are in the form of solids and liquids comprising different chemicals (Raghunath et al. 2016). As previously stated, these toxins are detrimental to the environment, threatening aquatic life and the flora surrounding the water sources and contributing to eutrophication. Due to this high volume of wastewater production and subsequent release of toxins, the dairy industry is considered one of the most polluting sectors among food industries (Borbón et al. 2014). Hence, the treatment of dairy waste water (DWW) is crucial in reducing overall damage to the environment.

In recent decades, studies have demonstrated that raw DWW composition fluctuates depending on the specific dairy product produced (Fig 2.1) and the processing techniques employed. These variations have led to significant environmental concerns due to the discharge of untreated effluent (Ahmad et al. 2019). Thus, it is essential to consider the type of dairy product being processed when planning an appropriate treatment design.

Figure 2.1 | Dairy products

Various factors influence the characteristics of DWW as shown in Figure 2.2, including the operational methods, the facility's scale, process parameters, and the cost of wastewater treatment (Joshiba et al. 2019). Consequently, the origin of the wastewater plays a crucial role in determining its properties, emphasizing the significance of selecting an appropriate treatment strategy. Notably, DWW possesses certain distinctive traits, such as a milky white appearance, high turbidity, and an unpleasant smell. Moreover, the temperature of DWW tends to be 7–15 °C higher than typical municipal wastewater, fostering faster biodegradation processes (Kolev Slavov, 2017). During dairy processing, the effluent undergoes significant alterations in various parameters, including pH, BOD, chemical oxygen demand (COD), total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), and FOGs. Notably, BOD and COD levels experience substantial increases in DWW, posing a significant challenge regarding its safe discharge into water bodies (Onet, 2010; Ahmad et al. 2019; Joshiba et al. 2019). These alterations exert a detrimental influence on ecosystems, leading to toxicological damage. The fats, oils, and greases (FOGs) emerge as a prominent concern among the pollutants, as they accumulate as oil droplets on the water's surface, impeding oxygen transfer. Consequently, this oxygen depletion emerges as a substantial threat to aquatic life and the myriad species dependent upon it (Mendes et al. 2010).

Considering the breadth of recent publications and acknowledging the adverse effects associated with the disposal of huge volumes of untreated DWW, there is a need to choose the most efficient, minimal-footprint wastewater treatment options whilst adhering to municipal standards and environmental guidelines and fostering a sustainable economy.

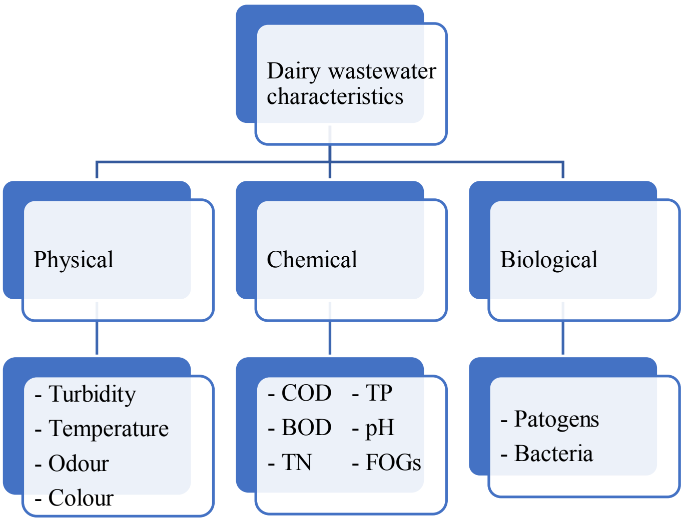


Figure 2.2 | Characteristics of Dairy Wastewater

However, it is worth noting that existing treatment technologies often fall short of consistently achieving desired effluent standards and may entail significant costs. Nevertheless, these processes exhibit efficacy only within a limited scope of operations. Hence, fluctuations in flow rate and waste concentration impact the technological processes. Consequently, wastewater pre-treatment is essential before discharge to guarantee the effectiveness of subsequent stages in wastewater treatment facilities. This literature review paper assesses the performance and effectiveness of utilizing enzymatic pre-treatment using a bioremediation agent coupled with an anaerobic digester (A.D) for DWW treatment. The review paper also discusses the background of enzymatic pre-treatment, its associated challenges, and its integration into biological treatment processes. Also included are recommendations on DWW treatment and future research directions.

2.2. Roles of pre-treatment in conventional wastewater treatment plants

DWW contains high levels of organic pollutants, nutrients, and sediments that can cause environmental pollution if not treated appropriately. The high contaminants in dairy wastewater, if allowed to be released into freshwater sources without any treatment, destroy the water's features, making it less habitable and non-potable. There are many harmful consequences of these actions, such as the water body becoming a breeding area for various insects and ultimately giving birth to diseases such as malaria, yellow fever, dengue, and others (Mohebzadeh et al., 2013; Al-Wasify, Ali & Hamed, 2018; Joshiba et al., 2019). According to Shams et al. (2018), the disposal of casein from dairy plants is a major culprit in freshwater contamination, leading to foul-smelling, black-coloured sludge that harms aquatic ecosystems. The excessive nutrient content triggers accelerated growth of algae and bacteria, depleting oxygen levels in the water and consequently causing a decline in aquatic life (Bhuvanendran et al., 2022).

Overall, selecting an appropriate treatment technology hinges on understanding the specific characteristics of the dairy wastewater, the level of contaminants, and the prevailing conditions for optimal mitigation. Table 2.1 highlights the main issues with DWW and the need to treat it to avoid environmental harm.

As outlined in Table 2.2, DWW undergoes a treatment train to ensure it meets the required discharge standards. The treatment train is a series of processes designed to eliminate different elements from the water, including nutrients, odour, colour, pathogens, and high organic content such as COD, etc. Typically, a treatment train comprises three stages: primary, secondary, and tertiary (Fig 3). The discharge standards for potable water is indicated in Table 2.2 showing the World Bank Report as well as SANS 214:2011.

DWW, known for its high-fat content, contains a significant amount of FOGs. DWW treatment encounters challenges like poor sludge settleability, nitrogen, and phosphorous removal complexity and substantial scum production (Andrade et al. 2013). Upon release into sewage systems, the presence of abundant FOGs, alongside solids such as cheese remnants, coagulated milk, curd fragments, and equipment cleaning residues like sand or soil, can engender blockages, potentially leading to pipeline bursts (Otsuka et al. 2020). Thus, removing these contaminants of high-fat content wastewater is imperative before subjecting it to any sustainable biological treatment approaches. By employing enzymes, it is possible to reduce the FOG contents of the raw DWW influent.

Table 2.1 | The need to treat Dairy Wastewater (Hansen, 2015)

Parameter	Need to treat
High organic content (COD & BOD)	When high organic loads are dispersed into water bodies, it can create "dead zones" where the water has almost no oxygen, and hence no fish will be found in these areas; the high organic load poses a considerable threat to aquatic life residing in the water body.
FOG's	Without treating the wastewater to reduce the FOG content, it can cause blockages within the reactor, ultimately leading to the reactor's malfunctioning.
Nutrient content	Wastewater has a nutrient content, which includes nitrogen and phosphates; when an excess amount is discharged into a water body, it will cause excessive algae to form, which utilizes more oxygen, thus putting aquatic life at risk due to a lack of oxygen.
Pathogens	Pathogens and bacteria are present in wastewater and cause severe problems in the water bodies that the wastewater will be discharged into, such as putting aquatic life at risk and causing a breakout of disease to communities who utilize the water from the water body.
Turbidity	Since the water is a milky colour, it puts the public off, so it needs to be treated with disinfection, such as chlorine, to reduce turbidity.

Table 2.2 | General DWW characteristics compared to effluent discharge standards.

	Types of dairy products (g/L)								Discharge standards (mg/L)	
Parameters	Fresh milk	Cheese whey	Cheese	Butter	Yoghurt	Ice cream	Cottage cheese	Buttermilk	World Bank Report	SANS 214:2011(Potable water quality)
COD	10.63	50-102.1	1-63.3	8.93	6.5	5.2	17.65	94.86-100.91	250	-
BOD	7.11	27-60	0.59-5	2.42	-	2.45	2.6	-	50	-
FOG	0.248	0.9-14	0.33-2.6	2.88	-	-	0.95	-	10	-
TSS	0.686	1.27-22.15	0.19-2.5	5.07	-	3.1	3.38	-	50	-
TN	0.21	0.2-1.76	0.018-0.83	-	-	0.06 (TKN)	-	-	10	13.4
TP	0.36	0.12-0.53	0.05-0.28	-	-	0.014	-	-	2	-
pH	4.217	3.92-6.5	3.38-9.5	12.08	3.93	5.1-6.96	7.83	4.7	6–9	9.7
References	-	(Kolev Slavov, 2017)	(Demirel, Yenigun & Onay, 2005)	(Carvalho, Prazeres & Rivas, 2013)	(Gok *et al.*, 2023)	(Karadag *et al.*, 2015)	(Kolev Slavov, 2017)	(Sevgi Kirdar & Gamze GENC, 2020)	(Shete, 2013)	

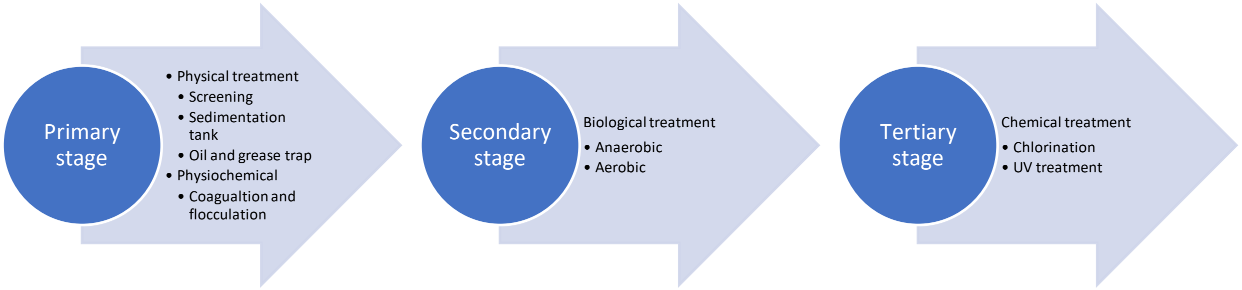


Figure 2.3 | Process train for treatment of wastewater

This reduction has a cascading effect, leading to a decrease in the organic content. Hence, the biological treatment process can operate without a significant energy requirement, reducing the overall treatment time. According to Bella & Rao (2023), pre-treatment reduces the FOG content, increases solubilization, improves the digestion rate, and decreases the raw influent’s organic content. Biological treatment of high-fat content wastewater, such as using an AD, is effective in the reduction of substrates and organic content. However, the FOGs pose numerous challenges, as mentioned above, thus requiring assistance from a pre-treatment stage to enhance the treatment of the raw influent (Mobarak-Qamsari et al. 2012; Harris & McCabe, 2015).

Pre-treatment plays a key role in enhancing the effectiveness of conventional wastewater treatment processes. Several studies have examined the different methods utilized in pre-treatment procedures, thereby supporting bio- diversity and ecosystems. Certain techniques within this process demand substantial energy consumption, increased air circulation, and are time intensive. However, to mitigate these challenges, integrating biological pre-treatment processes anchored to reactors has proven effective and efficient for treating DWW (Joshiba et al. 2019). An example often cited as representative of such a reactor is the Up-flow Anaerobic Sludge Blanket (UASB), which has been extensively documented. The UASB reactor stands out as a highly efficient and cost- effective solution for wastewater treatment. It has demonstrated remarkable efficacy in treating concentrated wastewater with high organic loads. Kaviyarasan (2014) describes the UASB as a system containing microbial granules, small agglomerations of microorganisms (0.5 to 2 mm in diameter), formed within the sludge blanket of the UASB. Due to their weight, these granules resist being washed out by the flow. Anaerobic digestion (AD) facilitated by bacteria in the sludge converts organic matter into biogas. The natural mixing of the sludge occurs through rising bubbles, eliminating the need for machinery while sloping walls push down material reaching the top of the tank. Wastewater flows into the UASB reactor from the bottom and flows upward. UASB reactors are particularly appealing because they can treat wastewater with broader strengths and higher sus- pended solids.

The advantages of using a UASB are that it is cost-effective and requires little space as it can be constructed underground, UASB has shown that it can reach high removal rates in high-strength wastewater, the biogas that is produced can be used as a source of energy, it produces small amounts of sludge, there are fewer CO_2 emissions from the system thus air pollution is reduced, there is no need for temperature control as heat is released during the methanogenesis stage and the effluent produced is high in nutrients thus it can be used for agricultural purposes (Kaviyarasan, 2014; Goli et al. 2019).

However, drawbacks highlighted by Goli et al. (2019) include a long startup period, substantial seed sludge requirement, dependence on skilled operators, partial pathogen removal, and hydrogen sulfide emission causing foul odors (Sinha et al. 2019). Accumulation of FOGs in the reactor can obstruct gas movement, affecting performance. A modified UASB reactor proposed by Bhuvaneshwari et al. (2022), equipped with a scum extraction device and a lamella settler, effectively reduces biomass washout, and eliminates FOGs, preventing blockages. Most UASB systems operate at mesophilic (25–45 °C) or thermophilic conditions (45–65 °C), but maintaining such temperatures requires significant energy, rendering it expensive and often impractical. Organic debris buildup during no wastewater inflow compromises system efficiency, emphasizing the need to reduce debris during downtime and intermittently run the system to enhance biological conversion efficiency. Combining the UASB system with another treatment, as Ji et al. (2020) demonstrated with an anaerobic baffled reactor (ABR), can achieve high COD removal rates.

When there is no inflow of wastewater, organic debris tends to build up within the sludge bed, which can cause the system to not operate at its optimum, thus reducing the organic debris in the sludge bed when there is no flow to increase the systems efficiency. Intermittently running the system also improves biological conversion efficiency. Another way of increasing the systems efficiency is to combine it with another treatment as well. Ji et al. (2020) reported that a combined system of an ABR and a UASB reactor achieved a COD removal rate of 98%.

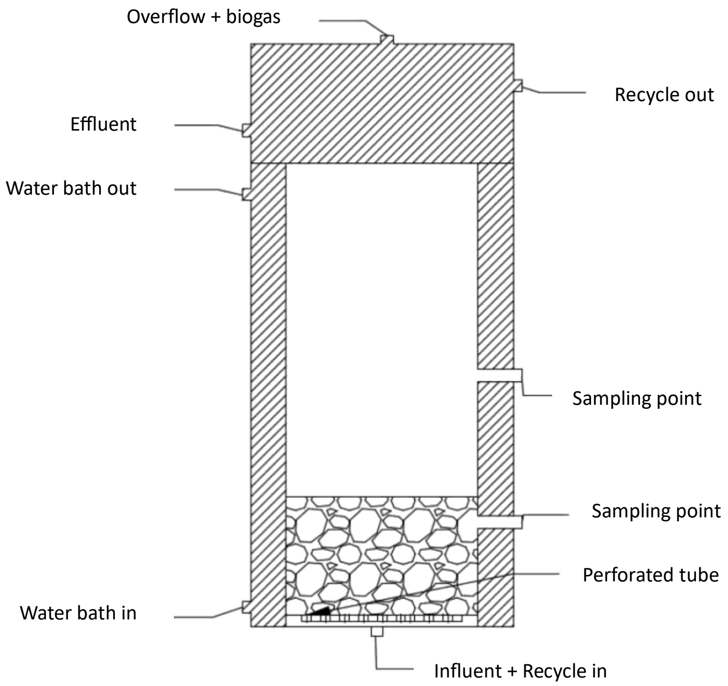


Figure 2.4 | UASB reactor setup

The pre-treatment stage involves a series of physical, chemical, and biological processes designed to remove or neutralize specific contaminants from wastewater (Goli et al. 2019). Physico-chemical pre-treatment has been researched and reported to be effective and fast. However, the drawbacks outweigh the former: the reagents needed are costly and hazardous, the reaction conditions are severe, and it requires a large amount of energy (Arvanitoyannis & Giakoundis, 2006; Crini & Lichtfouse, 2019; Musa & Idrus, 2021). Considering these challenges, the biological route for pre-treatment is a better option; this includes using enzymes that comply with environmental regulations and green economy goals (Cammarota & Freire, 2006; Mobarak-Qamsari et al. 2012; Adulkar & Rathod, 2015). A study investigating the pre-treatment of DWW with lipase Z coupled with the ultrasound irradiation technique by Adulkar & Rathod (2014), reported that coupling the enzymatic pre- treatment with ultrasound increased the rate of hydrolysis and lowered the reaction time. The optimum con- ditions used in this study were a 0.2% (w/v) enzyme load, a temperature of 30 °C, an ultrasonic power of 165 W, a frequency of 25 kHz, and a mixing speed of 200 rpm.

Hernández et al., (2015) reported that the combination of acid and enzymatic pre-treatment effectively broke down microalgal cell walls and converted carbohydrates into monosaccharides for bioethanol production. Acid hydrolysis is particularly effective at disrupting cell walls, increasing enzyme efficiency. The authors reported that the best results were achieved through a combination of H_2SO_4 with enzymatic hydrolysis, with the highest sugar release (S.R.) in *C. sorokiniana* (128 mg/g D.W.) and *N. gaditana* (129 mg/g D.W.). *S. almeriensis* had the highest S.R. from acid hydrolysis with H_2SO_4 for 60 min (88 mg/g D.W.). Enzymatic hydrolysis with amylases of *C. sorokiniana* previously suspended in 0% sulphuric acid released a significant concentration of monosaccharides (101 mg/g D.W.).

Gomes et al., (2011) reported that lipase pre-treatment increased the organic load of high-fat wastewater in a hybrid UASB reactor. Mendes, Pereira & de Castro, (2006) found that using pancreatic lipase drastically reduced the size of fat particles in pork increased LCFAs in the liquid phase and decreased the digestion time of slaughterhouse wastewater. Results of pancreatic lipase pre-treating high-fat wastewater showed that the fat particles were reduced by 60%, and a 4-hour pre-treatment increased the free LCFAs concentration to a maximum of 15.5 mg L^{-1}.

Adulkar & Rathod, (2015) reported that enzymatic pre-treatment using lipase Z of synthetic DWW before A.D. resulted in a reaction conversion of 75% and COD removal of 72% under optimum conditions. These conditions were reported to be 0.2% (w/v) enzyme load and a temperature of 30°C. sodium chloride (NaCl) was used as an emulsifying agent to promote the enzyme activity, increasing the reaction rate by 30%.

A study using lipase from Aspergillus niger in the pre-treatment of FOG-rich food waste found that biomethane production increased and the volatile solids decreased. Biomethane production was reported to be 0 mLg^{-1} at 0.5% (w/w) lipase concentration, and the digestion time was reduced by 10 – 40 d (Meng et al., 2015, 2017). Salama et al., (2019). stated that pre-treatment of FOG with the applicable lipase can increase the rate of hydrolysis and the production of biomethane.

Based on the case studies, it is evident that employing an enzymatic pre-treatment method for high-fat content wastewater yields multiple advantages and effectively manages the wastewater. In the biological pre-treatment approach, the energy expenditure is reduced, yet the use of enzymes leads to an increase in the overall operational cost (OPC). Considering the OPC, the expenses for chemicals and enzymes are significant, resulting in a higher combined biological and chemical OPC. Consequently, evaluating its energy consumption which directly affects the OPC is important. From Figure 2.5, it can be seen that the energy cost implications for chemical and physical pre-treatment are medium cost whereas enzymatic pre-treatment is of low energy consumption according to Preethi et al. (2023). This indicates that enzymatic pre-treatment is the best value for money amongst these pre-treatments. Table 2.3 provides further instances for reference. This enzymatic pre-treatment substantially mitigates the primary challenge associated with high-fat content wastewater, namely FOGs, thereby facilitating the smoother operation of the overall treatment process (Feng et al. 2021). Moreover, the byproduct generated during this treatment proves advantageous and boosts the production of biomethane, which can be utilized as an energy source.

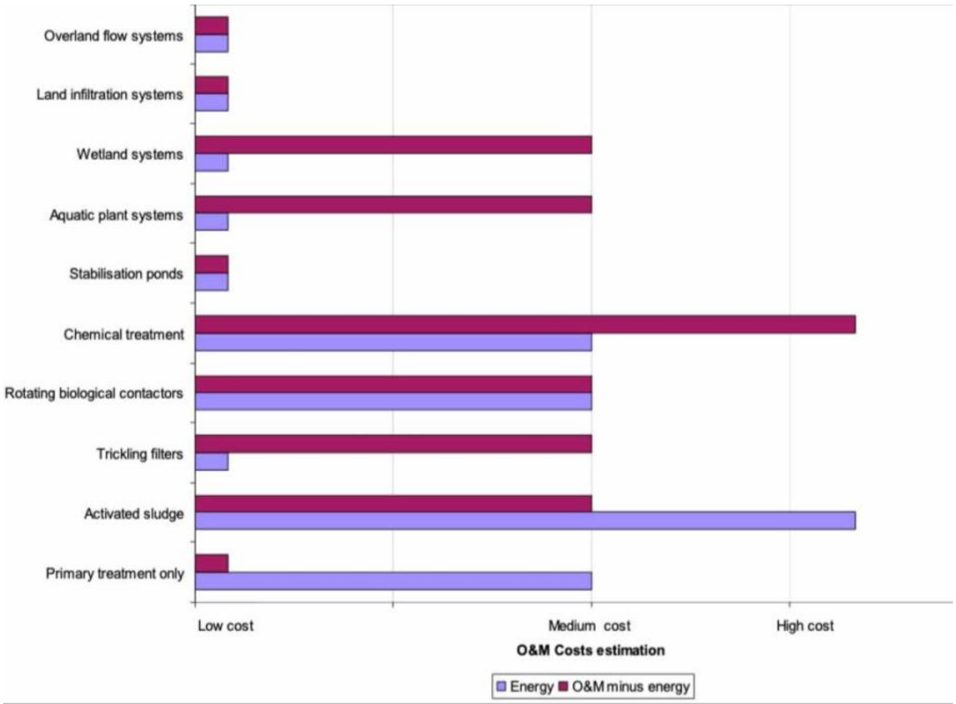


Figure 2.5 | Cost comparison between various treatments (Wendland & Ozoguz 2005)

Table 2.3 | Performance of enzymatic pre-treatment coupled with an A.D.

Type of enzyme	Source	Input		Output	Reference
		Dosage	Conditions		
Commercial Lipase	-	0.1% w/w	30 °C for 12 or 24 hr at a pH of 7.0	Increases the release of long-chain fatty acids	(Pascale et al., 2019).
Lipase	From raw milk and crude enzyme extract	4% w/v	37°C for 72 hr	A balanced pH, increased the removal of COD and T.S.	(Bhange & Suke, 2018)
Lipase	Porcine pancreas	0.05% w/v	37 °C for 4 hr at a pH of 8.0 and 200 pm	An increase of 1240% free fatty acid content, 39.5 ± 6.8% of lipids hydrolyzed, an increase in glycerol of 65%, 32.7% of proteins hydrolyzed, an increase of biogas production of 162 – 292% and an increase in COD removal of 30 – 40.9%.	(Mendes et al., 2010).
Lipase	Penicillium sp.	0.1% w/v	30 °C for 24 hr at a pH of 7.0	Removed COD 90.5 ± 3.4%	(Rosa et al., 2009).
Lipase	Rhizopus sp. microspores CPQBA 312–07 DRM	0.3% w/v	35 °C for 72 hr at 150 pm	Reduced FOGs by 76.9%, COD by 47% and BOD by 92%	(Alberton et al., 2010).
Protease	B. licheniformis	2g dry cell w/l	55 °C for 24 hr at 150 pm	A 27% reduction in solids, Biogas production increased by 310.6% and COD solubilized by 24%	(Kavitha et al., 2016).

2.3. Principles of Enzymatic Microbe Bioremediation (EMB)

Biological pre-treatments, also known as pre-hydrolysis and two-stage digestion, involve the modification or degradation of organic materials by using bacteria, fungi, or enzymes. The use of biological pre-treatment methods can have several advantages, including reduced energy consumption, milder operating conditions, and the possibility of generating value-added products from waste materials. The effectiveness of these methods depends on multiple factors, such as the composition of the material, the specific microorganisms or enzymes used, and the desired final product.

