

Chemical Engineering



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Evaluation of the performance of a multi-integrated lab-scale plant for the treatment of dairy wastewater

Dissertation

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Executive Summary

South Africa's dairy sector, producing 3.4 million tonnes of milk in 2019, generates significant wastewater, estimated at 2.5 times the volume of processed milk, or approximately 8.5 million tonnes annually. This wastewater poses serious environmental risks due to its high organic content, potentially leading to eutrophication and hypoxic zones in water bodies. Effective treatment methods are essential to mitigate these impacts. This study evaluates the performance of a lab-scale wastewater treatment plant, combining various biological processes previously used for abattoir wastewater (AWW), given the similarities between AWW and dairy wastewater (DWW) in terms of high organic and fat content. In anticipation of the challenge of clogging which has been prevalent in previous studies, an investigation was done into the use of okra as a biological coagulant which could possibly be integrated with the system.

The research focuses on optimizing individual treatment units and assessing their collective performance in treating DWW. Initial challenges included operational errors, such as the overuse of Morma in pre-treatment, leading to increased biomass production and elevated Chemical Oxygen Demand (COD). Despite subsequent corrections improving system stability, fluctuations persisted, particularly in the Expanded Granular Sludge Bed Reactor (EGSBR). COD removal rates ranged from 60% in week 3 to 26% in week 5, highlighting the system's potential but falling short of traditional anaerobic digestion (AD) systems, which can achieve up to 90% COD removal. However, the system performed better with higher raw COD levels, indicating its efficacy in treating wastewater with elevated COD content.

EGSBR stability issues were attributed to clogging and biomass washout, exacerbated by a reduced pre-treatment hydraulic retention time (HRT) of 12 hours. Analysis of fat, oil, and grease (FOG) removal revealed inconsistent performance, with the highest removal rates occurring in weeks with FOG content

exceeding 6000 mg/L DWW. FOG removal rates ranged from 27% to 94%, surpassing those of previous AD reactor studies. The observed correlation between FOG content and system performance warrants further investigation. Total Suspended Solids (TSS) removal rates served as indicators of reactor stability issues, with increased TSS indicating biomass washout. Decreasing pre-treatment HRT adversely affected reactor stability, highlighting the importance of optimizing pre-treatment parameters.

Additionally, the use of okra seeds as a coagulant was explored, showing promising results in reducing TSS and COD from DWW. The optimal dose was 5g per 2L of DWW, achieving the highest removal rates for both TSS and COD. This suggests that okra seeds offer an eco-friendly alternative to traditional coagulants like alum. Integrating coagulant treatments with biological agents could enhance the removal of solids and FOGs, addressing the stability issues observed in the EGSBR and improving overall system performance.

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Nomenclature

DWW	Dairy Wastewater
COD	Chemical Oxygen Demand
FOG	Fats, Oils, and Greases
TSS	Total Suspended Solids
TDS	Total Dissolved Solids
TN	Total Nitrogen
BOD	Biological Oxygen Demand
PT	Pre-Treatment
AD	Anaerobic Digestion
TOC	Total Organic Carbon

1 INTRODUCTION

1.1 Background information

The disposal of wastewater is a topic of great importance as improper disposal may lead to a detrimental effect on the lives of people as well as the degradation of the environment. Many laws and regulations have been put in place to ensure that the wastewater being released from various industrial processes meet a minimum standard of safety. Different industries release varying quantities of wastewater with different types of contamination. The dairy industry in South Africa is a relatively large industry producing more than 3 million tonnes of raw milk per year (van Heerdan, 2022) as previously stated. This indicates that the dairy industry will be a significant producer of wastewater as the processing of raw milk is estimated to produce 2.5 m³ of effluent per m³ of processed milk (Kolev, 2017).

Globally, the dairy sector is a substantial industry amounting to 900 million tonnes of milk being produced annually (*Dairy: World Markets and Trade*, 2022). However, this large-scale production raises concerns about the resulting wastewater. It is estimated that the volume of wastewater generated during milk processing is 2.5 times greater than the volume of milk processed, amounting to a staggering 2.25 billion tonnes of dairy wastewater produced annually (Dogan et al., 2020).

In South Africa, 40% of the population live in rural areas. According to recent estimates, 74% of the rural population rely on groundwater to meet their water needs. However, 19% of the rural population lack access to reliable water, and 33% lack basic sanitation. Inadequate water access is also a problem for 26% of all schools and 45% of clinics ("2016-06 - SA's farmers can benefit by reducing their water use - Wits University", 2016). Water scarcity is a significant issue in South Africa, where the agricultural industry uses 60% of the available water. This poses a challenge as a large portion of the population lack reliable access to water ("Water Access in South Africa | Water for all", n.d.). To exacerbate the problem, the dairy refinement industry alone consumes around 7 million tonnes of water per year (van Heerdan, 2022). However, there is an opportunity to address this issue by

implementing dairy wastewater (DWW) treatment strategies. By doing so, a substantial amount of water can be recovered and reintroduced into the water grid, thereby aiding in reducing the water shortage experienced by many South Africans.

The disposal of wastewater is a topic of great importance as improper disposal may lead to a detrimental effect on the lives of people as well as the degradation of the environment. Many laws and regulations have been put in place to ensure that the wastewater being released from various industrial processes meets a minimum standard of safety (Edna Molewa, 2013). Different industries release varying quantities of wastewater with different types of contamination. The release of DWW into water systems without proper treatment causes an array of environmental issues due to its high organic content. The decomposition of DWW in a water source causes the dissolved oxygen (O₂) inside the water body to drop as the microbes (especially algae) flourish in the nutrient-rich environment. This results in a hypoxic environment which is unsuitable for aquatic animal and plant life. The increased algal growth produces elevated levels of CO₂ which lowers the pH of the body of water, which in turn slows the growth of fish and shellfish. The addition of DWW into fresh water also provides a breeding ground for various insects, which can upset the balance of the surrounding ecosystem. The degradation of casein also causes the formation of a sludge which is harmful to aquatic organisms (Janet Joshiba et al., 2019). This impacts the health of aquatic life and the potential for fishing which has a negative economic impact. In addition to these environmental implications, the processing of dairy products and the release of DWW can lead to several social impacts. These stem from two main sources, namely, the discharge of untreated or partially treated wastewater into nearby water bodies and the odour produced by the plant and effluents. The contamination caused by the release of DWW can promote the spread of bacteria such as *Salmonella* and *E. coli*, which can cause illness in humans and animals which

use the water sources. Once contracted, these illnesses can easily be spread throughout the community.

1.2 Problem Statement

The environmental impacts of improper disposal of DWW into the environment necessitates effective treatment methods to be explored. Biological systems have traditionally been used to effectively treat DWW by reducing the BOD (biological oxygen demand), COD (chemical oxygen demand), FOG (fats oils & greases), TSS (total suspended solids), and TN (total nitrogen) content in the wastewater. The system to be studied is made up of a combination of various biological processes which has previously been used to treat abattoir wastewater (AWW)(Meyo et al., 2021). Both AWW and DWW are high strength organic wastewaters, with high fat content. These similarities between DWW and AWW suggest that the system will be effective at treating DWW. Both AWW and DWW are high strength organic wastewaters, with high fat content. These similarities between DWW and AWW suggest that the system will be effective at treating DWW.

1.3 Scope of study

This study evaluates the performance of a lab-scale wastewater treatment plant in treating DWW. The study additionally focuses on the optimisation of the individual units being utilised in this process and their operational feasibility. Finally, an overall analysis of the system is undertaken to determine if the unit optimisations work synergistically to improve the performance of the entire system.

2 LITERATURE REVIEW

Part of this literature review has been published as a review paper, detailed as follows:

Ramsuroop, J., Gutu, L., Ayinde, W.B., Basitere, M., & Manono, M.S. (2024). A Review of Biological Processes for Dairy Wastewater Treatment and the Effect of Physical Parameters Which Affect Their Efficiency. *Water*, 16(4), 537.

2.1 Introduction

The unique characteristics of dairy wastewater make it a challenging and complex category of industrial wastewater, namely, the high fat content and high levels of COD, which can be seen on Table 1. Within the dairy industry, different types of wastewaters are produced depending on the part of the process from which the water is sampled and the product being made. However, the contaminants contained in DWW which are of most relevance during treatment can be captured by the following parameters: BOD₅ (biological oxygen demand over 5 days), COD (chemical oxygen demand), FOG (fats, oils, grease), TSS (total suspended solids), TP (total phosphorous) and TN (total nitrogen). Table 2 shows information on the typical characteristics of effluents from different dairy products. In addition to elevated levels of problematic compounds, dairy wastewater is also warmer (17–25 °C) than municipal water (10–20 °C)(Kolev, 2017). This causes increased biological degradation rates, which can promote eutrophication if disposed of into water bodies. These warm temperatures can also negatively impact the growth of plants and organisms in these water bodies.