Enzymatic pre-treatment represents a potent and efficient solution for handling the complexities of DWW. Enzymes are biological catalysts that play an essential role in biological pre-treatment by catalyzing the breakdown of biomass components, thereby increasing the reaction rates. By utilizing the catalytic abilities of enzymes, specific organic compounds present in the wastewater can be efficiently broken down, resulting in improved biodegradability and a reduced environmental impact. Moreover, enzymatic pre-treatment can significantly enhance the performance of other treatment processes, such as biological treatment and membrane filtration, by breaking down complex organic compounds that would otherwise be difficult to degrade (Ahmad et al. 2019).

This section focuses on discussing enzymatic pre-treatment tailored for DWW. It will delve into the enzymes employed, their modes of action, and the parameters governing their activity in DWW. Additionally, the section will explore the myriad of benefits, and challenges associated with implementing enzymatic pre-treatment in DWW.

Once exposed to the wastewater, the enzyme interacts with large complex organic molecules; particularly during the pre-treatment of DWW, the enzyme targets specific substrates such as FOGs. During enzyme‑substrate interaction, the enzyme binds to the substrate at the active site, where this binding resembles a ‘lock and key’ mechanism (Spencer, 1944). More models have been put forward that better describe the binding of the enzyme and substrate. Daniel Koshland proposed the induced fit model, where the active site of the enzyme is reshaped to fit the substrate during the interaction. According to Tripathi & Bankaitis (2018), the ‘correct’ sub- strate naturally aligns itself with the active site residues of the enzyme, inducing the necessary conformational changes for the desired outcome. The Michaelis‑Menten theory of enzyme action is currently the most widely accepted concept in enzymatic research. This theory initially suggests a reversible combination of the enzyme and substrate, followed by an irreversible formation of products and the release of the enzyme (Park & Agmon, 2008; Bhatia, 2018) This process can be represented in equation form, as depicted in the following equation.

Enzyme + Substrate → Enzyme -Substrate Complex → Enzyme + Product

Equation 2.1: Enzyme substrate reaction

Once the enzyme‑substrate molecule is formed, the enzyme catalyzes the conversion of the large substrate into smaller molecules. The enzyme accelerates this reaction by weakening and breaking down the chemical bonds within the sizeable organic molecule (Bella & Rao 2023). These processes have high reaction kinetics and reduce the time required for the substrates to travel into cells, making the process more effective (Feng et al. 2021).

Ligand binding is the interaction between the ligand (molecule/ion) and the receptor but within in enzymes where the active sites are located deep within the protein structure an extra step is required. This is because potential substrates need to traverse through the protein’s interior to reach the active site. In contrast to enzymes with active sites exposed on the protein surface, this structural arrangement offers more interaction opportunities between proteins and ligands. This is because the substrate must navigate through a series of tunnels before bind- ing to the active site. The core idea of this model is that in enzymes with concealed active sites, the ligand binding process involves two distinct steps. First, the ligand must migrate through the protein’s body, and then it can bind to the active site. By breaking down the process in this manner, the theory suggests that, in addition to compat- ibility between the ligand and the active site, there must also be compatibility between the ligand and the binding tunnel (Kingsley & Lill 2015).

Enzymes can be categorized into hydrolases and oxidoreductases (Figure 2.6). The latter removes substances through oxidation, moving electrons from reductants to oxidants, producing CO_2 and Cl^{-} ions. Microorganisms use the energy or heat produced from this for biochemical activities. The former uses water to create biochemical reactions to break down chemical bonds. The substrate of interest is DWW, mainly comprising fats, carbohydrates, proteins, and nutrients such as nitrogen and phosphorous (Kwarciak-Kozłowska & Bień 2018). According to Facchin et al. (2013), hydrolytic enzymes such as lipase, amylase, and protease are highly recommended for use in the treatment of DWW.

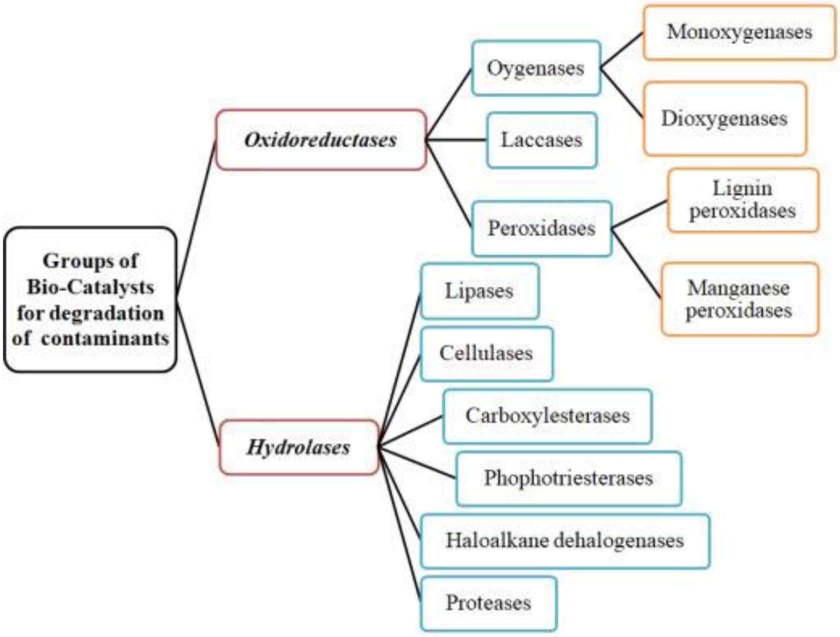


Figure 2.6 | Enzyme categories (Mishra et al., 2020).

Types of enzymes

Lipase is an enzyme that specifically targets the fats, oils, and grease molecules in wastewater, making it a vital enzyme in the treatment of high-fat content wastewaters such as dairy (Mobarak-Qamsari et al., 2012; Feng et al., 2021). It catalyzes the hydrolysis process of carboxyl ester bonds present in triacylglycerol into free long-chain fatty acids and glycerol. The process of lipase follows the ping-pong bi-bi mechanism (Fig 2.7). The initial step in this mechanism is the combination of the enzyme with an acyl donor, which is a triglyceride in this scenario which then forms a lipase-triglyceride complex. The isomerization process converts the lipase-triglyceride complex into an intermediate complex and produces glycerol. The binary complex is formed when the intermediate complex combines with three molecules of water. In the last step, fatty acids are produced by unimolecular isomerization, and then the enzyme is regenerated, and the process continues (Liew et al., 2020).

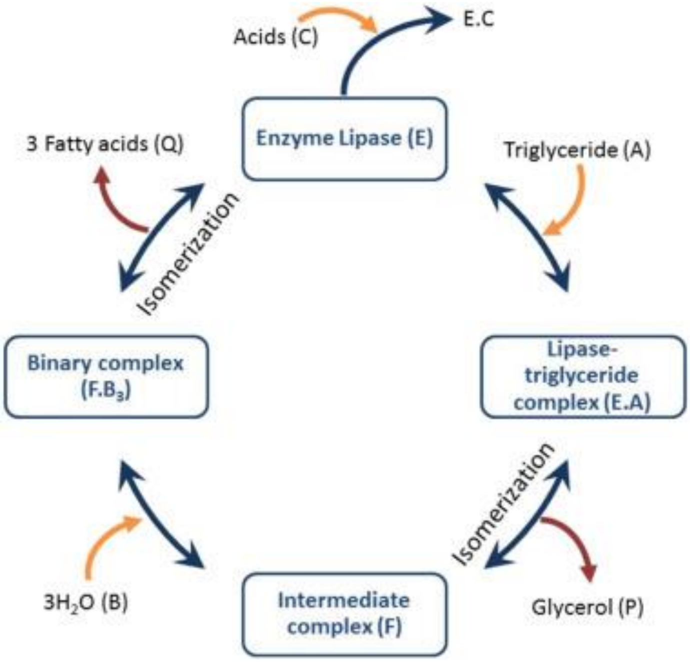


Figure 2.7 | Lipase process (Liew et al., 2020).

Amylase is introduced into the wastewater to break down carbohydrates, also called polysaccharides; they are made up of large chains of simple sugars. Amylase breaks these large chains of simple sugars into small polymers comprising glucose (Liew et al., 2020; Osho, Awe-Mathias & Onajobi, 2021).

Protease is an enzyme that breaks down the proteins within the wastewater. Proteins are polymers which comprise amino acids joined by peptide bonds. This enzyme hydrolyses these peptide bonds to break the protein down into simpler compounds such as polypeptides and amino acids. The protein in wastewater constitutes high amounts of total organic carbon and organic matter. Thus, a breakdown of proteins within the wastewater can reduce the TOC of the wastewater as well as produce simple sugars which can be used in the methanogenesis process in the A.D. (Facchin et al., 2013; Pandey et al., 2017; Liew et al., 2020).

Conclusively, these enzymes are pivotal in the treatment of dairy wastewater. Their enzymatic properties show great promise in effectively managing high-fat content wastewater, demonstrating their ability to degrade fats and greases and enhance anaerobic biodegradability (Mendes et al., 2010; Konkit & Kim, 2016). Consequently, they facilitate smoother and more efficient treatment processes, contributing to developing environmentally friendly and highly effective methods for treating dairy wastewater.

Factors affecting EMB

Enzymatic activity in wastewater treatments is subject to several limiting factors, including enzyme dosage, pH, temperature, FOG content, agitation, and NaCl content. To overcome these limitations, it is crucial to optimize the treatment process before implementation, which can be accomplished through a pilot plant.

Factors/mechanisms affecting enzyme-substrate complex.

In the realm of DWW treatment, the interaction of the enzyme-substrate complex is subject to a multitude of influences, encompassing protein dynamics, gating mechanisms, tunnel geometry, as well as cofactors (Prokop et al., 2012; Haggag et al., 2013; Kingsley & Lill, 2015). A cofactor is a non-protein substance that is used in the catalytic process of an enzyme. Cofactors can be introduced into a system as inorganic or organic molecules, and they can be strongly attached to the active site or loosely connected with the enzyme. In structural integrity, the cofactor can also play a vital role Illustrated in Figure 2.8 is an instance demonstrating how one of these elements can shape the lock and key model between an enzyme and a substrate (Richter, 2013). Various mechanisms, including covalent catalysis, catalysis by approximation, metal ion catalysis, and general acid-base catalysis, can modulate the active site to facilitate the formation of the enzyme-substrate complex (Bhatia, 2018; Lewis & Stone, 2020).

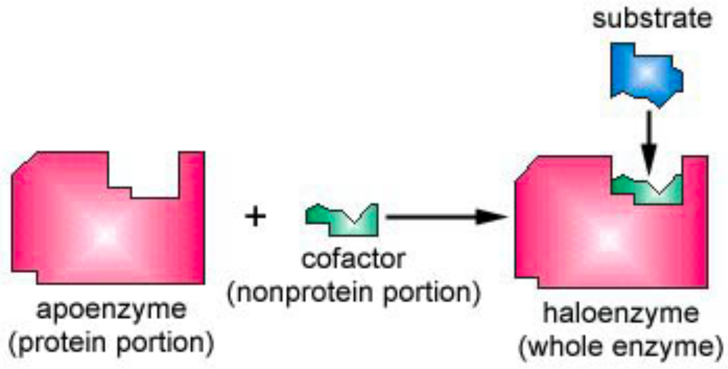


Figure 2.8 | Cofactor assisting the enzyme-substrate complex (Kaiser, 2022)

Environmental Factors

Temperature

Temperature is a crucial factor in the performance of enzymatic reactions as it is directly related to the solubility, the fluidity of the reactive media, and the viscosity of the substrates, which all affect the mobility of reactants (Baena et al., 2022). The optimum temperature range is between 37 and 40°C; if the temperature increases, then the enzyme activity slowly depletes until there is no activity and can lead to thermal denaturation as the internal bonds of the enzyme are sensitive to high temperatures (Liew et al., 2020; Baena et al., 2022). The enzyme will be inactive if the temperature is below the optimum temperature. Skliar et al., (2019) reported that the optimum temperature conditions of lipase *Rhizopus japonicus* depended on the correlation between (i) influence of the temperature on the speed of reaction and (ii) the effect on enzyme denaturation rate.

Enzyme concentration

When using enzymes for wastewater treatment, it is crucial to emphasize that increasing the enzyme dosage can speed up reactions by enhancing the number of available active sites. According to Mendes et al., (2010), an increase in lipase concentration led to a rise in free fatty acid levels. Yang et al., (2010) found that increasing the enzyme dosage from 3% to 6% (w/w) enhanced the reduction in Volatile Suspended Solids (VSS). However, the authors also observed that any dosage exceeding 6% (w/w) did not yield further reductions in VSS. Liew et al., (2020) suggested that the optimal enzyme dosage ranges between 1% and 2% (w/w).

Substrate concentration

In wastewater treatment, the substrate concentration plays a crucial role in the interaction between the substrate and the enzyme. As the substrate concentration increases, there are more frequent collisions between the substrate and the enzyme. This leads to a faster interaction between the substrate and the enzyme up to a certain point. However, further increases in substrate concentration do not bring any additional benefit beyond this point. This is because the enzyme's active site becomes fully saturated with the substrate. As a result, when a new substrate tries to attach itself to the enzyme, it has to wait for the enzyme-substrate complex to release the product, thereby freeing the enzyme for further interaction.

Electron acceptor/donor availability

The electron acceptor and donor molecules can significantly affect enzyme activity by influencing the redox (reduction-oxidation) reactions that enzymes facilitate. Electron acceptors are chemicals such as sulfates, nitrates, and high-valence metallic ions within the biochemical processes. The electron donor is usually comprised of organic matter, low valence metals, sulfides, and ammonia (Li, Xue & Xi, 2019; Tian & Yu, 2020; Ying et al., 2021).

The enzyme will increase the hydrolysis rate, leading to an increase in readily biodegradable substrates. Breaking the complex substrates into smaller substrates allows for an increase in electron donors. Thus, with the increase in the electron donors, the biomass will increase linearly.

pH

Establishing the ideal pH is crucial in enzymatic pre-treatment for DWW treatment. It is imperative to ascertain the optimal pH level, as the enzyme's functionality is compromised if the pH deviates from the optimal range of 7 to 8. According to Liew et al., (2020), when lipase is used at the optimum pH, it performs at its maximum and shows negative potential in the active site, which causes the ionized fatty acids to repel one another, which results in a fast release of lipolysis reaction products from the interface. This has a positive effect on the pre-treatment process because when the enzyme is at its optimum conditions there is an increase in the lipolysis reaction which helps in the breakdown of fats which is crucial in the removal of fats in the pre-treatment stage of treatment of high fat content wastewaters.

Inhibitors

Generally, and in the context of enzymes' role in DWW treatment, enzyme inhibition is distinct from denaturation as it involves the interference of a reagent with the active site region, leading to a specific action. This process is classified into two main types: (i) reversible inhibitors and (ii) irreversible inhibitors. The former, in turn, can be further categorized into distinct categories, including competitive, non-competitive, and uncompetitive inhibitors. In competitive inhibitors, the inhibitor mirrors a substrate and can bind to the same active site as the substrate, thus creating competition for the binding. Competitive inhibitors can be displaced from the active site if there is a high substrate concentration, thus restoring the enzyme activity.

Non-competitive inhibitors bind with the enzyme but at a different site away from the active site. It does not block the substrate from binding with the enzyme at the active site; it, however, reduces the catalytic ability of the enzyme, subsequently reducing the enzyme. Uncompetitive inhibition is rare and occurs after binding the enzyme and substrate. When an inhibitor forms a permanent bond with an enzyme, it is categorized as an irreversible inhibitor. Consequently, irreversible inhibitors possess high toxicity levels, an example of this inhibitor is organophosphorus compounds such as diisopropyl fluorophosphate (Haggag et al., 2013; Robinson, 2015; Bhatia, 2018).

2.4. Advantages and disadvantages of using EMBs

Enzymes play a pivotal role in the initial treatment of diary wastewater, significantly aiding in the reduction of FOGs, which are recognized as essential substrates for fostering biomethane generation. Salama et al., (2019) reported that the high content of lipids within FOGs causes a higher yield of biogas due to the higher convertibility of 94.8% to biogas compared to 50.4% due to carbohydrates and 71% due to proteins. Enzymatic pre-treatment also reduces the organic matter within the wastewater, thus reducing the organic load going into an A.D (Bella & Rao, 2023). A study by Samarasiri, Rathnasiri & Fernando (2019) demonstrated that the utilization of enzymes effectively reduces the energy input necessary to initiate a reaction. This not only accelerates the reaction rate but also enhances the hydrolysis process. The researchers also re-affirmed that pre-treatment with lipase decreased organic content within DWW effluent and facilitated biogas generation. Enzymes offer advantages over microbes in pre-treatment organic-rich wastes as they do not require an acclimatization phase to the biomass, leading to a faster process. Additionally, enzymes are biodegradable proteins that break down independently without generating new biomass. Enzymes can operate under mild conditions (i.e. low temperature and a neutral pH) and are effective in various environmental conditions like pH and temperature (Liew et al., 2020). Enzymatic pre-treatment stands out as a more environmentally sustainable and economically viable approach for wastewater treatment, particularly when juxtaposed with other methods reliant on chemical agents.

One significant drawback of utilizing enzymatic pre-treatment is the associated cost implications. As the usage of enzymes increases, there is a corresponding increase in the required purchase of enzymes. Thus, determining an optimal dosage becomes essential. According to Liew et al. (2020), while enzymes demonstrate functionality across various environments, such as different pH and temperatures, they are prone to denaturation and inactivity beyond their designated operational parameters. Consequently, careful monitoring and maintenance of the ideal conditions are consistently necessary. The enzymatic process's speed can be relatively slow, contingent upon the type of wastewater or the specific targeted substrate, necessitating frequent replenishment (Harris & McCabe, 2015; Rodriguez et al., 2015; Neumann et al., 2016). Baena et al. (2022). have identified a limiting factor, indicating a lack of compatibility between reactants and the bio-derived catalyst. To address this, using surfactants in the reactive media to achieve compatibility is recommended, albeit with some associated drawbacks.

2.5. Performance of genetically modified microorganisms (GMM) in DWW

Various studies have investigated the efficacy of different treatment methods for DWW. Among these, aerobic treatment approaches have been emphasized in research conducted by Scott & Smith, (1997); Carta-Escobar et al., (2004); Kolev Slavov, (2017); Joshiba et al., (2019). However, when considering DWW treatment, anaerobic digestion emerges as a more favorable option than aerobic digestion. This preference stems from its ability to yield biogas as a by-product, its reduced spatial requirements during construction lowered aeration demands resulting in decreased energy consumption, and the added advantage of reduced sludge and biomass generation, as highlighted by Adulkar & Rathod (2014). Notably, within the dairy sector, Carvalho, Prazeres & Rivas (2013) assert that anaerobic processes are particularly well-suited for addressing wastewater with high organic loads. Supporting this notion, Kolev Slavov (2017) presents findings indicating that anaerobic bacteria outperform aerobic bacteria in DWW treatment. Aerobic processes are compromised due to rapid acidification and filamentous growth attributed to elevated lactose levels and limited water buffer capacity, as discussed by Ahmad *et al.* (2019).

Analysing the performance of these A.D. reactors reveals their capability to treat wastewater with satisfactory removal rates, yet they encounter difficulties when treating high-fat content DWW. The high content of lipids within the wastewater causes blockages within the reactor, thus reducing the removal efficiency. The breakdown of these fats necessitates extended treatment periods, thereby escalating the time required and subsequently increasing the energy demand in the treatment process. Reports have indicated that pre-treating the wastewater with enzymes augments the removal rate, reduces suspended solids in the reactor, and increases biogas production. Figure 2.9 depicts the correlation between methane production and time, illustrating that methane production over time with wastewater without pre-treatment will plateau than when the wastewater is pre-treated with an enzyme. The performance of UASBs, particularly concerning methane production, highlights the challenges encountered when treating DWW with a high-fat content (Couras et al., 2015). A higher fat content in the wastewater corresponds to reduced methane production. From Table 2.3, according to Mendes *et al.* (2006), using an enzymatic pre-treatment to hydrolyze lipids by 39.5% produced an increase in biogas by 162-292%. Hence, minimizing the fat content becomes imperative to optimize methane production and prevent system clogging (Fig. 2.9).

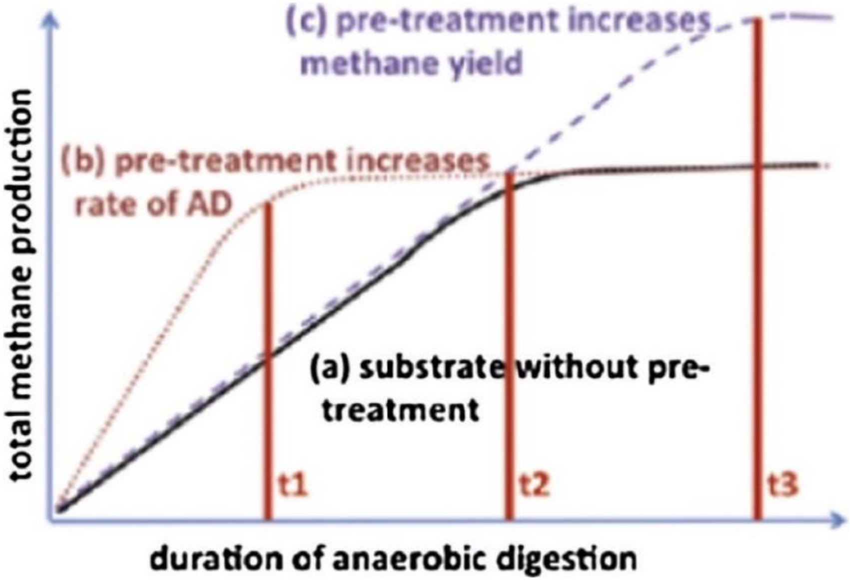


Figure 2.9 | Methane production over time (Harris & McCabe, 2015)

Leal et al. (2006). reported that when there is an increase in FOGs in the wastewater, the COD removal rate decreases in the UASB, but when the wastewater is pre-treated with an enzyme, the COD removal rate is above 90%. A study by Bhange & Suke (2018) investigated the effect of lipase of different dosages on high-fat dairy wastewater. They reported that there was an average removal rate of 97% of FOG, 67% of COD and 38% of T.S. According to Cammarota, Teixeira & Freire (2001), when DWW with high levels of FOGs is treated in a UASB reactor, the effluent is high in turbidity, volatile suspended solids (VSS) and achieves a COD removal rate of <50%. The authors reported that pre-treated DWW with Penicillium-restricted lipase showed a higher % COD removal rate of 90% when treated with the same UASB reactor. A.D treatment is prompt to release high amounts of nutrients which can be recovered using recovery technology such as struvite formation, which acts as a slow release fertilizer. In some areas of SA the struvite and stabilised sludge are used for growth of animal fodder (Nqayi et al., 2023), which is useful in the context of resource recovery from dairy waste.

According to existing literature, lipase stands out as the commonly employed enzyme in treating DW due to its ability to breakdown high-fat content (Ahmad et al., 2019). This enzyme effectively acts as a pre-treatment agent for DWW, augmenting the hydrolysis of FOGs within the wastewater before its introduction into the AD. The remaining FOGs further promote the production of biomethane. The performance analysis of lipase is outlined in Table 2.3, revealing its favourable impact on DWW and its assistance in the AD treatment process. Bella & Rao (2023) and numerous other researchers emphasize the necessity of pre-treatment techniques for complex dairy substrates, particularly to expedite the hydrolysis stage. Gutu et al. (2021) confirms that integrating an enzymatic pre-treatment step before AD enhances the reactor's efficiency, amplifies biogas production, and accelerates the breakdown of FOGs. Nonetheless, a key limitation associated with using enzymes is their cost implications, particularly when dealing with large volumes of DWW, necessitating a higher dosage and consequent continuous procurement of enzymes. Consequently, various scholars propose exploring new, more economical enzyme options to ensure the sustainability of enzymatic pre-treatment methods.

2.6. Applications of GMMs in other industries in South Africa (SA)

Genetically modified microbes (GMMs) find exclusive application in South Africa's agricultural industry, primarily driven by the urgent need to address challenges such as the burgeoning population, food scarcity, malnutrition, and soaring production costs. The GMMs are mainly used to enhance the cultivation of staple crops such as maize, soybeans, and cotton. Notably, the use of GMMs yields substantial benefits, an upsurge in the agricultural sector's revenue, thereby making a significant contribution to South Africa's overall economy. These modified microbes boost crop yields, minimise crop damage, and enhance food quality. Moreover, they contribute to reducing carbon dioxide emissions by curbing the requirement for insecticides and herbicides. Additionally, the adoption of genetically modified technology has led to a considerable reduction in fuel consumption on farms (Bothma et al., 2010; Muzhinji & Ntuli, 2020; Rozas, Kessi-Pérez & Martínez, 2022).