Due to the large variation in the characteristics of DWW, the choice of appropriate technology is crucial for achieving an effective treatment of different types of wastewater. This review highlights different biological treatment methods which can be applied to DWW, along with their strengths and weaknesses. It also considers possibilities to optimize the various types of processes by altering parameters or adding additional process steps. Most experimental studies focus on the effects of certain parameters on methane production during the anaerobic treatment of DWW. The effects of various parameters on the processes, specifically for the reduction of COD, BOD₅ and FOGs, have not been thoroughly investigated. Therefore, this review considers various optimization options that can be implemented and the predicted effects on process efficiency.

Table 2.1 Characteristics of types of wastewaters produced by various dairy products

Processing Effluent	pH	(mg/L)							Reference
		BOD ₅	COD	FOG	TS	TSS	TN	TP	
Mixed Dairy	3.38– 12.08	240– 5900	500– 10,400	20–1920	710– 7000	60–5800	10–660	0–600	(Geilman et al., 1992; Britz, van Schalkwyk & Hung, 2004; Demirel, Yenigun & Onay, 2005; Farizoglu et al., 2007; Yang et al., 2007; Gannoun et al., 2008; Janczukowicz, Zieliński & Debowski, 2008; Najafpour et al., 2008; Venetsaneas et al., 2009; Wang et al., 2009; Watkins & Nash, 2010; Carvalho, Prazeres & Rivas, 2013)
Milk	5–9.50	500– 1300	950– 2400	-	-	-	-	-	(Demirel, Yenigun & Onay, 2005)
Cheese	3.38– 9.50	590– 5000	1000– 63,300	330– 2600	1920– 53,200	190– 2500	18–830	5–280	[(Britz, van Schalkwyk & Hung, 2004; Demirel, Yenigun & Onay, 2005; Janczukowicz, Zieliński & Debowski, 2008; Watkins & Nash, 2010)
Cheese Whey	3.92– 9.50	27,000– 60,000	50,000– 102,100	900– 14,000	55,000– 70,900	1270– 22,150	200– 1760	120–530	[(Demirel, Yenigun & Onay, 2005; Gannoun et al., 2008; Najafpour et al., 2008; Venetsaneas et al., 2009; Carvalho, Prazeres & Rivas, 2013)
Whey	9–5	590– 1210	1070– 2180	-	-	80–440	-	-	(Geilman et al., 1992)
Milk Permeate	5.55– 6.52	-	52,940– 57,460	-	11,610– 15,390	300–450	350–450	2500	(Farizoglu et al., 2007; Wang et al., 2009)
Ice Cream	5.10– 6.96	2450	5200	-	3900	3100	-	14	(Yang et al., 2007)

Butter	12.08	220– 2650	8930	2880	-	700– 5070	-	-	(Janczukowicz, Zieliński & Debowski, 2008)
Washing Wastewater	10.37	3470	14,640	3110	-	3820	-	-	(Janczukowicz, Zieliński & Debowski, 2008)

TP—Total Phosphorus; TN—Total Nitrogen; TSS—Total Suspended Solids; TS—Total Solids; FOG—Fats, Oils, and Grease; COD—Chemical Oxygen Demand; BOD—Biological Oxygen Demand. All units are in mg per L of wastewater.

2.2 Treatment Options

Biological treatments have been highly effective in reducing the organic load in various types of wastewaters. The nature of microbial systems allows for biological treatment methods to be versatile with respect to the type of wastewater being remediated. However, the physical conditions of the system, such as pH, temperature, and O₂ levels, need to be carefully controlled to avoid damaging the microbial consortia. It is also necessary to ensure that certain compounds do not exceed the microbes' tolerance, as an excess would kill them. One of the downsides to biological treatment methods is that there are certain substances (such as heavy metals) which cannot be processed as they would poison the microbial colonies. There are two main types of biological treatment methods: aerobic and anaerobic treatment. Anaerobic and aerobic treatments are commonly used in conjunction with one another as they each treat different components of the DWW. A combination of aerobic and anaerobic is a suitable solution for treating different components of DWW, replacing traditional one-phase biological systems (Zhao et al., 2020). A summary of the below treatment options can be seen on Table 2.2

Table 2.2 Summary of the removal percentages of the previously mentioned biological systems

Process	Process Type	%Removal	Advantages & Disadvantages	Reference
Activated sludge	Aerobic	BOD ₅ : 95%	<ul style="list-style-type: none"> • Accumulation of sludge • Low resistance to environmental shock • Effective at removing complex compounds 	(Lateef et al., 2014)
		COD: 95–98%		
SBR	Aerobic & Anaerobic	TS: 60%	<ul style="list-style-type: none"> • Highly flexible system • Lower productivity due to being a batch system 	(Malaspina et al., 1995; Kolev, 2017)
		TN: 40%		
		NH ₃ : >90%		
		TP: >90%		
MBR	Aerobic & Membrane filtration	COD: 95%	<ul style="list-style-type: none"> • Susceptible to membrane fouling • Low energy usage • Compact design 	(Scott & Smith, 1997; Stepanov, Solkina & Stepanov, 2019)
		BOD ₅ : 95–99%		
		TN: 96%		
		NH ₄ : 99.9%		
		TP: 80%		
PBR	Anaerobic	COD: 96%	<ul style="list-style-type: none"> • Prone to clogging • Low biomass retention 	(Janet Joshiba et al., 2019)
		BOD ₅ : 93%		
		VSS: 90%		

AFs	Anaerobic	COD: 90% FOG: 47%	<ul style="list-style-type: none"> • Ineffective at treating high FOG content DWW 	(Omil et al., 2003; Rajagopal et al., 2013)
UASBR	Anaerobic	COD: 96.3%	<ul style="list-style-type: none"> • Suitable for high COD streams • Prone to accumulation of fats on the sludge blanket • Higher resistance to environmental shock due to granular sludge use 	(Buntner, Sánchez & Garrido, 2013)
AF + SBR	Aerobic & Anaerobic	COD: 90%		(Omil et al., 2003)
UASBR + Aerobic Denitrification	Aerobic & Anaerobic	COD: 95%		(Britz, van Schalkwyk & Hung, 2004)

2.2.1 Aerobic Treatment Processes

Aerobic treatment occurs when the microbes are exposed to air and thus oxygen whilst anaerobic occurs without the presence of air. Aerobic treatment is effectively used to reduce the BOD₅ as well as the phosphorous and nitrogen content in dairy wastewater. Aerobic processes are additionally more cost-effective than anaerobic ones for the removal of fats from DWW (Zhao et al., 2020). Importantly, the removal of nitrogen is largely associated with the oxidation of ammonia into nitrates, which in turn reduces the odour of the DWW. Aerobic systems are associated with high biomass growth and are susceptible to clogging if solely used to treat DWW (Zhao et al., 2020). Additionally, aerobic treatments suffer from the high energy demand due to aeration. The following are aerobic treatment methods which have been used to treat DWW, both in a lab setting and on an industrial scale.

2.2.1.1 Activated Sludge

Activated sludge treatment methods are processes which introduce microbes into the wastewater stream to both treat the water and stimulate the growth of microbes. The microbial colonies are then separated from the bulk stream using a clarifier or filter, with a portion of the sludge being reintroduced to the reactor. A schematic representation of an activated sludge system can be seen in Figure 1. These methods are preferred for the removal of carbon, nitrates, and ammonia (“Water Access in South Africa | Water for all”, n.d.). The constant recirculation of sludge allows for the microbes to acclimatize to the waste stream composition and increases the performance of the system, the longer it is operated for. These systems are also effective at breaking down complicated compounds such as lactose, FOG, and proteins (Bae, Han & Tak, 2003). Studies have shown a reduction

of BOD₅ in DWW of up to 95%, using activated sludge (Lateef et al., 2014), demonstrating its effectiveness at reducing organic compounds. Activated sludge processes tend to suffer from low resistance to environmental shocks and a build-up of toxins. In addition, sludge settling can be problematic, which reduces the recovery of biomass. These shortfalls have been overcome using granular sludge, i.e., a type of sludge which forms solid spherical granules from microorganisms and flocs. These granules have a higher resistance to shocks and better settling properties (Vashi, Iorhemen & Tay, 2018). Another downside to activated sludge processes is the disposal of sludge. This can be somewhat mitigated by reusing the sludge as fertilizer or as part of an animal feedstock.