2.7. Conceptualisation of EMBs in DWW treatment

Exploring new, affordable enzymes is crucial for improving the overall effectiveness of pre-treating wastewater. For instance, Ecoflush and Morma are prime examples that exhibit potential in the reduction of FOGs in wastewater containing high-fat content.

Eco-Flush, a South African product, serves the purpose of hydrolysing FOGs in kitchen sinks and drains. This biological treatment is facilitated by a specialized group of bacteria, ensuring a swift, efficient, and eco-friendly breakdown of the FOGs. The product's development revolves around a completely natural consortium of bacteria (Ergofito, 2019). The enzyme Eco-Flush is composed of microorganisms that have been cultivated and preserved in a dormant physiological state. The microorganism has a complex structure comprising various bacteria, such as anaerobic, aerobic, sulphur and nitrifying oxidized bacteria, which are combined with enzymes, fungi, and water (Dlamini et al., 2021; Dyosile et al., 2021). The natural constituents of the product come from amino acids and glucides, which stimulate the microorganisms to produce enzymes required to break down the FOGs of the wastewater oxidize NH_3 into NO_3^- and NO_2^- alongside the elimination of odour-producing organisms. Upon the introduction of Eco-Flush into the wastewater, the microorganisms initiate a reaction, instigating the production of enzymes that commence the biodelipidation process. This process involves the disruption of bonds between triglycerides and phospholipase, thereby separating the fatty acids from the wastewater (Dyosile et al., 2021).

On the other hand, Morma, a U.S.-based company, has developed a range of products, including a liquid bacteria digester designed to assist in treating effluents. This liquid bacteria digester is a combination of five different live spore-forming bacteria. This bacteria, when added to different sources of waste, will produce various enzymes, which include Protease (protein), Amylase (starch & carbohydrates), Lipase (fat & grease), Esterase (fat), Cellulase (cellulose, wood, paper), Xylanase (plant material), Urease (urea). Although it produces all these enzymes, the bacteria will only produce as many as needed to complete the waste source's digestion. This bacteria works best at a pH range of 5 - 9 and a temperature range of 12°C - 35°C. The advantages of employing this bacteria include its rapid activation within an hour of introduction to the waste source.

Furthermore, the aerobic and facultative bacteria within this liquid formula can function effectively in the presence of either oxygen or nitrates. Compared to other naturally occurring bacteria, it exhibits greater resistance to fluctuations in temperature and pH. Additionally, its non-toxic nature eliminates any harmful acids, and it offers a pleasant fragrance that counteracts the foul odour of the waste source.

In the realm of wastewater treatment, Eco Flush has been extensively studied, yielding significant results in the treatment of poultry slaughterhouse wastewater (Bingo *et al.*, 2021; Dlamini *et al.*, 2021; Dyosile *et al.*, 2021; Gutu *et al.*, 2021; Mdladla *et al.*, 2021; Meyo *et al.*, 2021). However, Morma, despite its capabilities, has not yet been utilized in any wastewater treatment procedures.

In conclusion, implementing innovative solutions such as Eco-Flush and Morma shows promise in boosting wastewater pre-treatment efficiency. Eco-Flush, a natural South African product, employs a diverse consortium of microorganisms to rapidly and ecologically degrade fatty, oily, and odorous compounds in wastewater. Its enzymatic approach effectively breaks down FOGs whilst complimenting other organisms that are common in high strength wastewaters and converts ammonia (NH_3) into less harmful by-products such as NO_3^- and NO_2^- (Meyo et al., 2021). On the other hand, Morma, a U.S. product, utilizes a versatile liquid bacteria digester that can adapt to different waste sources. This bacterium efficiently digests various waste components, adjusting its enzymatic activity based on the waste's composition. Its adaptability, quick action, and ability to thrive in diverse environmental conditions make it a compelling option for wastewater treatment. Overall, introducing these innovative solutions represents progress in wastewater management, promising improved purification results and a more sustainable approach to addressing contaminants and odour concern.

2.8. Future perspective and Recommendations

Although anaerobic treatment is typically preferred over aerobic treatment in managing dairy wastewater, it can still be employed as a preliminary step before anaerobic digestion. This preliminary aerobic treatment can accelerate hydrolytic activities during the initial stage, improving hydrolysis and increasing methane production. The use of aerobic treatment prior to a two stage AD process showcased that in the second stage the methane production was much higher than without aeration (Rafieenia et al., 2017). Furthermore, it can also be employed as a post-treatment measure following anaerobic digestion, aiding in the enhanced removal of nutrients. Some future recommendations for the potential advancement in the treatment of DWW as well as implementations in SA are highlighted below.

1. Enzymatic pre-treatment has proven to be highly effective in the removal of solids before the implementation of biological treatment, such as anaerobic digestion. The use of enzymes catalyses the hydrolysis process, demonstrating high substrate and reaction specificity. This specificity results in minimal side reactions and the generation of negligible or no waste by-products (Liu & Smith, 2021). Coupling biological treatment with enzymatic pre-treatment holds the potential for the development of more efficient wastewater treatment systems. Modern technologies can play a crucial role in improving the physiological conditions, including temperature and pH, ensuring that the enzymes operate optimally. Moreover, these advancements can reduce costs and decrease energy consumption (Pandey et al., 2017). Feng et al. (2021) suggest conducting further studies using various enzymes for treating different sources of natural wastewater rather than synthetic wastewater. Considering the potential cost constraints associated with treating large volumes of wastewater, the authors propose exploring new enzymes that are both efficient and economically viable.
2. Eco-flush has found application in the treatment of PSW, where it serves as a pre-treatment method. (Gutu et al., 2021) reported that Eco-flush degrades the FOGs present in the PSW before undergoing treatment in the anaerobic digestor. Meyo et al. (2021) observed that using Eco-flush during the pre-treatment stage reduces the TSS and COD of raw PSW. Subsequent treatment using the expanded granular sludge bed reactor (EGSBR) led to an over 80% removal of both FOGs and TSS, although the average removal of COD was only 60%. As PSW is characterized by a high-fat content, exploring the application of Eco-flush in DWW could significantly mitigate the high-fat content before the implementation of biological treatment. The rapid activation rate of Eco-flush enables the removal of FOGs within 24 hours, depending on the volume of wastewater being treated. Despite showing promising results, further optimization remains necessary and warrants continued investigation.
3. While Morma has traditionally been utilized as a drain cleaner in industrial settings to break down fat, dirt, and other substances, its potential application in wastewater treatment has yet to be explored. Morma contains hydrolytic enzymes that can potentially reduce FOGs in wastewater, making it a promising addition for treating high-fat content wastewater.
4. In South Africa, genetically modified microorganisms (GMMs) have primarily been

confined to the agricultural sector, exhibiting significant potential in enhancing crop yield and fostering industry growth. However, the adoption of GMMs for wastewater treatment has only gained momentum in the last decade, primarily through studies utilizing Eco-Flush for treating PSW. Given their cost-effectiveness and eco-friendly nature, further in-depth exploration of GMMs in wastewater treatment is imperative. Investigating the application of Morma in the pre-treatment of DWW for FOG reduction, coupled with subsequent biological treatment, warrants attention. Employing design software can aid in determining the optimal conditions, including dosage, time, and temperature, for utilizing Morma during the pre-treatment stage.

2.9. Conclusion

The dairy industry plays a significant role in global agriculture, but its processing activities generate substantial wastewater. Unfortunately, the high levels of contaminants in dairy wastewater, including BOD, COD, TSS, TN, TP, and FOGs, make it one of the most polluting sectors in the food industry. These contaminants pose a significant threat to the environment and aquatic life and contribute to eutrophication, characterized by an overabundance of nutrients and oxygen depletion in freshwater sources. The substantial amount of FOGs in DWW presents safe disposal and treatment challenges. However, FOGs exhibit high convertibility to biogas (94.8%), making them a valuable resource for biogas production compared to carbohydrates (50.4%) and proteins (71%). Studies on DWW treatment consistently favour anaerobic digestion over aerobic digestion due to its ability to yield biogas, reduced spatial requirements, lower energy consumption, and decreased sludge and biomass generation. Despite these advantages, the high-fat content in DWW can lead to blockages in the reactor, increasing treatment time and energy demand. Therefore, a pre-treatment stage is essential to reduce contaminants before entering the anaerobic digester. Enzymatic pre-treatment emerges as an efficient solution, as enzymes catalyze the breakdown of organic compounds, effectively reducing FOG content, increasing biogas production, improving digestion rates, decreasing organic content, and enhancing anaerobic biodegradability. However, enzymatic microbial bioremediation (EMBs) faces influencing factors and mechanisms, including the enzyme-substrate complex, temperature, enzyme concentration, substrate concentration, electron donor/acceptor availability, pH, and inhibitors. Commonly used enzymes in DWW pre-treatment are lipase, amylase, and protease. Lipase, particularly, proves effective in hydrolyzing FOGs before introducing the wastewater to anaerobic digesters, leading to enhanced performance and biogas production. A limitation of enzymatic pre-treatment is its cost, which can be significant for large volumes of wastewater. This necessitates further research into more economical enzyme options. Exploring affordable enzymes such as Eco-Flush and Morma, which exhibit potential in reducing FOGs in high-fat content wastewater, represents a way forward in advancing enzymatic pre-treatment of DWW. In conclusion, this review underscores the imperative for ongoing collaboration among researchers, industry experts, and policymakers to promote the adoption of enzyme-based pre-treatment as a transformative and sustainable approach to managing DWW in South Africa and beyond.

CHAPTER 3: METHODOLOGY

3.1. Methodology procedure

The Figure below provides a graphical representation of the experiment's process. The first stage involves collecting DWW at the local dairy factory. The second stage is the pre-treatment process, where the raw DWW is dosed with the enzyme and left to undergo pre-treatment. Following this stage, the wastewater moves on to the DEGBR for further treatment. Samples are collected from both the effluent produced by the DEGBR and the pre-treatment process. Analysis, including COD and TSS, is conducted on the day of sampling. Additionally, a weekly 1L sample of effluent is sent to Bemlab for FOG analysis. Bemlab is a water analysis laboratory in Cape Town, Western Cape province, South Africa (SA).

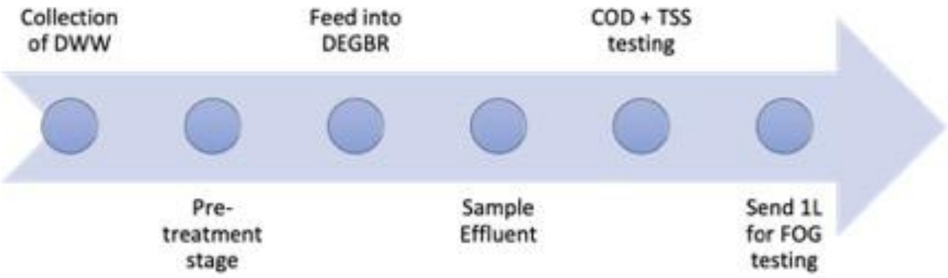


Figure 3.1| Flow diagram of procedure

3.2. Materials and methods

3.2.1. Experimental setup

The equipment utilized for the experiment included a 20L pre-treatment tank, a 20L feed tank, a peristaltic pump, a magnetic stirrer, a water bath, and the down-flow anaerobic reactor. The arrangement of this equipment is illustrated in Figure 3.2 below. The magnetic stirrer operated at 100 revolutions per minute (RPM) to ensure a homogeneous feed of DWW into the AD reactor. This action aimed to prevent the settling of solids in the DWW, which could potentially lead to tubing blockages. Subsequently, the feed was pumped from the feed tank to the AD reactor using the peristaltic pump.

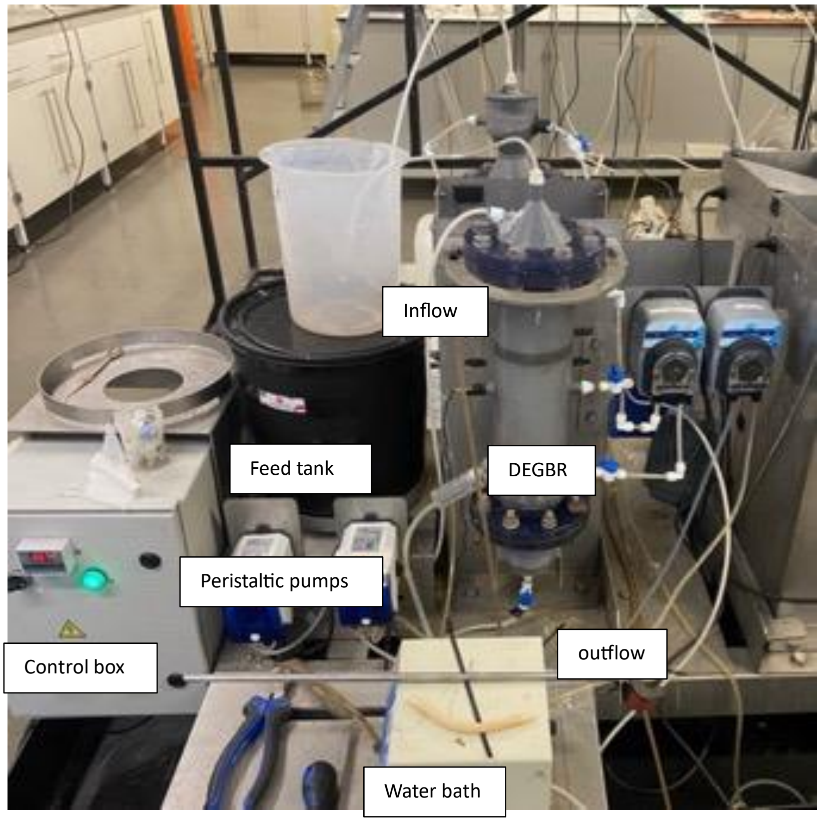


Figure 3.2 | Experimental setup

3.2.2. DWW sampling

The DWW used in this study was collected at a local dairy factory in Cape Town Western Cape province, SA. Collection occurred during the peak production periods, which varied throughout the day. A pump was employed to transfer the DWW into 25L polypropylene containers. Once brought back to UCT, the collected wastewater was stored in a cold room at 4°C to prevent fermentation and mold formation. Twelve 25L containers were filled with DWW during the initial collection, providing a supply that would last for a month before the need for another collection arose.

Figure 3.3 | Pumping of raw DWW

Figure 3.4 | Storage of collected DWW in cold room

3.2.3. Momar – Enzymatic pre-treatment agent

In previous studies conducted by Njoya et al., 2019; Bingo et al., 2021; Dlamini et al., 2021; Dyosile et al., 2021; Mdladla et al., 2021; Meyo et al., 2021, with PSW using this lab-scale plant, an aerobic enzyme called Eco-Flush was utilized during the pre-treatment stage to assist in the removal of FOGs. However, numerous authors noted that a major issue encountered was the high levels of DO within the wastewater. In response to this, the current study opted for an anaerobic enzyme, Momar, to address the elevated DO levels entering the AD reactor.

Momar, developed by a U.S.-based company, offers a range of products, including a liquid bacteria digester designed to aid in treating effluents. This digester comprises five different live spore-forming bacteria. When introduced to various waste sources, these bacteria produce various enzymes, such as Protease (protein), Amylase (starch & carbohydrates), Lipase (fat & grease), Esterase (fat), Cellulase (cellulose, wood, paper), Xylanase (plant material), and Urease (urea). While capable of producing all these enzymes, the bacteria generate only as many as needed to complete the digestion of the waste source. This bacterium functions optimally within a pH range–of 5 - 9 and a temperature range of 12°C - 35°C. One of the notable advantages of employing this bacterium is its rapid activation, occurring within an hour of introduction to the waste source.

3.3. Operating conditions

3.3.1. Pre-treatment and sample preparation

Before initiating the pre-treatment stage for the lab-scale plant, a batch test with Momar was conducted to determine the optimal operational parameters. The batch test considered parameters such as temperature, enzyme dosage, and HRT. Temperatures of 23°C, 30°C, and 37°C were tested, along with enzyme dosages of 10ml/L, 30ml/L, and 50ml/L. HRT variations included 12hr, 24hr, and 36hr. These parameters were inputted into the Design Expert software. See Appendix A for full table of runs. Upon executing the experiments suggested by Design Expert, the optimum conditions were identified and applied in the lab-scale plant.

The pre-treatment stage in the lab-scale plant involved a 20L bucket, a magnetic stirrer, and heating elements. The raw DWW was treated with Momar and allowed to undergo the treatment process. After treatment, it was transferred to the feed tank, where a 0.45 µm sieve was employed to screen the DWW and remove solids post pre-treatment. Subsequently, it was fed into the DEGBR through a peristaltic pump. The primary focus of the pre-treatment stage was the removal of FOGs, given the high FOG content in the DWW, which had the potential to cause reactor clogging if not reduced.

Figure 3.5 | Pre-treatment tank setup

Figure 3.6 | Adding PT DWW to Feed tank

3.3.2. DEGBR setup, operation, and inoculation

The DEGBR setup is illustrated in Figure 3.8, constructed from PVC with dimensions of 0.62 m in height, an internal diameter of 0.08 m, an external diameter of 0.1 m, and a volume of 2.2 L. Surrounding the DEGBR is a water jacket, securely sealed with nuts and bolts. At the bottom of the reactor, there is a stainless-steel mesh with a pore size of 2 mm. Activated sludge (AS) was obtained from the SAB Brewery in Newlands, Cape Town, in liquid form and stored in a water bath to ensure the sludge's viability.

Above this sieve, a material with a smaller pore size than the metal sieve is positioned to aid in the filtration process of the DWW. Pumice stones, displayed in Figure 3.7, are utilized to assist in sludge retention, promoting bacterial growth. Three handfuls of these stones are placed in the reactor. To mitigate clogging within the reactor, a recycle stream is connected to the side of the reactor. The stones have an average porosity of 0.66 (Njoya, Basitere & Ntwampe, 2019). contributing to the overall efficiency of the system.

The reactor inoculation began by filling one-third of the reactor with AS (0.73L), while the remaining portion was filled with DWW (1.47L, operating volume). Unlike previous studies on PSW that included milk powder to aid bacterial growth, the use of DWW eliminates the need for this additive. A water bath circulated water through the water jacket, maintaining an average temperature of 39 ± 2°C. This served to uphold mesophilic conditions and ensure the viability of the sludge. The inoculation period extended for 30 days, allowing the microbes to acclimatize. Throughout this period, the pre-treatment continued to be fed into the reactor using a peristaltic pump with an inflow and outflow of 0.15 RPM which was a flow rate of 5.34l/d. The DEGBR operated continuously for 14 weeks (98 days), excluding the inoculation phase. It ran at three different HRTs during the study, showcasing its versatility and efficiency in treating dairy wastewater.

Figure 3.7 | Pumice stones

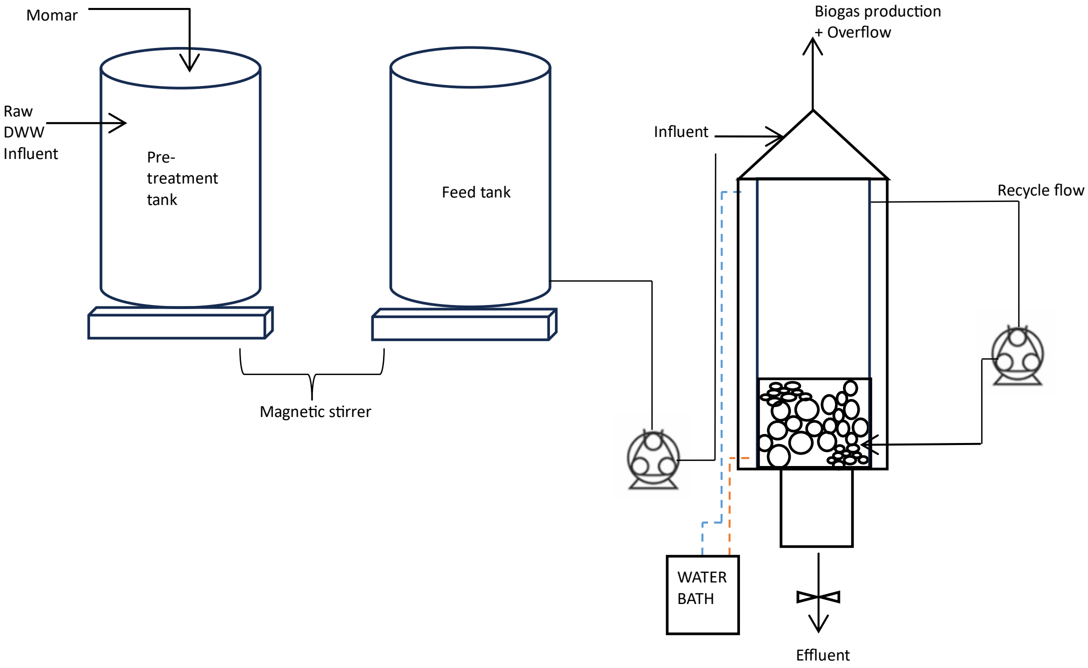


Figure 3.8 | Schematic of experimental setup

3.3.3. Sampling and Analysis

Throughout this experiment, samples were collected from various stages, including the raw DWW, the pre-treated DWW (Influent), and the effluent of the DEGBR. A volume of 1L was taken at each stage for analysis. Sampling occurred three times a week, and once a week, the collected samples were mixed to obtain the average of the three sampling days. A 1L sample was then sent to Bemlab for FOG analysis.

The collected samples underwent testing for COD, FOG, TSS, and pH. While the lab conducted the COD, TSS, and pH testing, the FOG analysis took place at Bemlab, as the lab did not possess the necessary equipment for FOG testing. This comprehensive testing regimen ensured a thorough analysis of the wastewater at different stages of the treatment process.

Figure 3.9 | 1L sampling containers

Table 3.1 | Summary of sampling points and tests conducted at each point

Sampling point	Tests		
	COD	TSS	FOG
Raw	X	X	X
Pre-treatment	X	X	X
Effluent	X	X	X

X = Unfiltered
COD = Chemical Oxygen Demand
TSS = Total Suspended Solids
FOG = Fats, Oils and Greases

Figure 3.10 | Multiparameter for pH testing

COD testing was conducted by obtaining a 10 ml sample of the wastewater at each stage. Subsequently, the sample was diluted 100 times with deionized water due to the high COD of the wastewater, making it challenging to detect using the lab equipment. After dilution, 10 ml of the wastewater was transferred to boiling tubes, where it was mixed with 5 ml of potassium dichromate and 15 ml of H_2SO_4. The mixture underwent thorough mixing using a vibration mechanism and was then transferred to a digestor set at 180 ± 10°C for 2 hours.

Upon completion of the digestion process, the sample was placed in a cool bath for 10 minutes and then transferred into conical flasks containing two drops of a ferroin indicator. Subsequently, it underwent titration with ferrous ammonium sulphate (FAS) completes the COD testing process. This detailed procedure ensures accurate and reliable measurements of the COD in the wastewater samples at each stage of the treatment process.

Figure 3.11 | Potassium dichloride and H_2SO_4

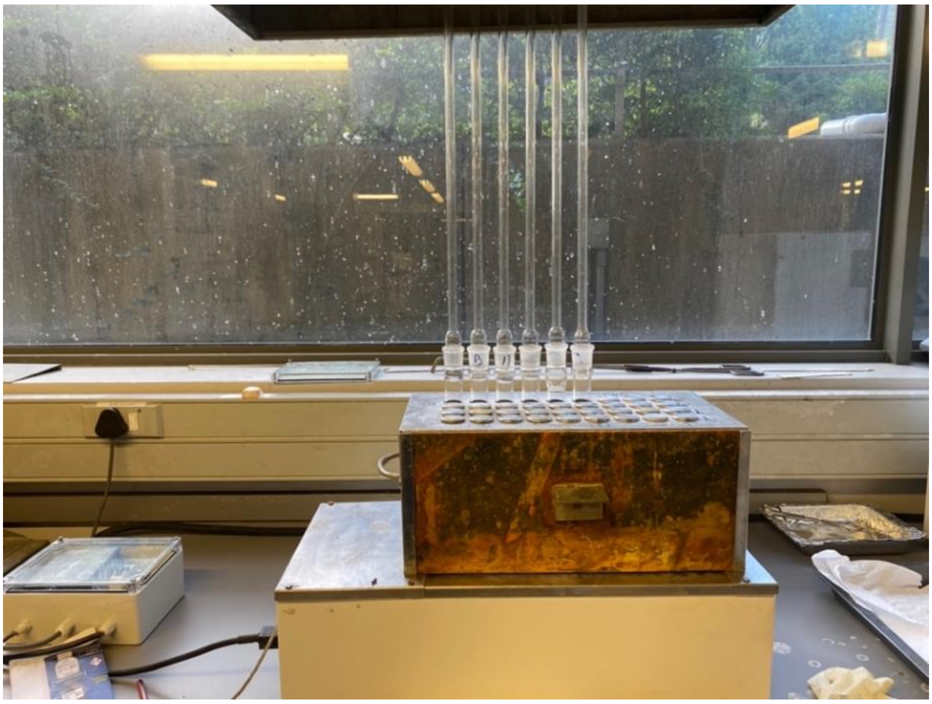


Figure 3.12 | Boiling tubes in digestor

TSS testing involves an initial step of weighing the crucibles on a scale. Following this, 40 ml of 10x diluted wastewater is added to these crucibles and transferred to an oven set at 105°C for a period of 24 hours. After this duration, the crucibles are placed into a desiccator to cool down, and they are then weighed once again. This meticulous process ensures accurate measurements of TSS in the wastewater samples, providing valuable information about the particulate matter present in the tested samples.

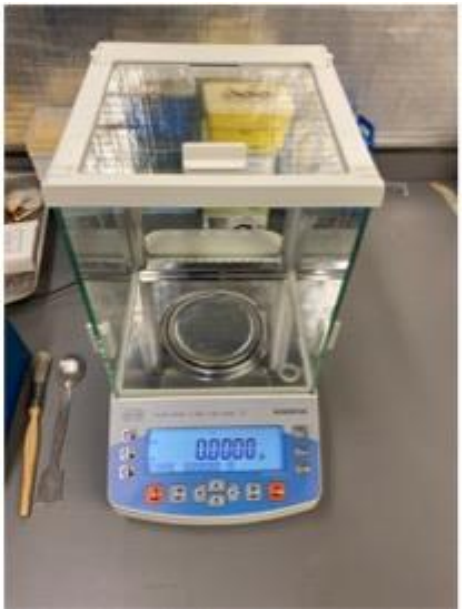


Figure 3.13 | Scale for weighing crucible

Figure 3.14 | Crucibles with DWW

Figure 3.15 | Oven for TSS testing

This chapter illustrated the methods and equipment that will be used in this investigation. All tests conducted besides the FOG testing will be done within the lab. The following chapter will depict the results as well as discuss the results that was achieved from the pre-treatment stage and in the lab scale plant.

CHAPTER 4: RESULTS AND DISCUSSIONS

Part of this chapter will be submitted for publication.

4.1.Introduction

The current population of South Africa is 61.02 million and is projected to increase by 0.95% each year. It is estimated that the population in 2030 will reach 64.7 million ("*South Africa population (live)"*. n.d). With this ongoing population growth, the demand for water is expected to rise as well, estimated to increase by 17.7 billion m^3 in 2030. However, the water supply is projected to reach only 15 billion m^3 by 2030 (*South Africa* (2022) *2030 Water Resources Group*, n.d.). This indicates that water is being utilized at a faster rate than it can be supplied.

One of the major contributors to freshwater consumption in South Africa is the agricultural sector, which includes the meat and dairy industry. The dairy industry, in particular, consumes a substantial amount of fresh water, ranging between 1 and 10 m^3 of water per m^3 of processed milk (Stasinakis, Charalambous & Vyrides, 2022). During the processing of dairy products, several parameters, including pH, BOD, COD, TSS, TN, TP, and FOG, undergo significant alterations in the effluent produced. BOD and COD levels, in particular, are notably high (Onet, 2010; Joshiba et al., 2019).

According to Chaudhary et al., (2023) there is an annual release of 4-11 million tons of dairy waste into the environment, posing a threat to aquatic and land biodiversity due to its high organic content. This elevated organic content has the potential to decrease dissolved oxygen levels in water bodies, posing a serious risk to aquatic life (Mendes et al., 2010). Furthermore, these contaminated water bodies become breeding grounds for insects and are susceptible to diseases such as yellow fever and malaria (Ahmad et al., 2019).

The wastewater, with its high concentration of nutrients like nitrogen and phosphorus, contributes to an excess of nutrients in water bodies. This excess can lead to eutrophication, promoting the growth of algae and aquatic plants (Henze et al., 2019). This process has significant implications for the ecological balance of aquatic ecosystems.

DWW is classified as high-fat-content wastewater, containing a significant amount of FOGs within the produced wastewater. This elevated FOG content can lead to substantial issues within the central treatment system. The potential impacts of untreated raw influent wastewater with high FOGs include blockages within the system, resulting in malfunctions or even damage to the equipment. Therefore, it is essential to pre-treat high-fat-content wastewater before any biological treatment.

As noted by Bella & Rao, (2023), pre-treatment not only reduces the FOG content but also enhances solubilization, improves the digestion rate, and decreases the organic content of the raw influent. Biological treatment of high-fat-content wastewater, such as using an anaerobic digester, proves effective in reducing substrates and organic content. However, the presence of FOGs poses numerous challenges, as mentioned earlier, necessitating assistance from a pre-treatment stage to enhance the treatment of the raw influent (Mobarak-Qamsari et al., 2012; Harris & McCabe, 2015).

An essential factor that can contribute to the treatment of DWW involves the use of an enzyme known as Momar during the pre-treatment stage. This is followed by the implementation of the DEGBR. The pre-treatment stage is crucial for breaking down contaminants, including FOG and organic content.

Similar studies were conducted using this lab-scale plant for the treatment of Poultry Slaughterhouse Wastewater (PSW) with a bioremediation agent, Eco-Flush. However, issues arose with Eco-Flush as it increased the DO levels in the wastewater. Consequently, a new enzyme is under investigation to address this issue and enhance the effectiveness of the treatment process.

The intention of this study is to assess the efficacy of an integrated system that treats DWW by using a DEGBR in conjunction with a biological pre-treatment to achieve high removal efficiencies of FOG, TSS, and COD. To accomplish this goal, the subsequent objectives were created.

4.1.1. Objectives

- Optimize the reduction of FOGs in the DWW has it has a high fat content.
- Determine the performance of the pre-treatment stage with respect to TSS, COD and FOG removal efficiency with various HRTs, temperature and enzyme dosages.
- Determine the performance of the DEGBR in terms of FOG, COD and TSS removal efficiency at a set HRT and organic loading rate (OLR).

- Determine the overall system (PT – DEGBR) performance in terms of pollutant removal.

4.2. Pre-treatment (PT) Stage Performance

The purpose of this stage was to determine the optimal conditions for running Momar, as it had not been used previously as a pre-treatment for wastewater. Given the high fat content in DWW, the primary objective was to reduce the FOG content. This was crucial due to the significant problem of clogging in the AD. Previous studies on the same lab-scale plant, albeit with PSW, experienced clogging despite a reduction in FOG content.

During the pre-treatment stage, measurements of pH and DO were taken. Previous studies highlighted DO as a major concern when using Eco-flush as the pre-treatment enzyme. Therefore, one of the key observations with Momar was the monitoring of DO levels. Over 26 samples, the average pH of the influent was 4.22, whereas the pH of the effluent was 3.89. The pH indicated that the raw wastewater (influent) was acidic to begin with, and this was due to the addition of acidic chemicals during the cleaning of the manufacturing equipment before production, altering the raw DWW's pH level. The pre-treatment (PT) wastewater (effluent) became more acidic after the addition of Momar, this could be due to Momar which was reducing the pH of the wastewater or due to lactic acid formation (i.e. fermentation occurring). This comprehensive evaluation aimed to address potential clogging issues and optimize the pre-treatment conditions for Momar in handling high-fat-content wastewater. The DO levels can be seen in Figure 4.1, and it indicated an average 42% decrease in DO levels. The majority of the DO levels leaving with the effluent (DO out) are lower than what it began with (DO in), showing that Momar is reducing the DO level of the wastewater, a key observation when considering an alternative enzyme to Eco-flush. In cases where the DO increased, this was due to the sampling of the effluent; when sampling into containers, there is an addition of oxygen during that period, thus increasing the DO.

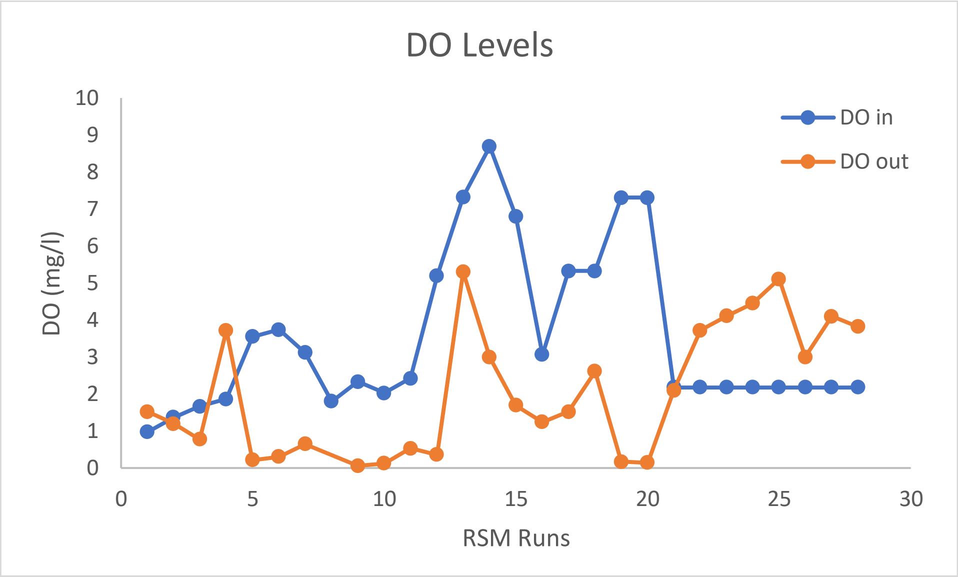
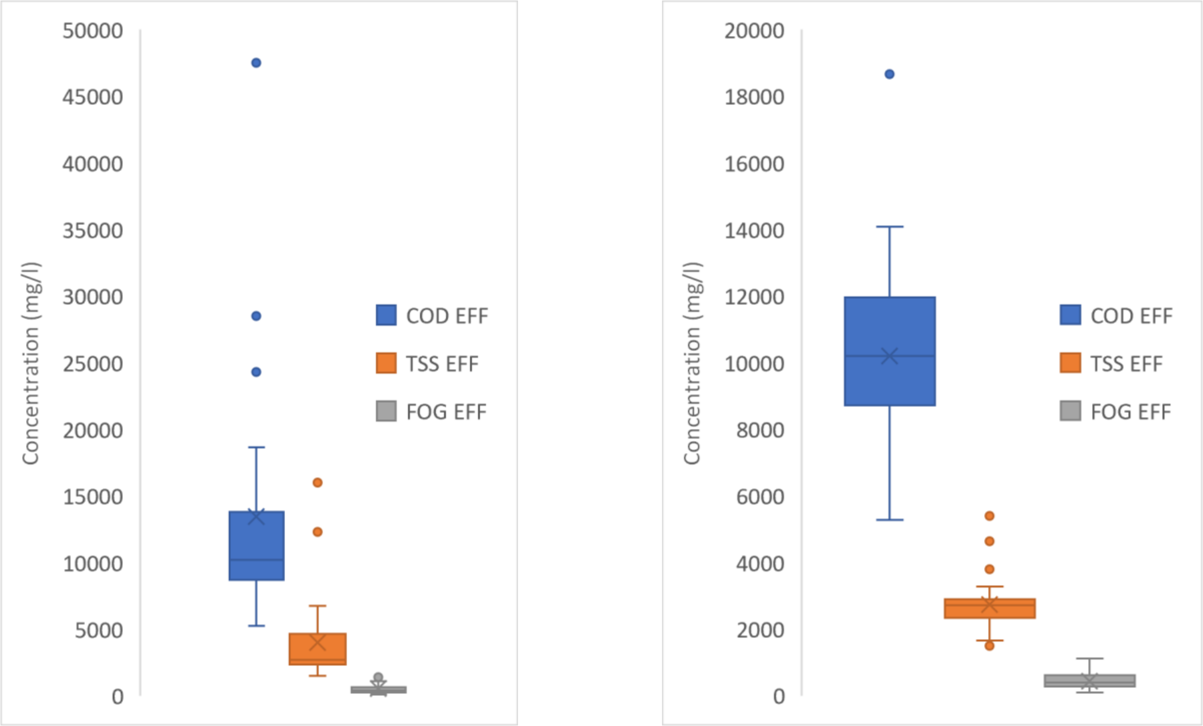


Figure 4.1 | DO comparison

To assess and interpret the performance distribution of the pre-treatment, it was necessary to identify outliers. Figure 4.2 presents the boxplot (a) before outlier replacement and (b) after outlier replacement. To rectify the distribution for a more homogeneous dataset and eliminate noise, various data processing methods, such as the standard scalar, Z-score, or the interquartile rule, can be employed. In this paper, the interquartile rule was used to identify and replace outliers, considering the size of the dataset.

From these diagrams, it is evident that the distribution of data points aligned well, with outliers being replaced by the median value for each observed parameter. The occurrence of outliers in this experiment could be attributed to variations in the raw wastewater used on specific days. During collection periods, the DWW did not consistently exhibit a milky color, and some 25L containers might have stored wastewater with cleaning chemicals or a higher proportion of freshwater than wastewater. Adjusting these outliers facilitates the interpretation of data and enhances the assessment of outcomes at each stage of DWW treatment.



a) Boxplot before outlier replacement

b) Boxplot after outlier replacement

Figure 4.2 | Boxplots of the investigated parameters in the pre-treatment stage

Figures 4.3 and 4.4 depict the performance of COD, TSS, and FOG, with the former illustrating the performance before outlier replacement and the latter after outlier replacement. A comparison of the two graphs reveals a noticeable improvement in the graphical representation of the observed parameters. The replacement of outliers has resulted in a smoother presentation, rectifying any skewing that was previously present in the graphs. This adjustment enhances the clarity and accuracy of the performance assessment for COD, TSS, and FOG.

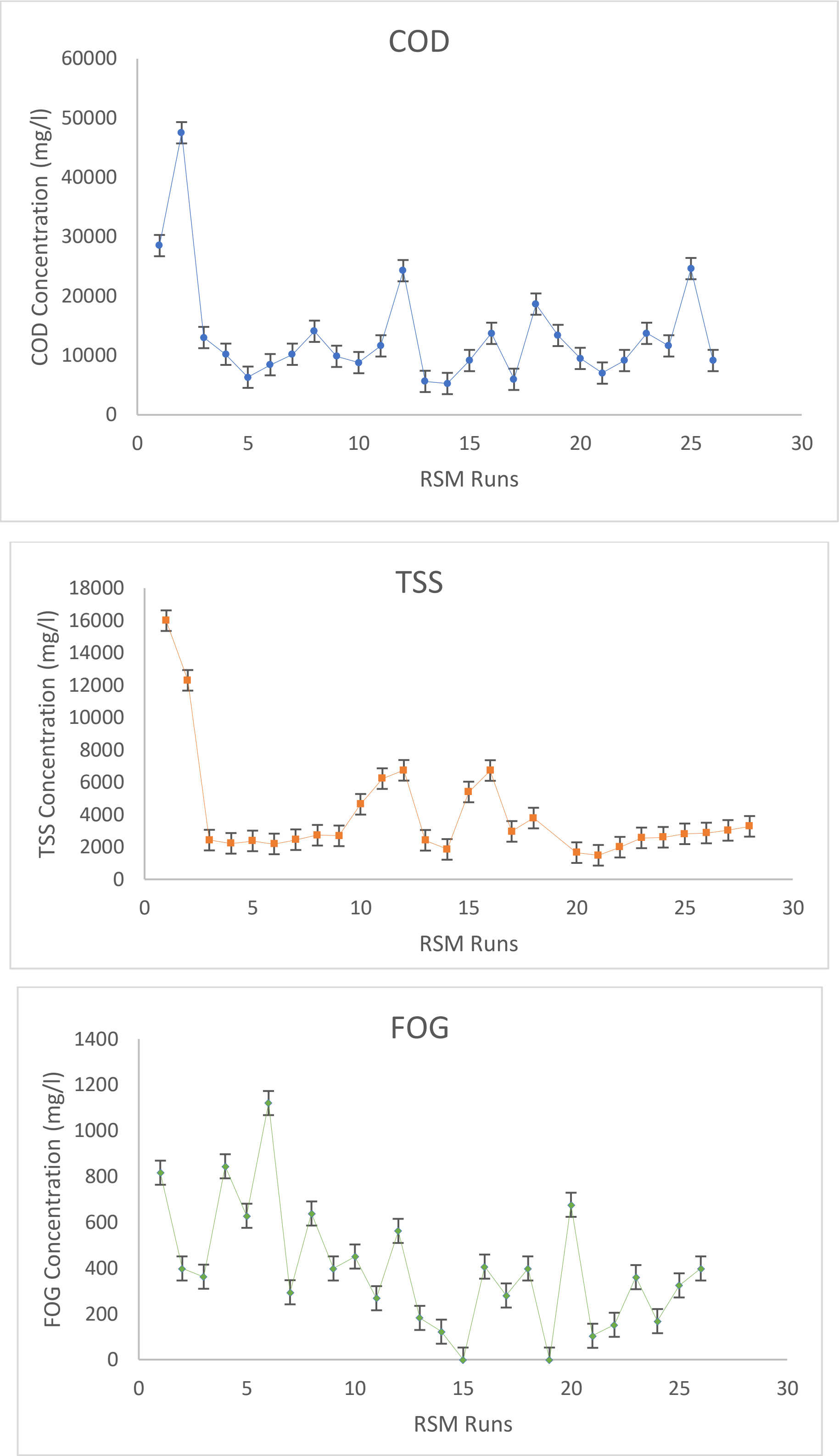


Figure 4.3 | Performance of the pre-treatment before outlier replacement with respect to COD, TSS and FOG.

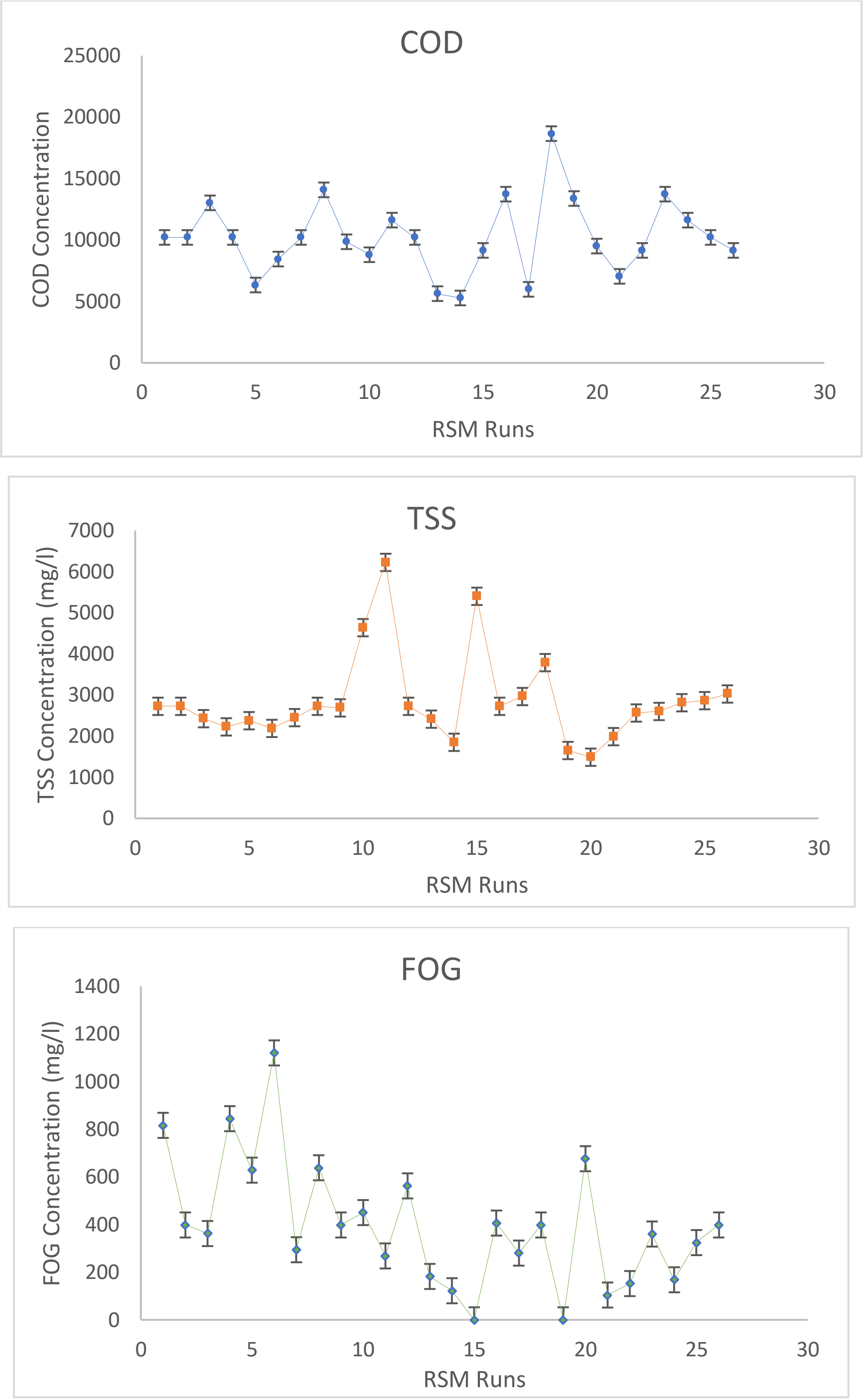


Figure 4.4 | Performance of pre-treatment after outlier replacement with respect to COD, TSS and FOG.

The comparison graphs for the influent and effluent were created using a baseline mark set at the influent, where the average of the raw samples was taken to establish a baseline. This approach facilitated the identification of effective and ineffective runs. Figures 4.5, 4.7, and 4.10 illustrate the comparison between the influent and effluent, also showcasing the corresponding removal efficiencies. Detailed information on the runs can be found in Table 4.1. This method of comparison provides a clear visual representation of the treatment efficiency for each parameter, aiding in the assessment of the overall effectiveness of the system.

Table 4.1 | Detailed RSM runs for the pre-treatment stage

Runs	Time (hr)	Dosage (ml)	Temp(°C)
1	24	90	22
2	24	120	22
3	24	150	22
4	24	90	24
5	24	30	38
6	12	30	38
7	36	90	30
8	36	150	30
9	24	30	22
10	24	30	30
11	36	30	22
12	12	90	38
13	24	150	30
14	12	90	22
15	36	30	38
16	12	150	22
17	12	90	30
18	12	150	30
19	12	30	30
20	12	150	38
21	12	30	22
22	12	150	38
23	24	150	30
24	24	150	38
25	36	90	38
26	36	30	22

FOG Removal

All tested runs returned FOG levels below the baseline (marked in orange), signifying the enzyme's effective reduction of FOG content. FOG removal varied from 43% to 93%, with the highest removal observed at run 21. The average removal efficiency reached 70%. However, operational issues during runs 15 and 19 resulted in zero values for FOG removal, highlighting specific challenges encountered during these runs. Despite these exceptions, the overall trend indicates successful FOG reduction in most of the experimental runs.

Figure 4.6 illustrates the effects of each variable changed on FOG. These graphical depictions showcase which changes have the most significant impact on FOG by using the line of best fit. At an HRT of 12 hours, the correlation between FOG and HRT is 0.71, the highest among the three HRTs. This suggests a substantial reduction in FOG at an HRT of 12 hours compared to 36 hours, where the correlation is only 0.22. This demonstrates that at lower HRTs, the reduction in FOG is most pronounced. At a temperature of 38°C, the FOG reduction is the greatest, with a strongly positive correlation of 0.97, while the lowest FOG reduction occurs at 22°C. This indicates that at higher temperatures, the fats within the DWW tend to break up and can be more effectively removed from the wastewater. At an enzyme dosage of 10ml, the highest correlation of 0.69 is observed, suggesting that lower enzyme dosages of Momar have a positive effect on FOG reduction. In conclusion, for optimal results in reducing FOG, the recommended operating conditions are an enzyme dosage of 10ml/L, an HRT of 12 hours, and a temperature of 38°C.