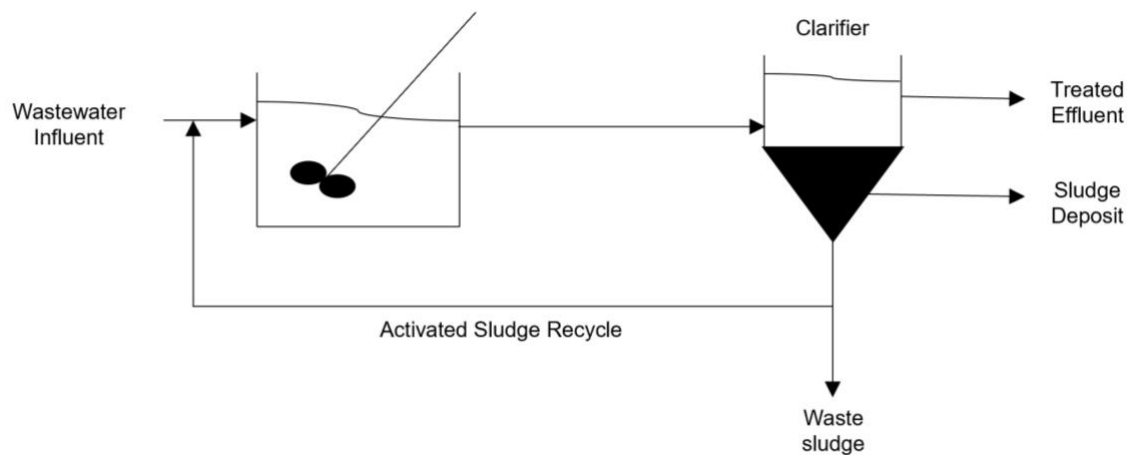


Figure 2-1 . Schematic diagram of an activated sludge process(Kolev, 2017).

2.2.1.2 Sequencing Batch Reactors

Sequencing batch reactors (SBRs) comprise a series of process steps within a single bioreactor. These steps occur in the sequence of filling, reacting, settling, decanting, and idling. The filling stage is the addition of the DWW and liquor, which contains microbes. The reaction stage can consist of cycles of aerobic and anaerobic phases.

This can be achieved by alternating between periods of aerating and no aeration. For the settling stage, the aeration and mixing are stopped for the suspended solids to settle and be easily separated from the treated water. The decanting stage involves the removal of the supernatant fluid (treated wastewater). The idle stage is mostly needed for multi-reactor systems where there is a time delay between the filling stage in between units (Zhao et al., 2020). A schematic representation of a SBR system can be seen in Figure 2. SBRs have been observed to reduce COD by up to 95%, total solids by up to 60%, and up to 40% of the total nitrogen (Janet Joshiba et al., 2019) from DWW. These systems do suffer from relatively low removal rates for phosphorous, nitrogen, and limited solid removal. A study (Malaspina et al., 1995) utilizing a hybrid up-flow–downflow reactor was able to achieve a stable treatment of DWW with an average organic loading rate of 10,000 mg_{COD}/L/day. This system demonstrates the flexibility of an SBR system as it contains both downflow pre-acidification chambers and up-flow methanation chambers. It achieved a COD conversion into biogas of 98%, with an additional removal of NH₃ and TP of over 90% (Bae, Han & Tak, 2003). Studies have also been carried out with an SBR coupled with membrane filtration to remedy these limitations. However, the inclusion of a membrane brings the new challenge of membrane fouling, which requires additional strategies to mitigate this risk. SBRs also have the drawback of being a batch system, which necessitates the loading and unloading of the reactors along with cleaning for each batch. This results in lower productivity than that of a continuous system.