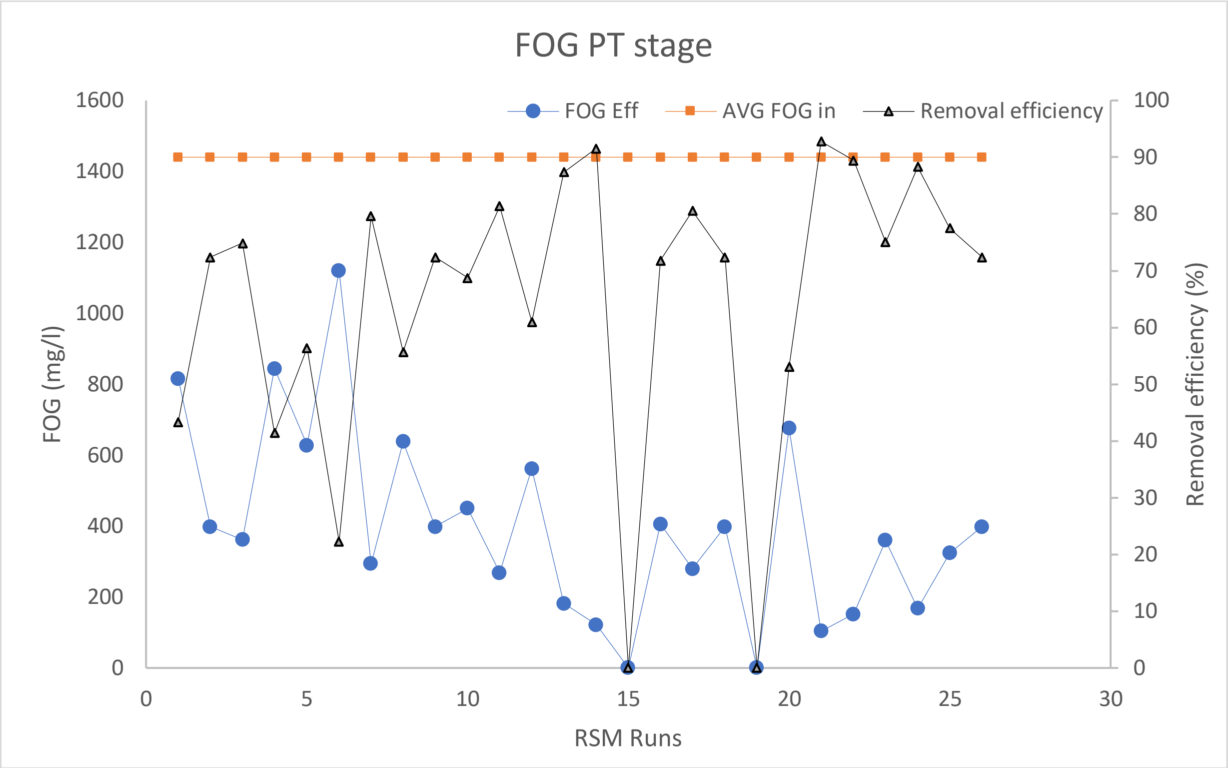
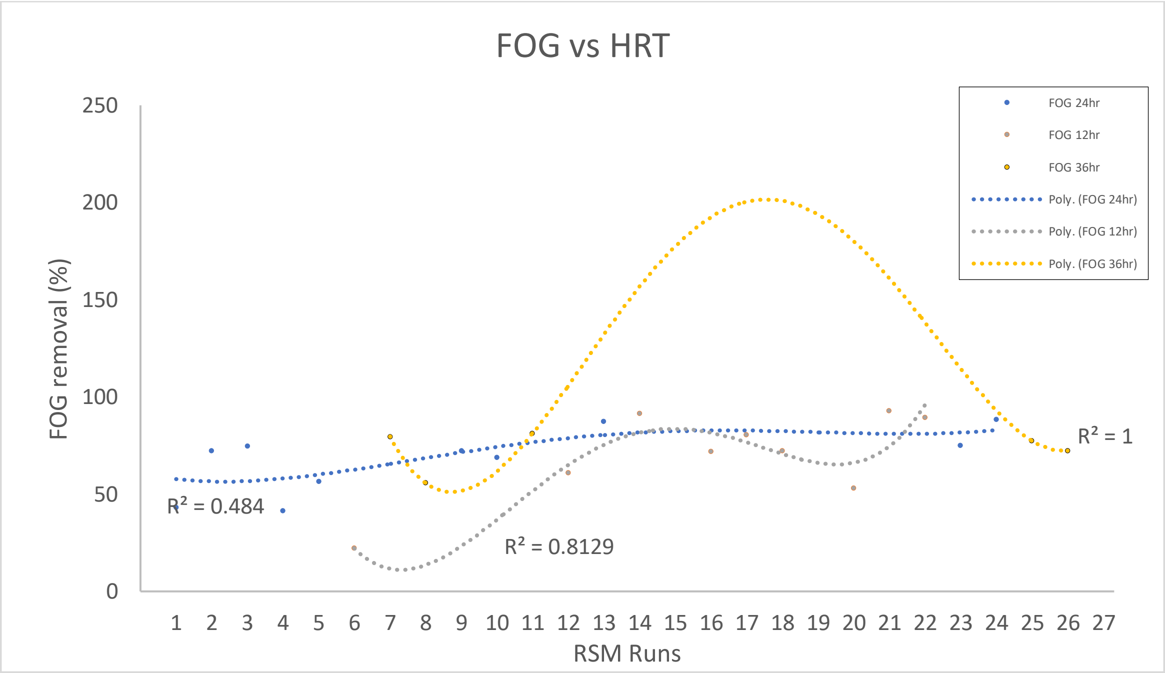


Figure 4.5 | FOG comparison graph



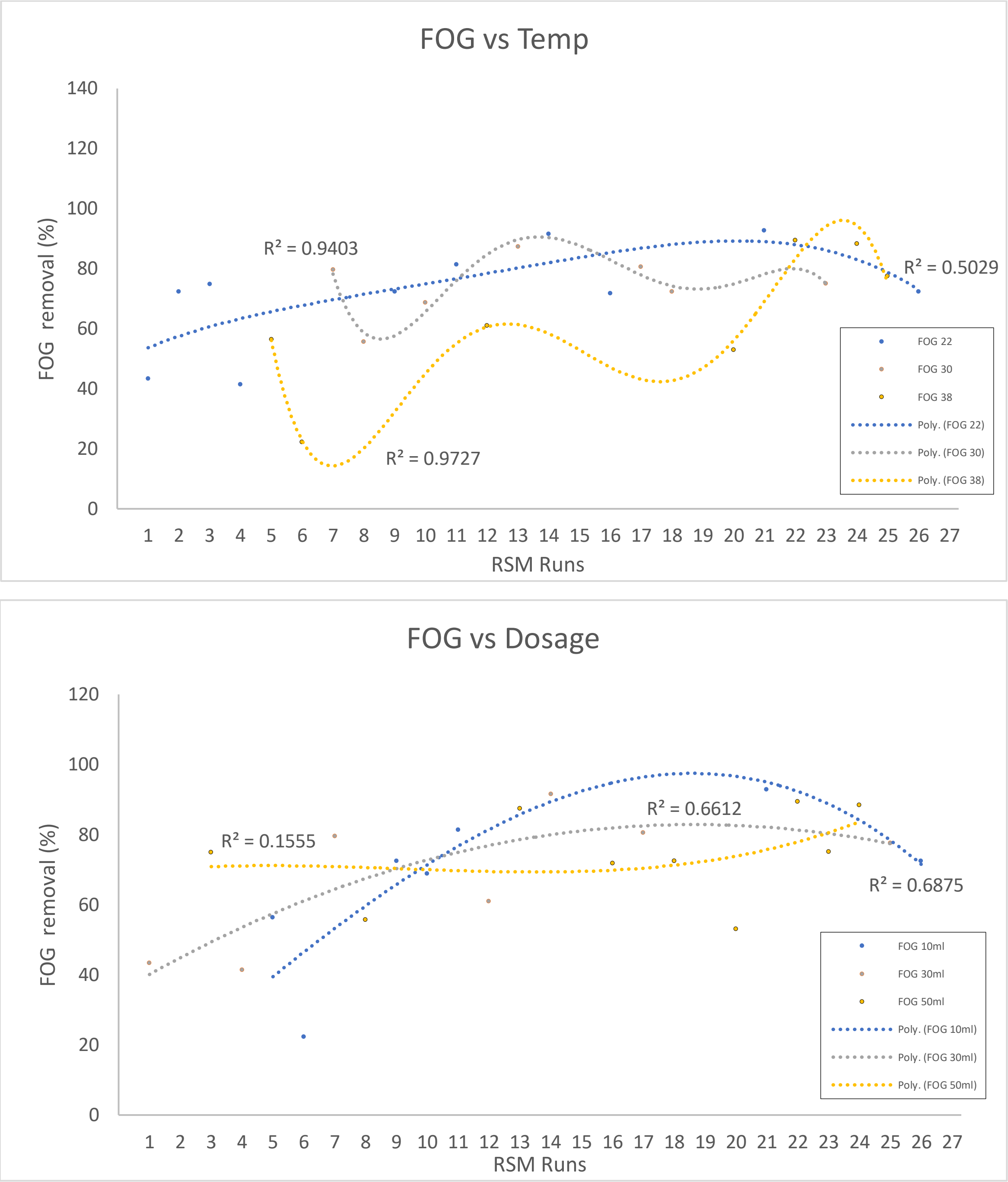


Figure 4.6 | Effect of HRT, temperature, and dosage on FOG

COD Removal

In Figure 4.7, it is easy to discern the runs that effectively reduced COD; those below the baseline (marked in orange) indicate runs with a lower COD than the influent. The average removal efficiency for COD was 16%. The highest removal percentage occurred at run 14, reaching 49%. Negative percentages were generated due to additional COD introduced to the system by Momar. The overdosing of the enzyme contributed to COD, resulting in these negative values. A 1L sample of Momar tested at Bemlab yielded a COD value of 1270 mg/L and a TP value of 1.4 mg/L. Observations made from this was that when using Momar, the correct amount to dose is vital for the removal of COD because if overdosed it will add more COD to system. A potential reason behind this is that due to the various bacterium within the enzyme the bacteria that is not contributing to the removal of COD, i.e. dissolving the organic content, is being left as residue and escaping with the effluent. In Figure 4.9 a COD mass balance can be seen with a 2L sample. Runs with negative percentages were promptly flagged, indicating the need to exclude them from the process of determining optimum conditions. This scrutiny ensures the reliability of the data and the accuracy of the assessment of COD removal efficiency.

In Figure 4.8, a graphical comparison among various parameters concerning COD is presented. Upon analysing the data and constructing the line of best fit, the following observations can be made. The greatest COD reduction occurs at a high HRT, specifically 36 hours, as confirmed by a robust positive correlation of 0.78. This indicates that a more extended treatment duration allows the bacterium within the enzyme to effectively break down and reduce the COD content of the raw wastewater. At a temperature of 38°C, it was established that COD reduction is most significant, showing a correlation of 0.88. This suggests that COD exhibits a higher removal rate at elevated temperatures compared to lower ones. Regarding enzyme dosage, there was a similar correlation for 50ml/L and 10ml/L, which were 0.58 and 0.55, respectively, whereas at a dosage of 30ml/L, there was a correlation of 0.8. For optimum COD removal, the recommended conditions are an enzyme dosage of 30ml/L at an HRT of 36 hours and at a temperature of 38°C.

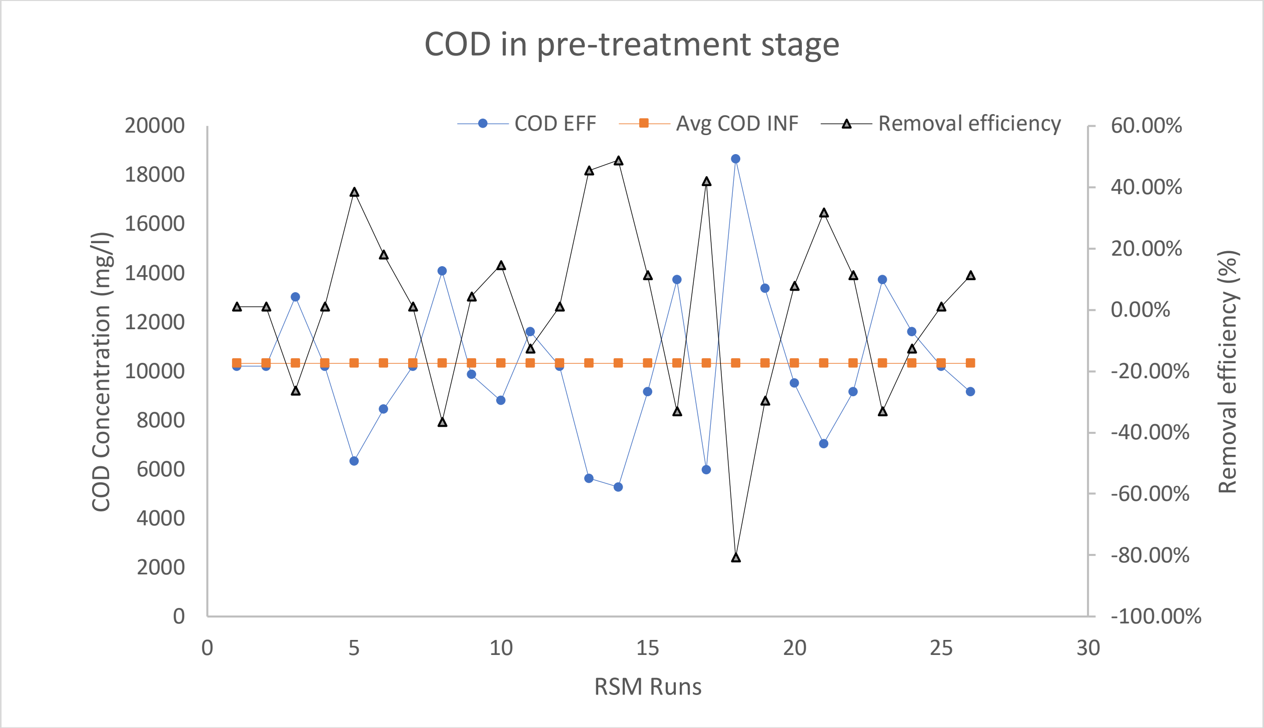
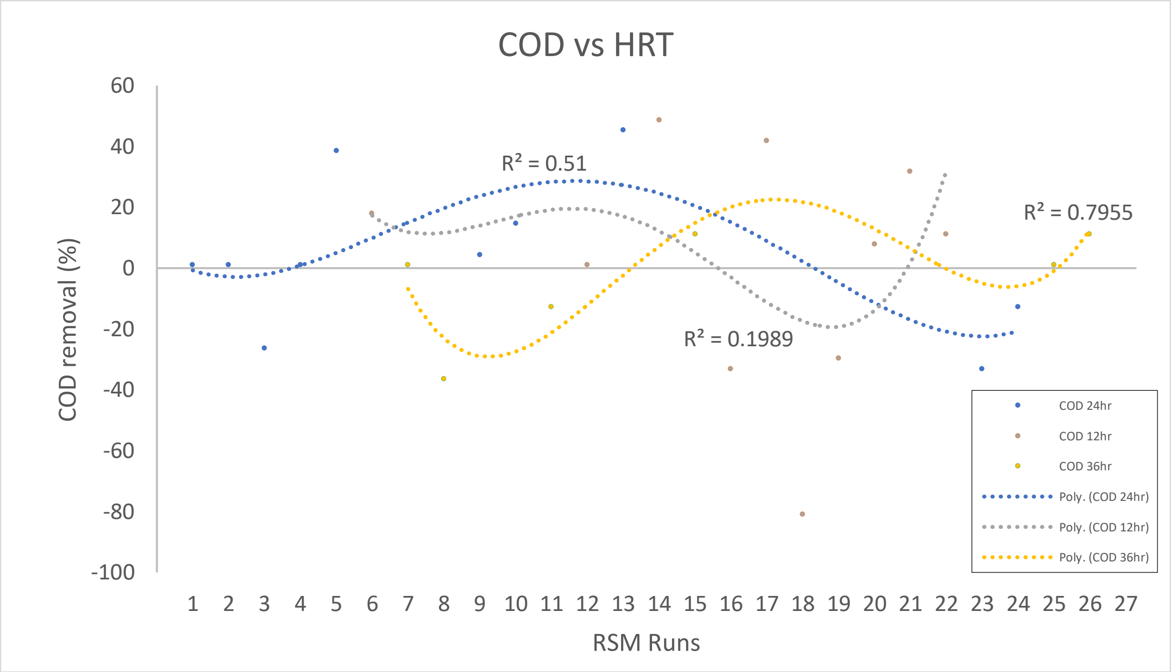


Figure 4.7 | COD comparison graph



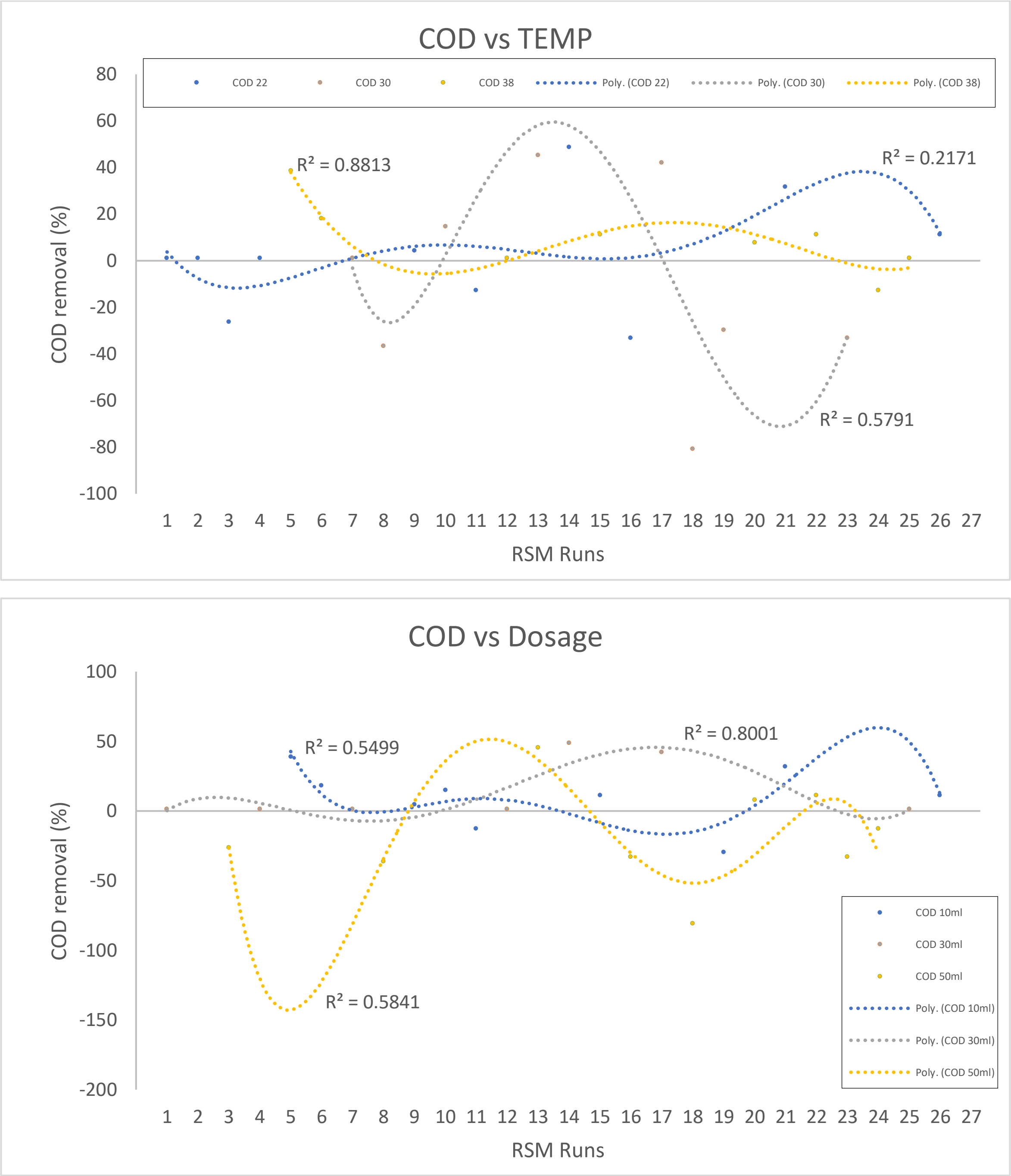


Figure 4.8 | Effect of HRT, temperature, and dosage on COD

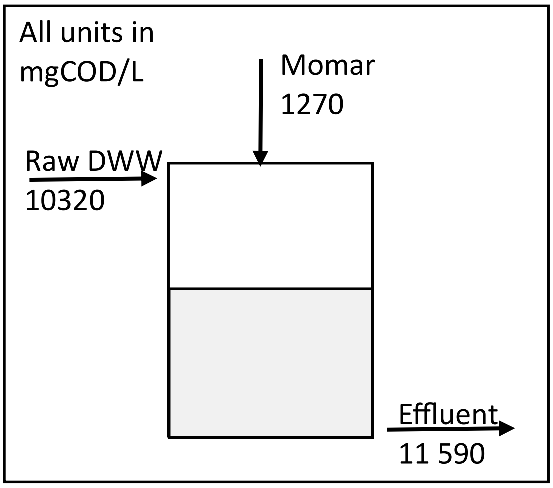


Figure 4.9 | Example of a 2L sample COD balance for pre-treatment with Momar

TSS Removal

The average removal efficiency for TSS was 20%. The highest TSS removal efficiency was achieved at run 20, reaching 85%. In instances where negative removal efficiencies were observed, potential factors contributing to this outcome could include variations in the wastewater composition for that run, overdosing of the enzyme, an extended HRT, and/or the temperature conditions during that specific run. These factors highlight the need for a thorough examination of the experimental conditions to understand the variations in TSS removal efficiencies across different runs.

Figure 4.11 showcases the impact of various parameters on TSS with the aid of constructing the line of best fit. TSS demonstrated the greatest removal at a high HRT of 36 hours, showing a very strong positive correlation of 1. This indicates that the longer the wastewater is allowed to be treated, the higher the removal of TSS. At 30°C, it was deemed to be the optimal temperature for the removal of TSS, showing a strong correlation of 0.83. The enzyme dosage with the highest correlation, at 10ml/L, demonstrated a correlation of 0.91. To maximize the reduction in TSS, one should explore the following conditions: an enzyme dosage of 10ml/L at an HRT of 36 hours and at a temperature of 30°C.

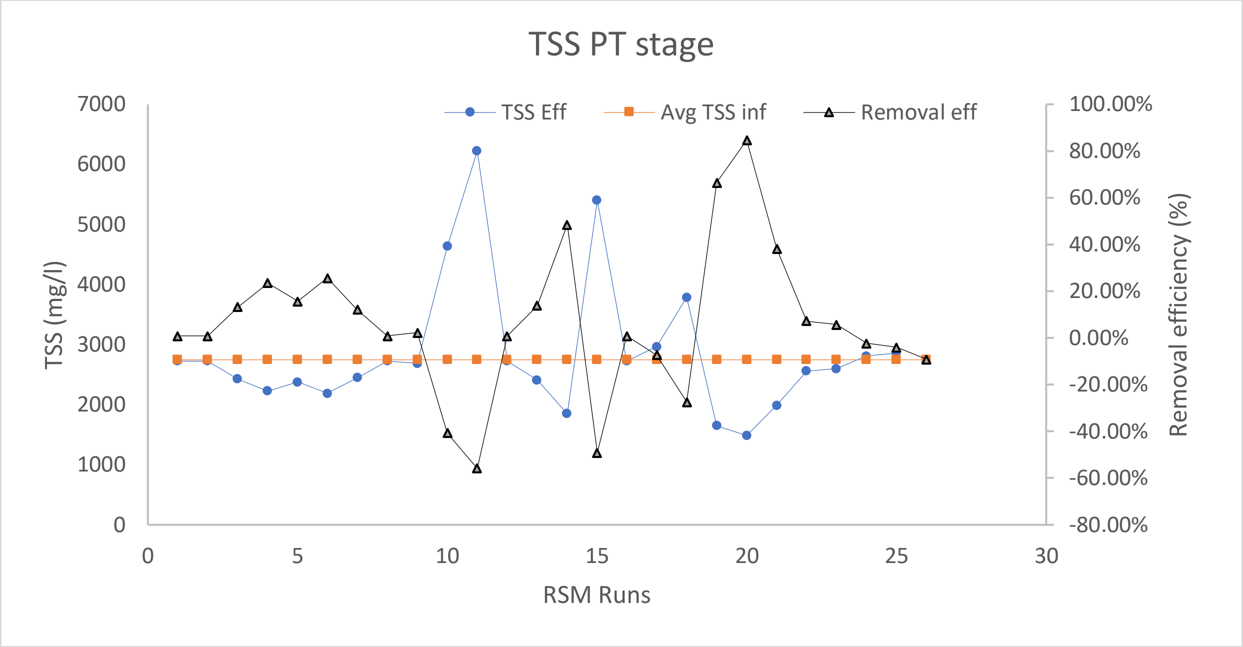
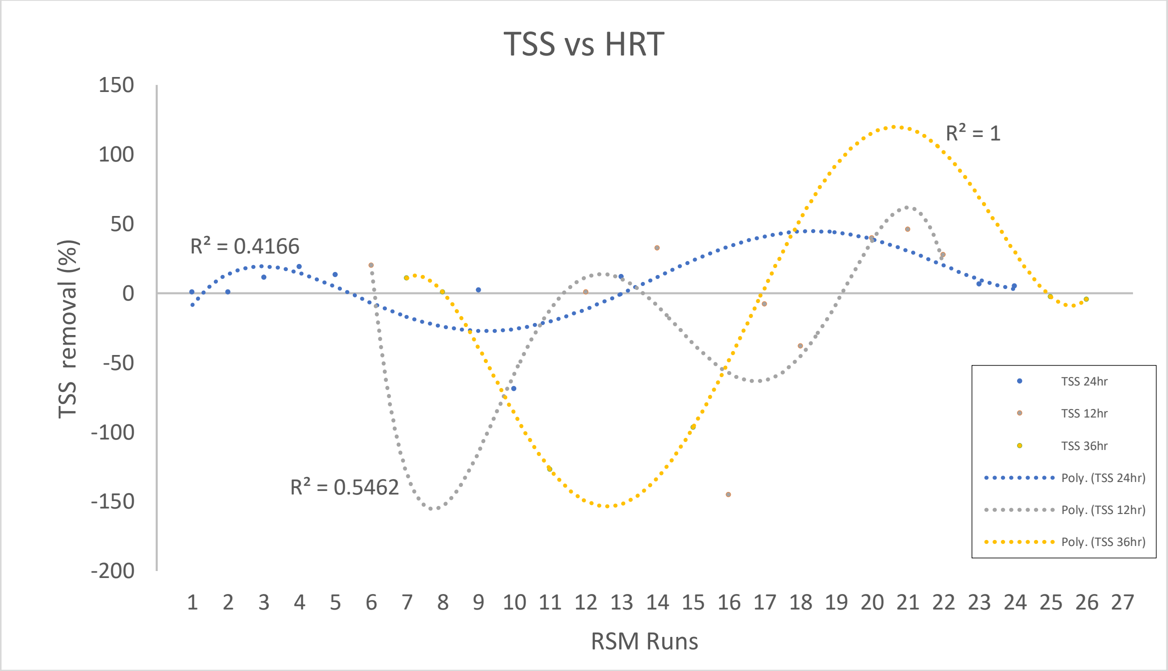


Figure 4.10 | TSS Comparison graph



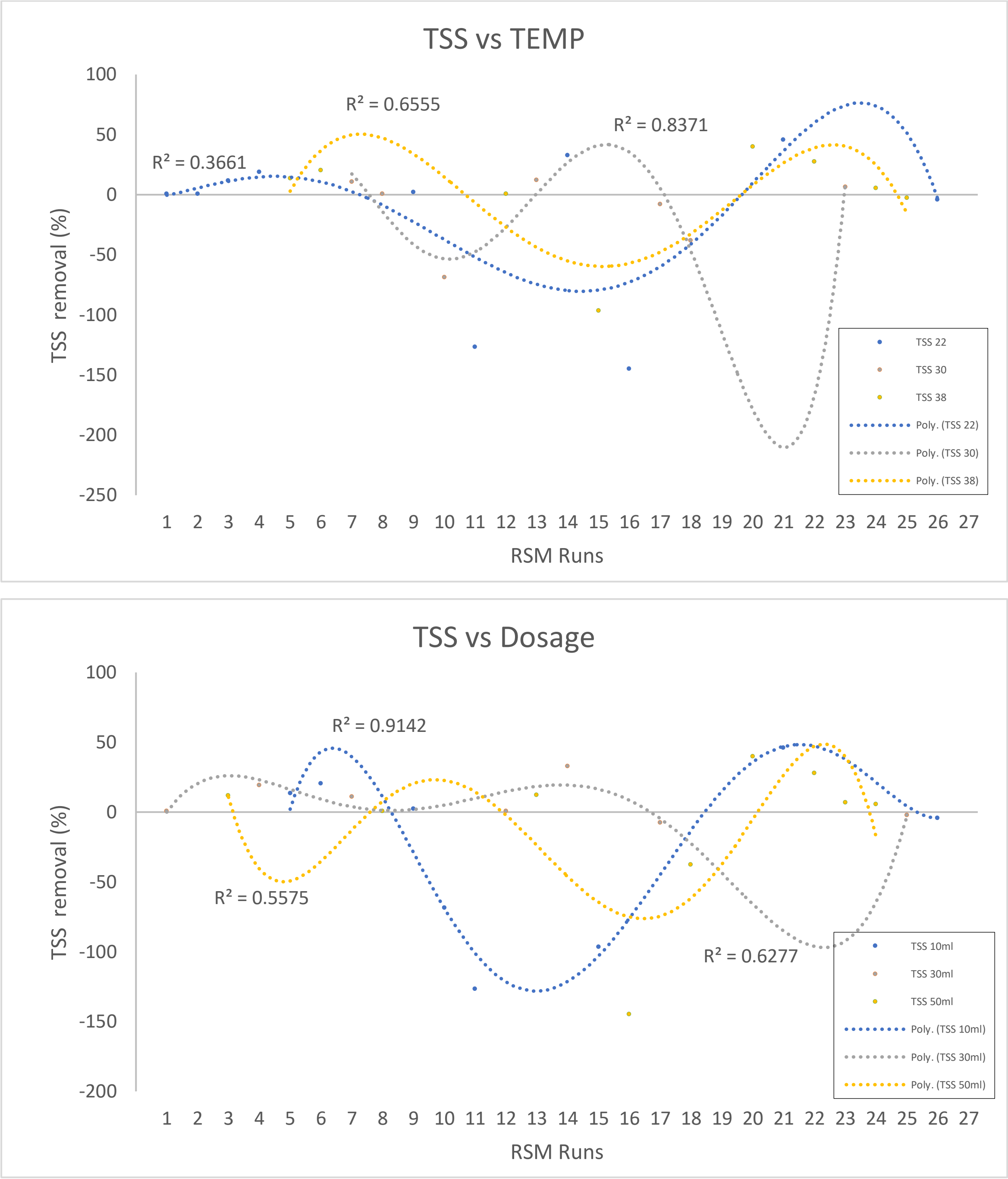


Figure 4.11 | Effect of HRT, temperature, and dosage on TSS

Optimum conditions for the removal of FOGs

To determine the optimal conditions to enter the DEGBR, Response Surface Methodology (RSM), a design software, was employed. Data for the three parameters under investigation were inputted, and graphs were generated in the software. When creating the desirability graph, FOG was assigned the highest importance due to its critical role in mitigating the risk of clogging in the DEGBR. As FOG is a significant concern in managing high-fat wastewater, it took precedence over COD and TSS. The DEGBR is designed to further reduce COD and TSS, with preventing system clogging deemed crucial.

The data for FOG, COD, and TSS is available in Appendix A. Desirability graphs for temperatures of 22 °C, 30 °C, and 38 °C are depicted in Figures 4.12, 4.13, and 4.14, respectively. These graphs assist in identifying the optimal operating conditions that prioritize FOG reduction and overall system efficiency for the DEGBR.

When determining the optimum conditions, both the desirability graphs and energy consumption were considered. From the desirability graphs, it was observed that the optimum conditions, with FOG being of the utmost importance, were 30°C, 36hr, and with a 10ml enzyme/L DWW. Even though some conditions differed when looking at the three parameters separately, energy consumption made them less compatible. At high temperatures, the heating element wore out more quickly, resulting in damage. These established conditions were used to run the pre-treatment, which was fed into the DEGBR.

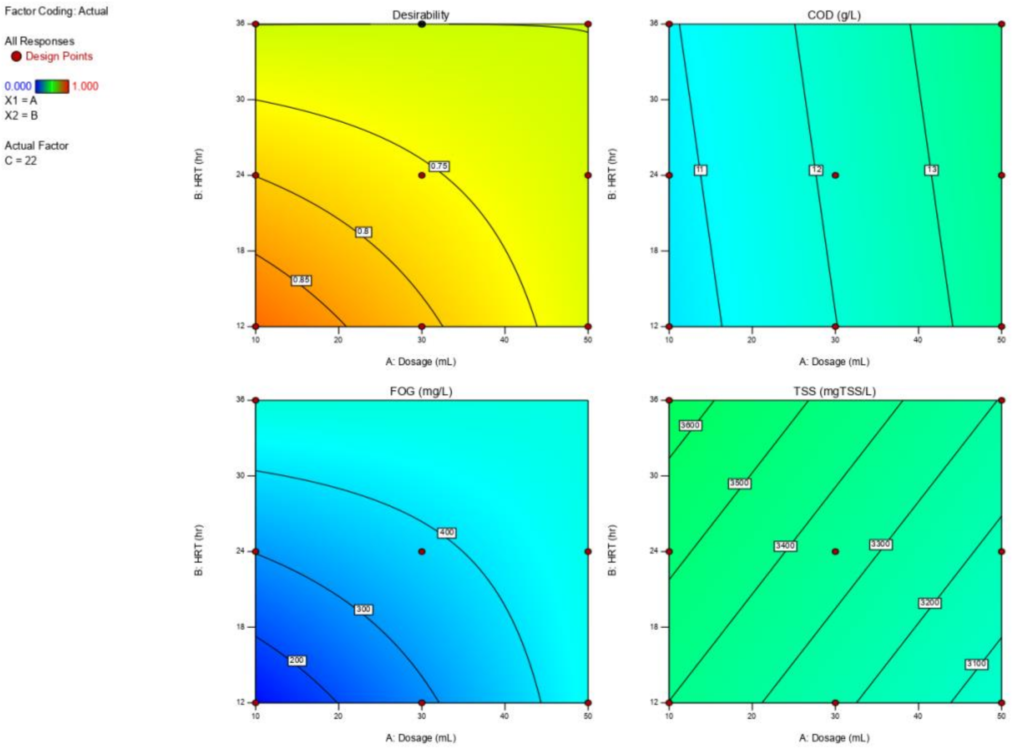


Figure 4.12 | Desirability graph at 22°C

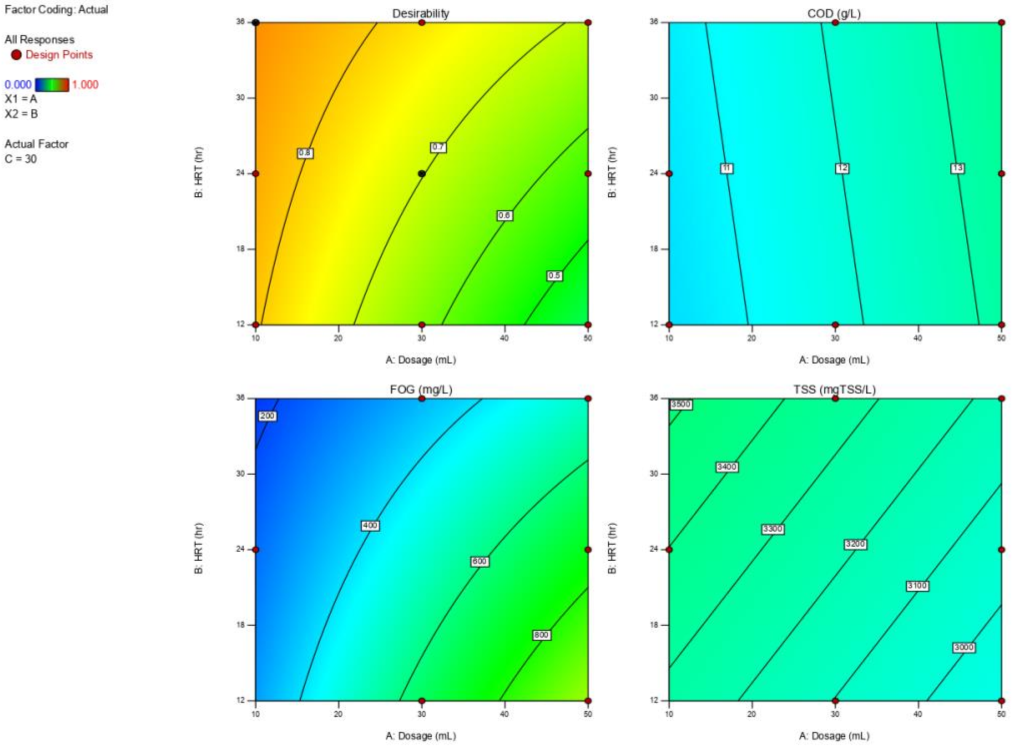


Figure 4.13 | Desirability graph at 30°C

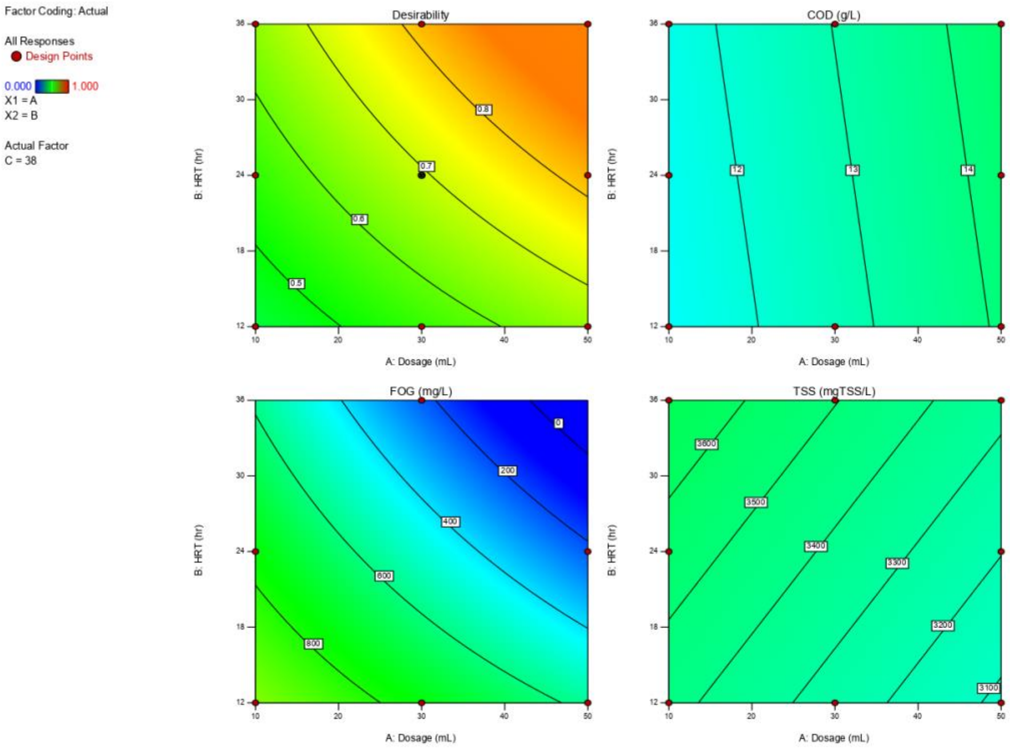


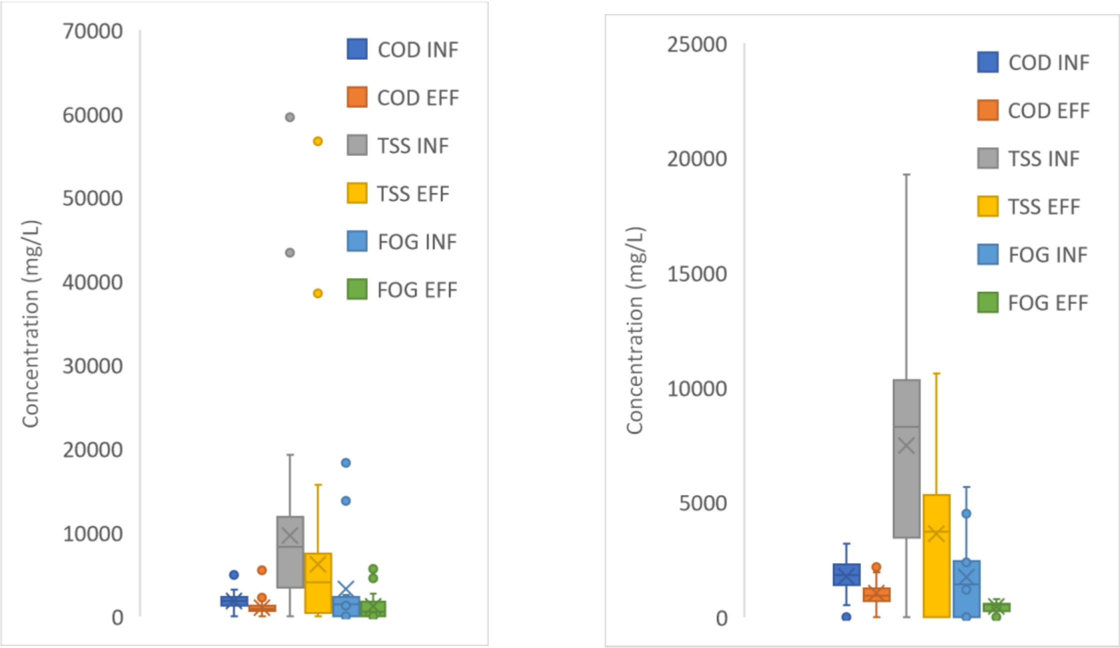
Figure 4.14 | Desirability graph at 38°C

4.3. DEGBR performance

The product from the pre-treatment stage was continuously fed into the DEGBR with the aim of reducing the concentration of contaminants in DWW. The DEGBR's performance in terms of COD, TSS, and FOG removal efficiencies was investigated across a range of organic and hydraulic loading rates and temperature conditions. The HRT was varied: the first 33 days at 36 hours, the next 33 days at 24 hours, and the last 33 days at 12 hours. Organic Loading Rates (OLR) of 0.052 gCOD/L.hr, 0.078 gCOD/L.hr, and 0.156 gCOD/L.hr were applied, while the reactor's temperature remained constant at 39 ± 2°C. The DEGBR's start-up HRT was 36 hours, ensuring continuous feeding to allow microbes to acclimatize. After the inoculation period, the system ran successfully for a week before encountering operational issues. After resolving these issues, the system ran continuously for the next 7 weeks before facing a clogging problem.

The Interquartile Rule (IQR) method identified outliers, and instead of deletion, the median rule was applied to maintain data size. Figure 4.15 (a) boxplot shows outliers in the influent and effluent COD and TSS distributions, replaced by median values in Figure 4.15 (b). This step was crucial in reducing data skewing, facilitating further analysis of DEGBR performance.

Figure 4.16 illustrates the COD, TSS, and FOG concentrations' variations over the experimental period at the DEGBR's inflow and outflow, including removal efficiencies for each observed parameter.



a) Boxplot before outlier replacement

b) Boxplot after outlier replacement

Figure 4.15 | Boxplots of the investigated parameters in the DEGBR treatment stage

Figures 4.16 and 4.17 compare graphical data illustrating the performance of the DEGBR concerning COD, TSS, and FOG. Figure 4.16 presents the data without outlier replacement, while Figure 4.17 reflects the performance after replacing outliers.

In Figure 4.17, it is evident that the overall average removal efficiencies for COD, TSS, and FOG are 39%, 49%, and 73%, respectively. The highest percentage removals for COD, TSS, and FOG are 76%, 82%, and 95%, respectively. At an HRT of 12 hours, the average removal efficiency for COD, TSS, and FOG is 48%, 43%, and 74%, respectively. These results indicate that at 12 hours, there is a similar removal rate for COD and TSS and a higher removal rate for FOG.

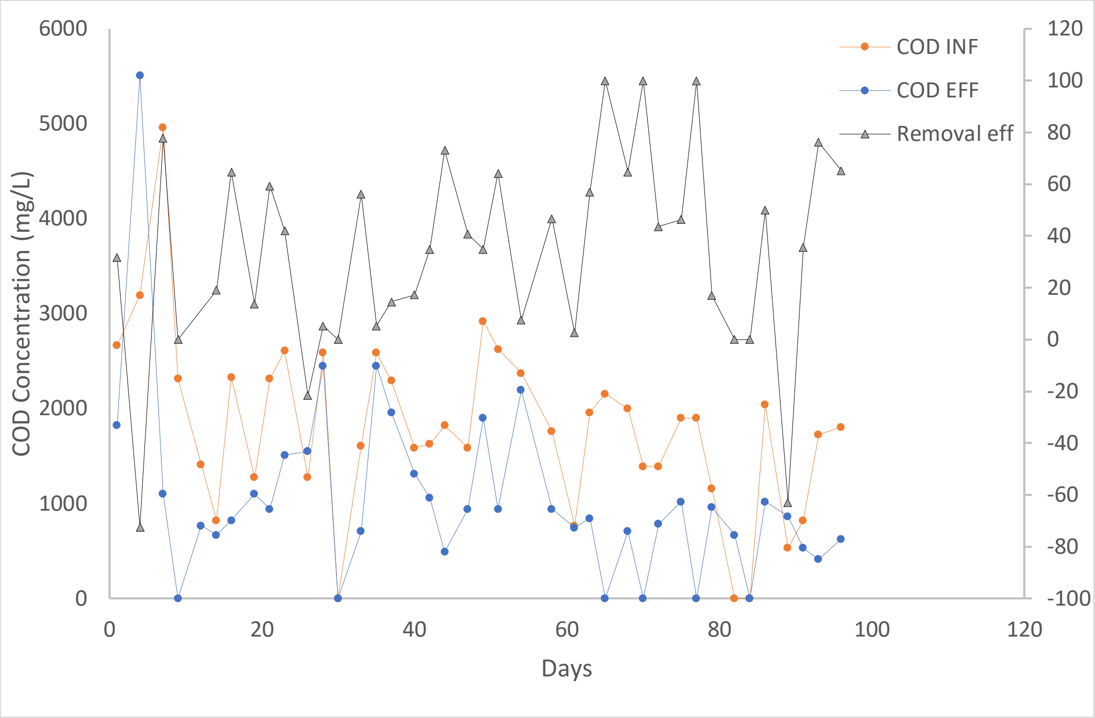
At an HRT of 24 hours, the average removal efficiency for COD, TSS, and FOG is 36%, 48%, and 68%, respectively. At this HRT, it is evident that the TSS removal increases while the COD and FOG drop, respectively. This could be due to the raw wastewater used during this period; the raw wastewater could have had less initial FOG and COD, resulting in a very small decrease in removals.