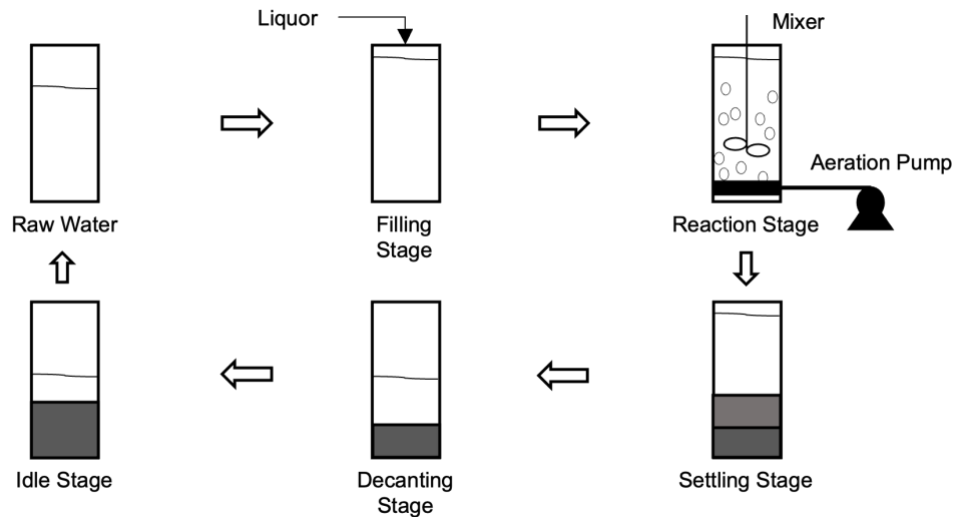


Figure 2-2 Schematic of an SBR treatment system (Kolev, 2017).

2.2.1.3 Membrane Bioreactor

Membrane bioreactors (MBR) incorporate the principle of membrane filtration with a bioreactor. A schematic representation of an MBR can be seen in Figure 3. There are two main configurations of MBR, one where the membrane is submerged within the reactor and one where the membrane is located externally with a recycle back into the reactor. MBRs with submerged membranes are more popular as they are compact and have low energy usage (Goli et al., 2019). One of the downsides of submerged membranes is that they are more prone to fouling than the external configuration. This is an issue that must be dealt with for most membrane systems. However, with an MBR, soluble microbial products add to the risk of fouling. This can be somewhat mitigated by treating the membranes with sodium metabisulfite to prevent microbial growth on the membrane (Meyo et al., 2021). In lab-scale operations, when treating DWW, an aerobic MBR has been observed to decrease the BOD₅ level by up to 99% and the ammonium levels by up to 99.9% (Stepanov, Solkina & Stepanov, 2019). Another study, treating high-strength ice-cream wastewater (13,300 mg_{COD}/L & 6500 mg_{BOD₅}/L), was able to

achieve a COD and BOD₅ reduction of over 95%, TP by 80% and TN by over 96% (Scott & Smith, 1997).

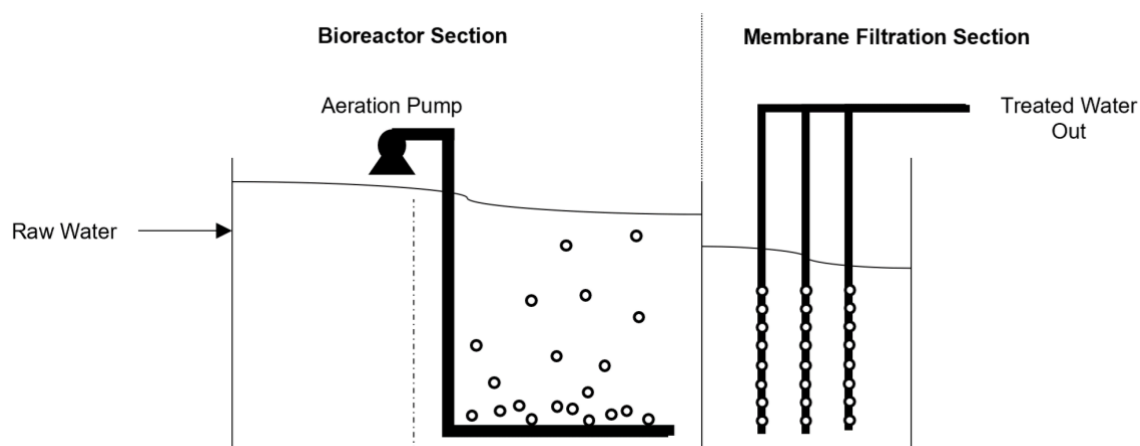


Figure 2-3 Schematic diagram of a membrane bioreactor (Stepanov, Solkina & Stepanov, 2019).