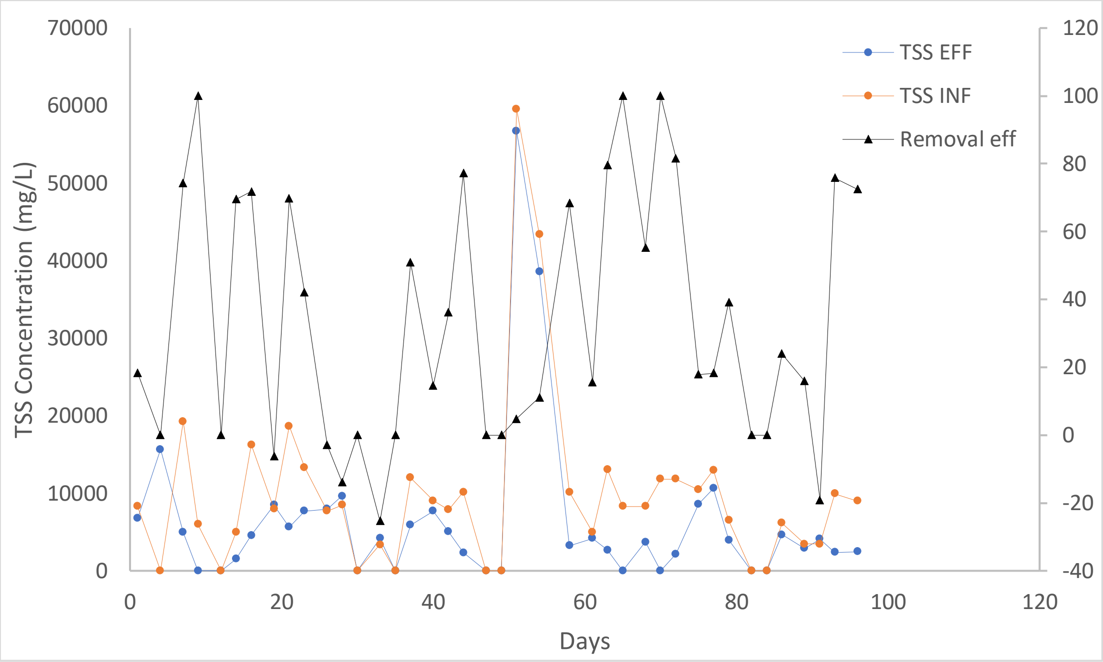
At an HRT of 36 hours, the average removal efficiency for COD, TSS, and FOG is 38%, 55%, and 76%, respectively. At this HRT, all the observed parameters increased in removal efficiencies, making this HRT ideal for higher removals in TSS and FOG. The inconsistent characteristics of the raw wastewater have a significant impact on the experiment, as some weeks had more dilute wastewater than others, resulting in lower removal rates for the parameters.

System issues were encountered on Day 9, as explained earlier in this chapter. Between days 28 and 32 (week 5), on day 65, day 70, day 77, and day 83, the system experienced failures due to clogging. This resulted in an overflow from the top of the DEGBR, with the overflowing wastewater recycled back into the feed tank. The system's temperature was maintained at 37°C to ensure the sludge remained alive and active.

To address the clogging problem, a backwash was performed using the same pre-treated DWW, and a heating element was added to break down the fats. The backwash lasted for a minimum of 6 hours to remove all blockages. The collected backwash had a COD test result averaging 2300 mgCOD/L. On Day 26, a negative removal efficiency occurred due to sludge washout, caused by the outflow pump operating at a higher RPM than the inflow pump. Once noticed, flowrates were recalculated, and a new set of flowrates were used to reduce sludge washout which was 3.4 l/d.

Sludge washout led to a reduction in biomass within the digester, causing decreased microbial activity in the reactor, ultimately affecting COD removal. Since biomass also contributes to COD, its washout adds to the effluent's COD value. The negative value was excluded in the calculation of the average removal efficiencies. This detailed analysis provides insights into the challenges and corrective measures taken during the DEGBR operation.


6000
5000
4000
3000
2000
1000
0
COD Concentration (mg/L)
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
20
40
60
80
100
120
Days
COD INF
COD EFF
Removal eff


70000
60000
50000
40000
30000
20000
10000
0
TSS Concentration (mg/L)
120
100
80
60
40
20
0
-20
-40
0
20
40
60
80
100
120
Days
TSS EFF
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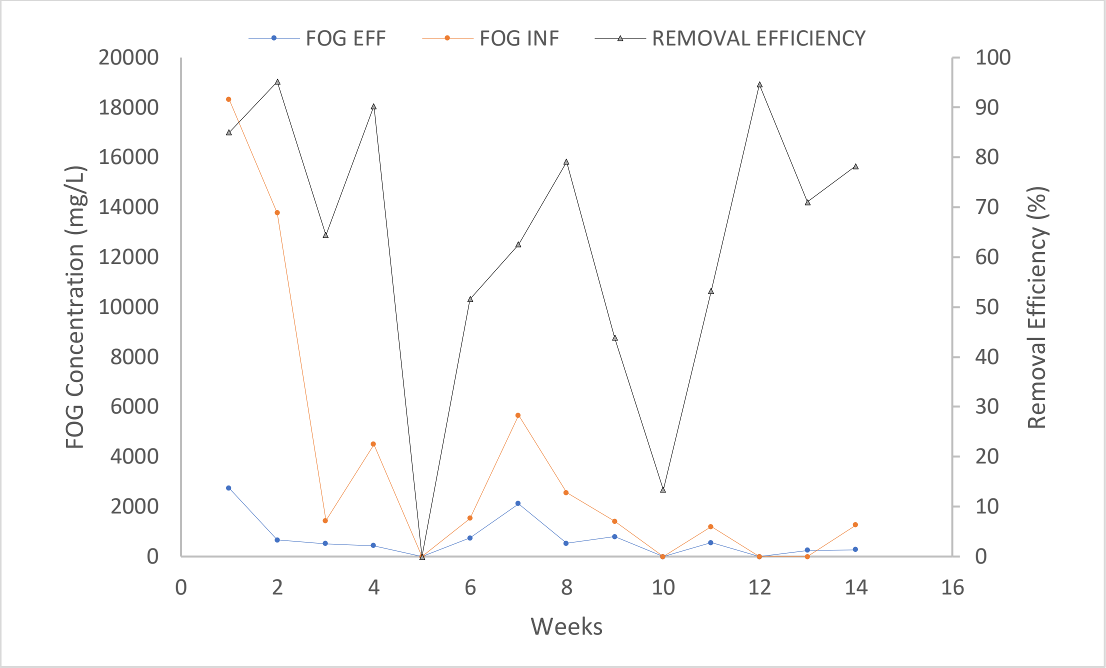
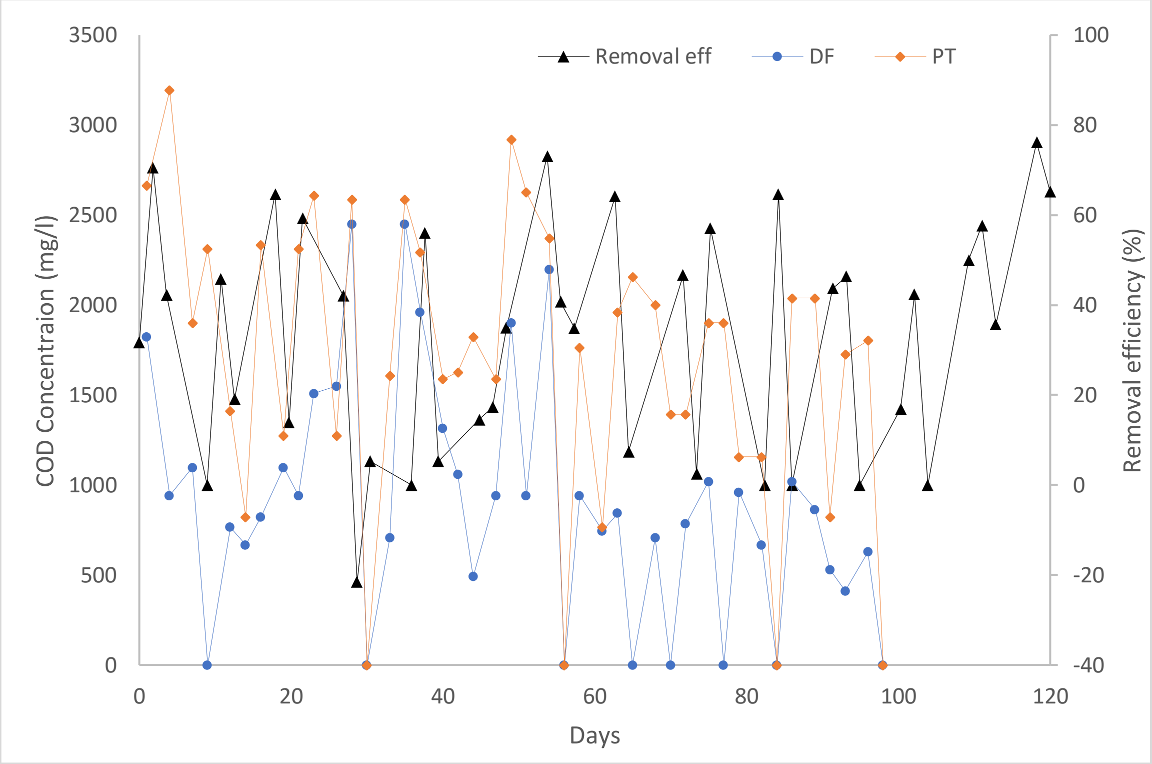


Figure 4.16 | Performance of DEGBR before outlier replacement with respect to COD, TSS and FOG



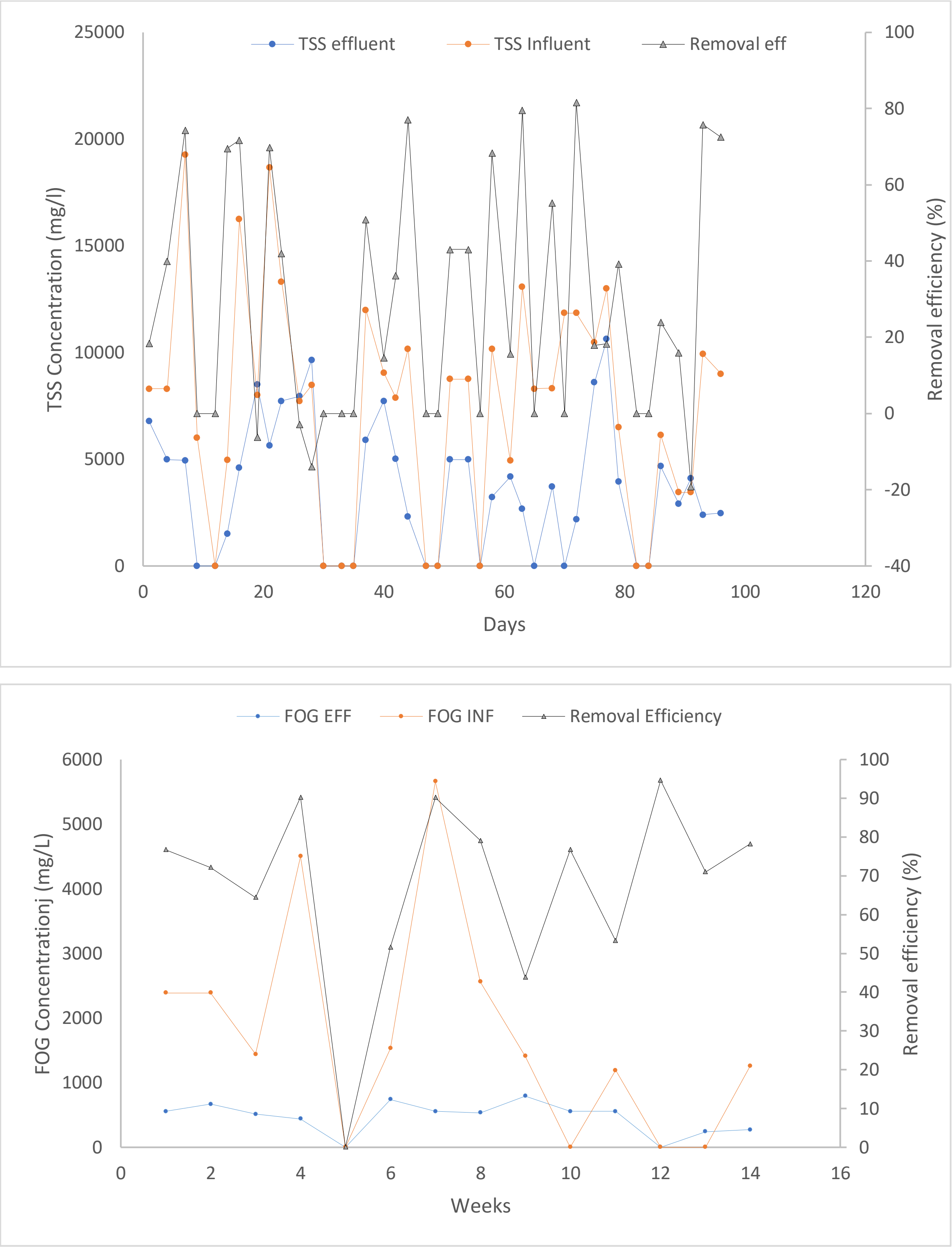


Figure 4.17 | Performance of DEGBR after outlier replacement with respect to COD, TSS and FOG

The total COD values for the influent, effluent and backwash was used to generate a COD mass balance. The following assumption was made when generating the mass balance that 90% of COD removed was converted into methane and the remaining 10% of COD removed was utilized for biomass synthesis (Oh, 2012). These assumptions can be made has the author conducted a range of experiments to allow for these assumptions to be made. The schematic below shows the distribution of COD.

From the COD mass balance, just more than half of the COD coming in was leaving through the effluent. Even though the effluent had much less COD than when it came in it requires further treatment to bring down the organic content of the effluent. The difference between the influent COD and the sum of the backwash and effluent COD was then spilt with 90% going towards methane and the 10% towards retention of COD via biomass. Backwashing was not conducted regularly in the beginning stages of the experiment has it was operating optimally; in latter the stages of the experiment backwashing was conducted every two weeks has clogging became a major issue. Backwashing was done by pumping the pre-treated wastewater (feed into the DEGBR) through the effluent chamber to dislodge any blockages within the packed granular sludge bed at a steady flow to reduce disruption to the microbes. The biomass was retaining 3.8% of the COD that was coming in, this was for biomass synthesis. The remainder of the COD was 34% which was converted to methane. In this experiment the biogas was not captured.

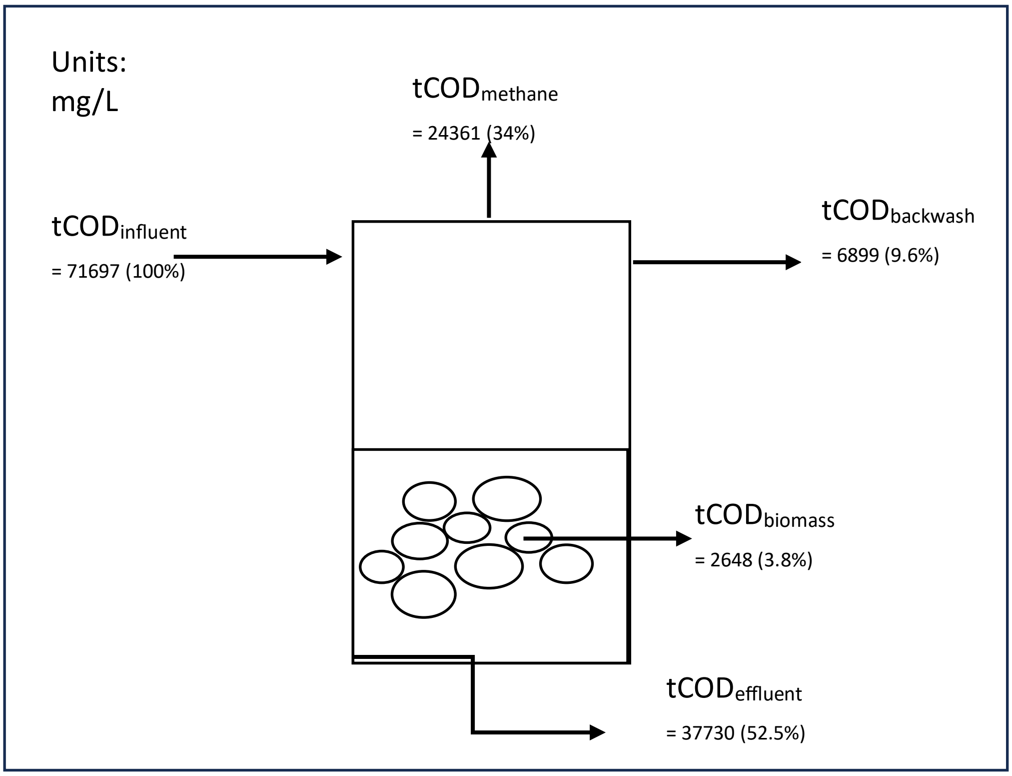


Figure 4.18 | Overall COD balance of the DEGBR

For TSS testing there were experimental issues experienced where there are values of zero. In the early stages of the project there was small traces of washout experienced hence the negative percentage removals. After the system stabilized there was no washout experienced. The negative percentage removals were excluded from the average removal efficiency.

Overall, the fluctuations in COD and TSS is mainly due to the raw wastewater used in that week, each 25L of DWW was not entirely the same, some had more solids than others, some were more diluted, and there could be potentially more cleaning chemicals than others. Another cause for these fluctuations is the problem of load shedding, when it occurs sometimes the power supply trips leaving the system off for hours sometimes.

4.4. Comparison of results

Table 4.2 | System comparisons

System	**COD removal %**	**TSS removal %**	**FOG removal %**	**References**
Overall PT-DEGBR	48%	56%	71%	-
PT(Enzyme)-AD	75%	-	-	(Adulkar & Rathod, 2015)
PT(Enzyme)-AD	67%	38%	97%	(Bhange & Suke, 2018)
PT(Enzyme)-UASB	90%	-	-	(Cammarota, Teixeira & Freire, 2001)
PT(Enzyme)-AD	30-40.9%	-	39.5%	(Mendes et al., 2010).
PT(Enzyme)-AD	47%	-	76.9%	(Alberton et al., 2010).
PT(Enzyme)-AD	Solubilize COD by 24%	27% decrease in solids	-	(Kavitha et al., 2016).

From Table 4.2 the overall removal efficiencies of the overall system, PT-DEGBR, is compared to other PT-AD systems where the pre-treatment stage involved the use of an enzyme, such as protease or lipase, to treated high fat wastewaters before entering an AD. This overall system can produce similar results to some studies. The differences could be due to the enzyme dosages used or the HRT in the studies where the results generated were higher. It can be seen though that the removal efficiencies of the overall system are very similar to the removal efficiencies that was calculated by Alberton et al., (2010) indicating that this experiment was ran well and that there is potential that Momar can produce similar results to that of lipase. This overall system produced greater results than that of Kavitha et al., (2016) where the author used protease in the enzymatic pre-treatment phase this indicates that Momar can produce greater results than that of protease.

4.5. Summary

The investigation into treating DWW using a biological pre-treatment coupled with the DEGBR was successfully conducted. In the pre-treatment stage, COD, TSS, and FOG exhibited removal efficiencies of 16%, 20%, and 70%, respectively. In the DEGBR, the average removal efficiencies achieved for COD, TSS, and FOG were 39%, 49%, and 73%, respectively. With the main objective in the pre-treatment stage to remove the FOG, the pre-treatment stage was successful in this manner.

Overall, the DEGBR proved effective in handling high-fat-content wastewater, such as DWW. However, it encountered significant clogging issues. The DWW would solidify in the tubing itself during clogging, and the recycle flow was unable to agitate the sludge bed to reduce clogging. Further work is needed to improve the system's performance when treating dairy wastewater.

CHAPTER 5: CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1. Conclusion

The combination of using an enzymatic pre-treatment coupled with the DEGBR was found to be satisfactory in reducing the COD, FOG, and TSS of dairy wastewater over the duration of the project, which was 14 weeks (98 days). From the pre-treatment stage, it was observed that the DO levels dropped by 42%, which was an improvement from previous enzymes such as Eco-flush that required an aerobic environment that was detrimental to the connected AD stage of treatment that followed FOG removal. This was evident from the COD, TSS, and FOG in the pre-treatment stage, that showed removal efficiencies of 16%, 20%, and 70%, respectively. With satisfactory removal efficiencies achieved at sufficiently low DO levels, it can be concluded that the usage of Momar had a positive effect on the pre-treatment of raw DWW.

The DEGBR operated with a hydraulic retention time (HRT) of 36 hrs at an average OLR of 0.052 gCOD/L.hr. The DEGBR produced sufficient average removal efficiencies for COD, TSS, and FOG, which are 39%, 49%, and 73%, respectively. The diverse HRTs exhibited distinct removal efficiencies, highlighting the crucial role of HRT in the treatment of dairy wastewater. At 36 hours, COD, TSS, and FOG removal rates were 38%, 55%, and 76%, respectively; at 24 hours, they stood at 36%, 48%, and 68%, and at 12 hours, the values were 48%, 43%, and 74%. The initial two HRTs displayed an inversely proportional relationship, with an increase in one parameter corresponding to a decrease in the other. Conversely, the last HRT demonstrated a more balanced removal efficiency. Depending on the characteristics of the raw wastewater, different HRTs can be employed to enhance the targeted parameter's removal efficiency.

Over the period of this study, the system had a few drawbacks, which will be discussed in the subsection. Overall, the combined system produced satisfactory results with removal efficiencies of COD, TSS, and FOG, which are 48%, 56%, and 71%, respectively. In conclusion, the system is able to achieve its target of reducing the organic content and FOG of the DWW.

5.2. Limitations

During the course of the project, many issues were experienced. The main concern was the clogging of the system, particularly within the downflow reactor at the bottom. Significant clogging occurred, initially assumed to be caused by an excess of stones and sludge within the reactor. It was thought to be the overgrowth of biomass that clogged up the system. The reactor was drained, and a new ratio of sludge to DWW was used. Instead of using 1 litre of sludge, it was halved to 500ml, and half of the stones were used in the new setup. The new configuration worked efficiently for a while until another clogging was experienced, this time due to the fat buildup of the DWW itself hardening on the steel mesh at the bottom of the reactor, preventing effluent from passing through and causing clogging and an overflow of the DWW at the top of the reactor.

Another significant factor experienced in this study was load shedding. With load shedding occurring every day, the system had to be put on a UPS alongside other projects. However, with this configuration, the adapter would trip, leading to the system switching off, and no one switched it back on. Due to these unforeseen circumstances, some of the microbes in the DEGBR would die due to the temperature drop.

Another issue encountered was the breaking of the water jacket input socket on the side of the reactor. This breakage caused leakage, leading the tray beneath the system to constantly fill up and drain the water from the water bath. As a result, excessive amounts of water had to be refilled daily. Under optimal operation, the water bath required 1 litre of water every second day, but due to the leakage, it needed approximately 5 litres of water each day.

Another issue encountered was the cracking of the pre-treatment bucket and the feed bucket at the bottom, which caused significant leakage and resulted in the complete loss of that day's pre-treatment. This occurred because the magnetic stirrer was left on continuously, weakening the bucket until it eventually broke. Consequently, a new bucket was purchased and used.

Other minor issues experienced were that the inflow socket broke as well, but that was fixed by filling the crack with adhesive to bind the crack, and silicone was put on top of it to add extra protection. Tubing of the outflow and inflow required maintenance as it would clog up if there was an issue with power, i.e., load shedding, preventing the movement of liquid to occur.

Overall, the system required continuous maintenance, from fixing ports and replacing tubing in the peristaltic pumps to improvising methods to prevent leakages.

5.3. Recommendations

- In the future, the lab-scale plant utilizing DWW as its source should be run for a longer period, and the VFAs should be examined to establish if the system is stable or not.
- The sludge used should be investigated with lower pH levels, as DWW has a pH value of 4.22 before treatment, and Momar was observed to drop the pH.
- The system should be run with a lower HRT and a higher OLR to achieve higher COD removal efficiency.
- An investigation should be conducted more in-depth for the optimization of the pre-treatment stage to observe the effects of temperature, enzyme dosage, and HRT. This should be done by keeping two constants at a time, with one changing, and repeated for all three parameters.
- In the future, run the system again with the same DWW conditions but with fewer issues to verify the results in this paper.
- The actual ratios between sludge, wastewater, and stones should be established and used for all other wastewaters with this system.
- A different approach to filtering the effluent at the bottom of the DEGBR should be considered instead of using a piece of material.
- In the future, the biogas produced should be trapped and investigated for use as an energy source for the system itself to become self-sufficient.
- When dealing with high-fat wastewater in the future, it is recommended to conduct weekly backwashes to ensure there are no blockages.
- It is recommended to conduct an investigation looking at an SBR using Momar and Eco-Flush; this can potentially achieve high levels of removal efficiencies for the parameters in question.
- A thorough analysis should be conducted on the microbes in the bioremediation agent, Momar, to identify its impacts on wastewater.
- For the pre-treatment, a bucket with a metal attachment should be used to reduce the risk of the magnetic stirrer breaking the bucket.
- The system itself can be improved, and it is recommended that a new recycle flow should be explored; a concept design of a new system is shown in Appendix C.

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Appendix

Appendix A

Analytic methods

pH and DO

2) Calibrate the multiparameter with the pH solutions provided.
3) Calibrate the DO probe by placing in deionised water for 10mins.
4) Press the on button and place pH probe into the wastewater and keep there until the reading is stable.
5) Place DO probe next and following the same procedure as the pH probe.
6) Observe and record result.

COD

$$COD\left(\frac{mg}{L}\right) = \frac{(B - A) * N * 8000 * D}{V}$$

Where: B = blank (ml)

A = Average titration of sample (ml)

N = Normality (0.049)

D = Dilution factor

V = Volume of sample (ml)

TSS

$$TSS\left(\frac{mg}{L}\right) = \frac{(mass\ 1 - \ mass\ 2) * \ 1000000}{V}$$

Where: Mass 1 = Crucible with sample residue (g)

Mass 2 = Dry Crucible (g)

V = Volume of sample (ml)

Organic Loading Rate:

$$OLR = COD_{influent} * (\frac{1}{HRT})$$

Removal efficiency (RE):

$$RE = \frac{influent - effluent}{influent} * 100$$

Detailed runs for pre-treatment

DM	Time	Dosage	Temp
1	24	90	22
2	24	120	22
3	24	150	22
4	24	90	24
5	24	30	38
6	12	30	38
7	36	90	30
8	36	150	30
9	24	30	22
10	24	30	30
11	36	30	22
12	12	90	38
13	24	150	30
14	12	90	22
15	36	30	38
16	12	150	22
17	12	90	30
18	12	150	30
19	12	30	30
20	12	150	38
21	12	30	22
22	12	150	38
23	24	150	30
24	24	150	38
25	36	90	38
26	36	30	22

Outlier identification and replacement for Pre-Treatment stage

COD				TSS				FOG			
5280	FALSE	Q1	8888	1487,5	FALSE	Q1	2384,375	104	FALSE	Q1	277
5632	FALSE	Q3	13728	1650	FALSE	Q3	3659,375	122	FALSE	Q3	647,5
5984	FALSE	IQR	4840	1850	FALSE	IQR	1275	152	FALSE	IQR	370,5
6336	FALSE	LOWER	1628	1987,5	FALSE	LOWER	471,875	168	FALSE	LOWER	-278,75
7040	FALSE	UPPER	20988	2187,5	FALSE	UPPER	5571,875	182	FALSE	UPPER	1203,25
8448	FALSE	MEDIAN	10208	2225	FALSE	MEDIAN	2725	268	FALSE	MEDIAN	398
8800	FALSE			2375	FALSE			280	FALSE		
9152	FALSE			2412,5	FALSE			294	FALSE		
9152	FALSE			2425	FALSE			324	FALSE		
9152	FALSE			2450	FALSE			360	FALSE		
9504	FALSE			2562,5	FALSE			362	FALSE		
9856	FALSE			2600	FALSE			398	FALSE		
10208	FALSE			2687,5	FALSE			398	FALSE		
10208	FALSE			2725	FALSE			406	FALSE		
11616	FALSE			2812,5	FALSE			450	FALSE		
11616	FALSE			2862,5	FALSE			562	FALSE		
13024	FALSE			2962,5	FALSE			628	FALSE		
13376	FALSE			3025	FALSE			638	FALSE		
13728	FALSE			3275	FALSE			676	FALSE		
13728	FALSE			3787,5	FALSE			816	FALSE		
14080	FALSE			4637,5	FALSE			844	FALSE		
18656	FALSE			5400	FALSE			1120	FALSE		
24288	TRUE	x		6225	TRUE	x		1430	TRUE	x	
24640	TRUE	x		6725	TRUE	x		1780	TRUE	x	
28512	TRUE	x		6737,5	TRUE	x					
47520	TRUE	x		12300	TRUE	x					
				15987,5	TRUE	x					

Pre-treatment data

Runs	Avg COD inf	COD eff	removal	Avg TSS inf	TSS eff	removal	Avg FOG inf	FOG eff	removal
1	10320	10208	1,09%	2747	2725	0,81%	1440	816	43,33
2	10320	10208	1,09%	2747	2725	0,81%	1440	398	72,36
3	10320	13024	-26,20%	2747	2425	13,28%	1440	362	74,86
4	10320	10208	1,09%	2747	2225	23,46%	1440	844	41,39
5	10320	6336	38,60%	2747	2375	15,66%	1440	628	56,39
6	10320	8448	18,14%	2747	2187,5	25,58%	1440	1120	22,22
7	10320	10208	1,09%	2747	2450	12,12%	1440	294	79,58
8	10320	14080	-36,43%	2747	2725	0,81%	1440	638	55,69
9	10320	9856	4,50%	2747	2687,5	2,21%	1440	398	72,36
10	10320	8800	14,73%	2747	4637,5	-40,77%	1440	450	68,75
11	10320	11616	-12,56%	2747	6225	-55,87%	1440	268	81,39
12	10320	10208	1,09%	2747	2725	0,81%	1440	562	60,97
13	10320	5632	45,43%	2747	2412,5	13,87%	1440	182	87,36
14	10320	5280	48,84%	2747	1850	48,49%	1440	122	91,53
15	10320	9152	11,32%	2747	5400	-49,13%	1440	0	0,00
16	10320	13728	-33,02%	2747	2725	0,81%	1440	406	71,81
17	10320	5984	42,02%	2747	2962,5	-7,27%	1440	280	80,56
18	10320	18656	-80,78%	2747	3787,5	-27,47%	1440	398	72,36
19	10320	13376	-29,61%	2747	1650	66,48%	1440	0	0,00
20	10320	9504	7,91%	2747	1487,5	84,67%	1440	676	53,06
21	10320	7040	31,78%	2747	1987,5	38,21%	1440	104	92,78
22	10320	9152	11,32%	2747	2562,5	7,20%	1440	152	89,44
23	10320	13728	-33,02%	2747	2600	5,65%	1440	360	75,00
24	10320	11616	-12,56%	2747	2812,5	-2,33%	1440	168	88,33
25	10320	10208	1,09%	2747	2862,5	-4,03%	1440	324	77,50
26	10320	9152	11,32%	2747	3025	-9,19%	1440	398	72,36

Appendix B

Outlier identification and replacement for DEGBR

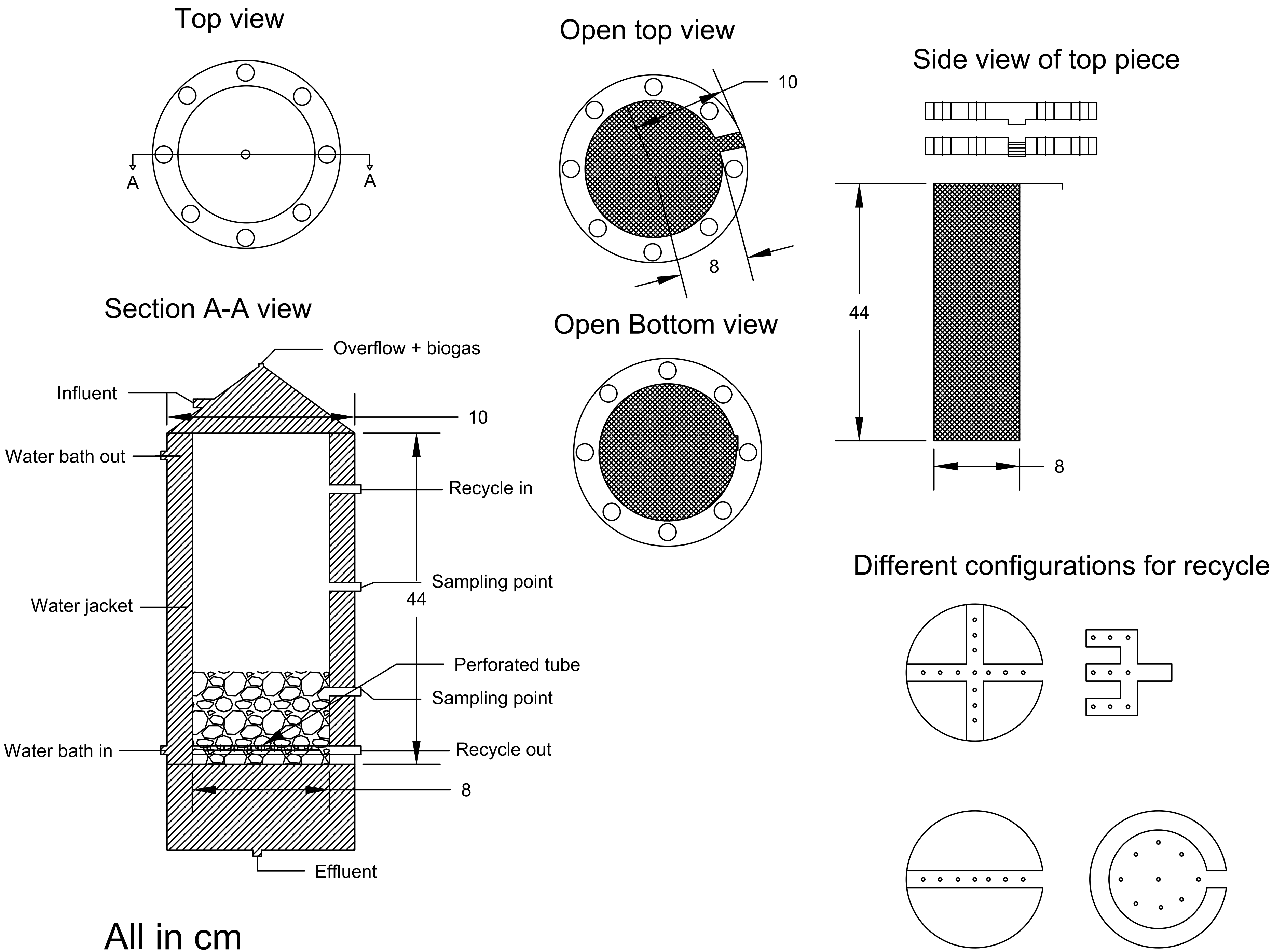
COD								TSS								FOG							
COD eff				COD inf				TSS eff				TSS inf				FOG EFF				FOG inf			
411,6	FALSE	Q1	749,7	529,2	FALSE	Q1	1538,6	1516,1	FALSE	Q1	3893,8	3350,0	FALSE	Q1	6806,2	0,0	FALSE	Q1	438,0	0,0	FALSE	Q1	1410,0
490,0	FALSE	Q3	1460,2	764,4	FALSE	Q3	2342,2	2175,0	FALSE	Q3	8087,5	3450,0	FALSE	Q3	12750,0	244,0	FALSE	Q3	779,0	842,0	FALSE	Q3	5370,0
529,2	FALSE	IQR	710,5	823,2	FALSE	IQR	803,6	2325,0	FALSE	IQR	4193,7	3450,0	FALSE	IQR	5943,8	274,0	FALSE	IQR	341,0	1190,0	FALSE	IQR	3960,0
627,2	FALSE	LOWER	-316,1	823,2	FALSE	LOWER	333,2	2675,0	FALSE	LOWER	-2396,9	4950,0	FALSE	LOWER	-2109,4	320,0	FALSE	LOWER	-73,5	1260,0	FALSE	LOWER	-4530,0
666,4	FALSE	UPPER	2526,0	1156,4	FALSE	UPPER	3547,6	2900,0	FALSE	UPPER	14378,1	4975,0	FALSE	UPPER	21665,6	438,0	FALSE	UPPER	1290,5	1410,0	FALSE	UPPER	11310,0
666,4	FALSE	MEDIAN	940,8	1274,0	FALSE	MEDIAN	1901,2	3225,0	FALSE	MEDIAN	4987,5	6000,0	FALSE	MEDIAN	8762,5	512,0	FALSE	MEDIAN	556,0	1440,0	FALSE	MEDIAN	2390,0
705,6	FALSE			1274,0	FALSE			3725,0	FALSE			6150,0	FALSE			534,0	FALSE			1530,0	FALSE		
705,6	FALSE			1391,6	FALSE			3950,0	FALSE			6500,0	FALSE			556,0	FALSE			2390,0	FALSE		
744,8	FALSE			1391,6	FALSE			4111,1	FALSE			7725,0	FALSE			666,0	FALSE			2560,0	FALSE		
764,4	FALSE			1587,6	FALSE			4175,0	FALSE			7875,0	FALSE			740,0	FALSE			4500,0	FALSE		
784,0	FALSE			1587,6	FALSE			4200,0	FALSE			8000,0	FALSE			792,0	FALSE			5660,0	FALSE		
823,2	FALSE			1607,2	FALSE			4590,9	FALSE			8300,0	FALSE			2070,0	TRUE	x		5980,0	FALSE		
842,8	FALSE			1626,8	FALSE			4675,0	FALSE			8300,0	FALSE			2120,0	TRUE	x		13770,0	TRUE	x	
862,4	FALSE			1724,8	FALSE			4950,0	FALSE			8325,0	FALSE			2740,0	TRUE	x		18320,0	TRUE	x	
940,8	FALSE			1764,0	FALSE			5025,0	FALSE			8475,0	FALSE										
940,8	FALSE			1803,2	FALSE			5650,0	FALSE			9050,0	FALSE										
940,8	FALSE			1822,8	FALSE			5900,0	FALSE			10175,0	FALSE										
940,8	FALSE			1901,2	FALSE			6775,0	FALSE			10175,0	FALSE										
960,4	FALSE			1901,2	FALSE			7725,0	FALSE			10475,0	FALSE										
1019,2	FALSE			1960,0	FALSE			7725,0	FALSE			11850,0	FALSE										
1019,2	FALSE			1999,2	FALSE			7950,0	FALSE			11850,0	FALSE										
1058,4	FALSE			2038,4	FALSE			8500,0	FALSE			12000,0	FALSE										
1097,6	FALSE			2156,0	FALSE			8600,0	FALSE			13000,0	FALSE										
1097,6	FALSE			2293,2	FALSE			9650,0	FALSE			13075,0	FALSE										
1313,2	FALSE			2312,8	FALSE			10625,0	FALSE			13325,0	FALSE										
1509,2	FALSE			2312,8	FALSE			15650,0	TRUE	x		16250,0	FALSE										
1548,4	FALSE			2332,4	FALSE			38575,0	TRUE	x		18675,0	FALSE										
1822,8	FALSE			2371,6	FALSE			56700,0	TRUE	x		19275,0	FALSE										
1901,2	FALSE			2587,2	FALSE							43375,0	TRUE	x									
1960,0	FALSE			2587,2	FALSE							59550,0	TRUE	x									
2195,2	FALSE			2606,8	FALSE																		
2450,0	FALSE			2626,4	FALSE																		
2450,0	FALSE			2665,6	FALSE																		
5507,6	TRUE	x		2920,4	FALSE																		
				3194,8	FALSE																		
				4958,8	TRUE	x																	

DEGBR performance

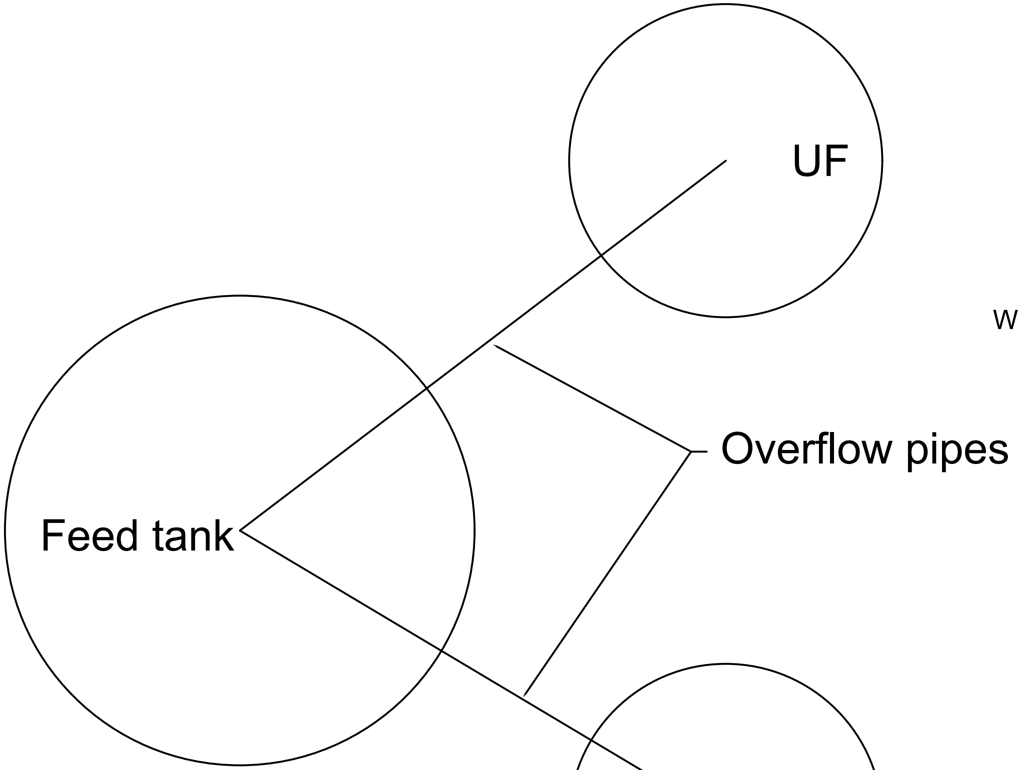
		COD (mg/l)			TSS (mg/l)			FOG (mg/l)		
weeks	Days	DF (eff)	PT (inf)	Removal (%)	DF (eff)	PT (inf)	Removal (%)	DF (eff)	PT (inf)	Removal (%)
1	1	1822,8	2666	32	6775	8300	18,37	556	2390	77
	4	940,8	3195	71	4988	8300	39,91			
	7	1097,6	1901	42	4950	19275	74,32			
2	9	0	2313	0	0	6000	0,00	666	2390	72
	12	764,4	1411	46	0	0	0,00			
	14	666,4	823	19	1516	4975	69,53			
3	16	823,2	2332	65	4591	16250	71,75	512	1440	64
	19	1097,6	1274	14	8500	8000	-6,25			
	21	940,8	2313	59	5650	18675	69,75			
4	23	1509,2	2607	42	7725	13325	42,03	438	4500	90
	26	1548,4	1274	-22	7950	7725	-2,91			
	28	2450	2587	5	9650	8475	-13,86			
5	30	0	0	0	0	0	0,00	0	0	0
	33	705,6	1607	56	0	0	0,00			
	35	2450	2587	5	0	0	0,00			
6	37	1960	2293	15	5900	12000	50,83	740	1530	52
	40	1313,2	1588	17	7725	9050	14,64			
	42	1058,4	1627	35	5025	7875	36,19			
7	44	490	1823	73	2325	10175	77,15	556	5660	90
	47	940,8	1588	41	0	0	0,00			
	49	1901,2	2920	35	0	0	0,00			
8	51	940,8	2626	64	4988	8762,5	43,08	534	2560	79
	54	2195,2	2372	7	4988	8762,5	43,08			
	56	0	0	0	0	0	0,00			
9	58	940,8	1764	47	3225	10175	68,30	792	1410	44
	61	744,8	764	3	4175	4950	15,66			
	63	842,8	1960	57	2675	13075	79,54			
10	65	0	2156	0	0	8300	0,00	556	2390	77
	68	705,6	1999	65	3725	8325	55,26			
	70	0	1392	0	0	11850	0,00			
11	72	784	1392	44	2175	11850	81,65	556	1190	53
	75	1019,2	1901	46	8600	10475	17,90			
	77	0	1901	0	10625	13000	18,27			
12	79	960,4	1156	17	3950	6500	39,23	320	5980	95
	82	666,4	1156	42	0	0	0,00			
	84	0	0	0	0	0	0,00			
13	86	1019,2	2038	50	4675	6150	23,98	244	842	71
	89	862,4	2038	58	2900	3450	15,94			
	91	529,2	823	36	4111	3450	-19,16			
14	93	411,6	1725	76	2400	9925	75,82	274	1260	78
	96	627,2	1803	65	2475	9000	72,50			

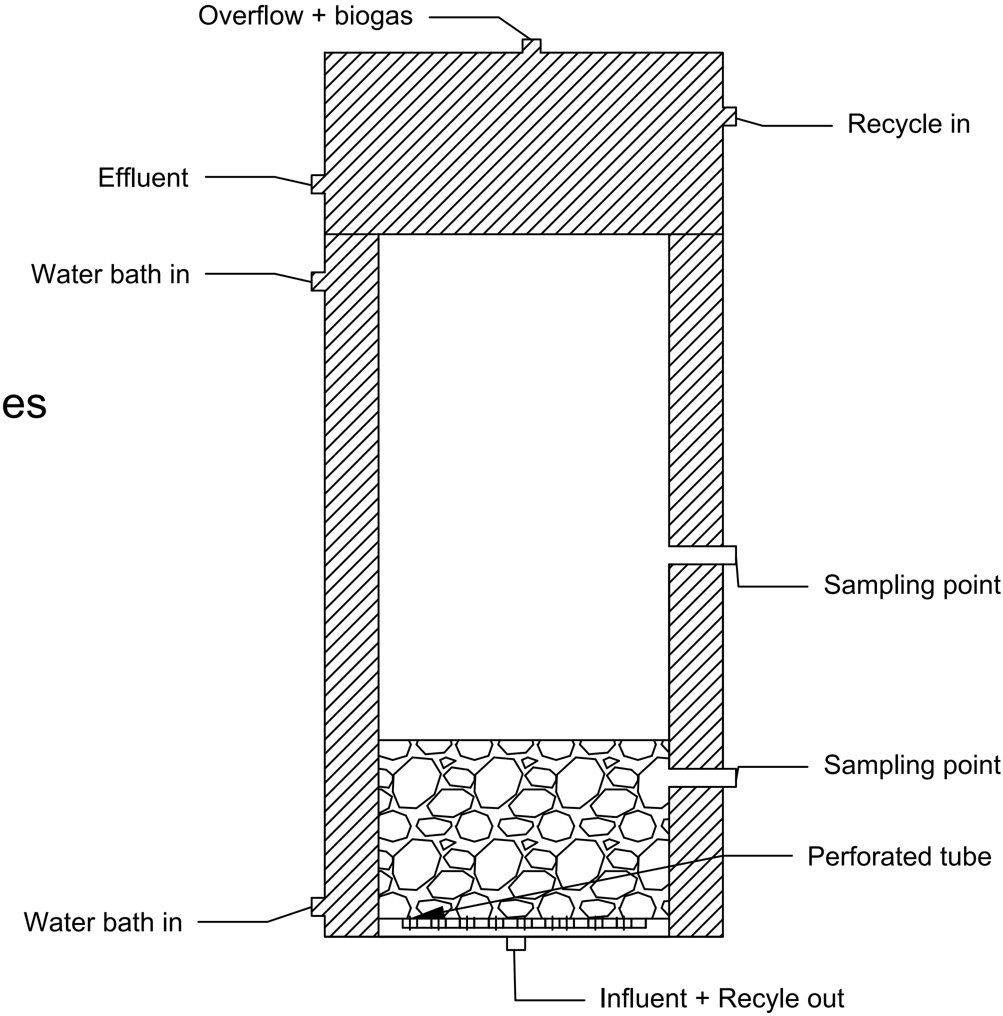
Appendix C

System concept design


Top view
A
A
Open top view
10
8
Side view of top piece
44
8
Section A-A view
Open Bottom view
Overflow + biogas
Influent
10
Water bath out
Recycle in
Sampling point
44
Water jacket
Perforated tube
Sampling point
Water bath in
Recycle out
8
Effluent
Different configurations for recycle

All in cm


UF
Overflow pipes
Feed tank
DF


Overflow + biogas
Recycle in
Effluent
Water bath in
Sampling point
Sampling point
Perforated tube
Water bath in
Influent + Recyle out