2.2.2 Anaerobic Processes

Anaerobic treatment processes are primarily aimed at reducing the organic content of wastewater and are suited for the remediation of wastewater with high organic loads (Zhao et al., 2020). Anaerobic systems are also effective at decreasing the nitrites and nitrates by reducing them into nitrogen gas. These systems are, however, less effective at removing the FOGs, ammonia, and phosphorous (“Water Access in South Africa | Water for all”, n.d.) and may require further downstream treatment or a suitable pre-treatment method to meet discharge standards. FOGs and other complex polymeric compounds are difficult to break down using anaerobic consortia and usually require a hydrolysis or oxidation step to break them down into smaller, soluble molecules such as short-chain fatty acids and alcohols (AS & NN, 2001). Hydrolysis is the process whereby a complex polymeric organic compound is broken down into smaller soluble compounds in the presence of water (Hanrahan, 2012; Tocchi et al., 2012). This process can be catalysed by the addition of acids or enzymes to the solution. This is relevant for

anaerobic processes as the lipid content is usually a limiting factor for performance (Pascale et al., 2019). Anaerobic treatment also has the potential of methane production from organic-rich wastewater. Common uses of anaerobic technology include anaerobic filters, sludge blanket reactors, and packed bed digestors.

2.2.2.1 Anaerobic Filters

Anaerobic filters (AFs) are reactors that are packed with a porous support media with biomass in the packing void. AF reactors contain at least two filtration chambers within the system, with the filter media used to prevent biomass from passing through. A side effect of these filtration chambers is enhanced solid removal, which can be observed (Janet Joshiba et al., 2019). A schematic representation of an AF system can be seen in Figure 4. This treatment method is most appropriate for wastewater with low organic loading and struggles with high-strength wastewater streams (Karadag et al., 2015). As the organic material in the wastewater stream degrades, it adheres to the support media, which acts as a physical barrier. The selection of media is an important aspect of the AF system, as the characteristics of the media will affect the microbial activity and, ultimately, the performance of the system. Studies have shown that a reduction in COD of up to 90% can be achieved when treating a DWW stream (Rajagopal et al., 2013). However, it is not effective at dealing with high-fat DWW, showing low FOG removal rates of around 47% (Omil et al., 2003). This highlights the need for the further downstream treatment of the effluent and sludge produced by AF systems when treating wastewater with high organic loading. Alternatively, appropriate pre-treatment systems can be implemented to overcome these difficulties. This has been successfully implemented by the addition of an SBR to remedy the low FOG removal, where a 99% overall FOG removal was achieved (Omil et al., 2003).

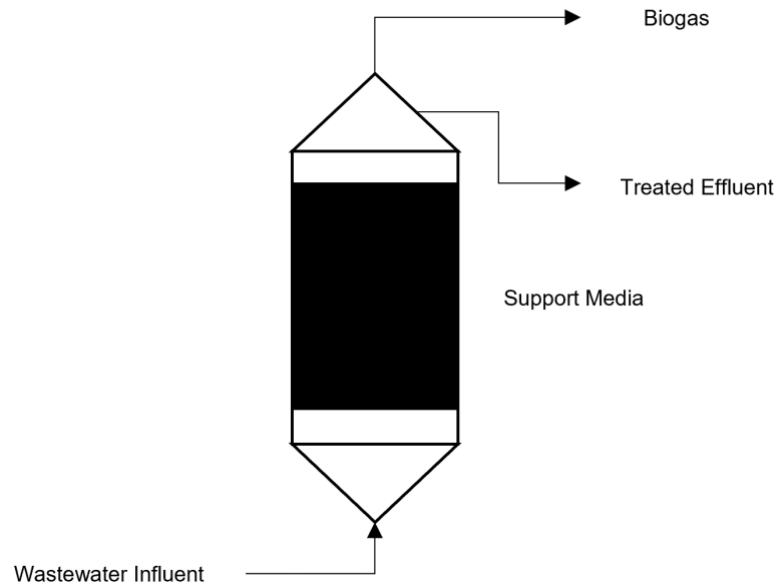


Figure 2-4 Schematic diagram of an anaerobic filter (Kolev, 2017).

2.2.2.2 Anaerobic Packed Bed Reactors

Anaerobic packed bed reactors (APBRs) utilize sludge which has been immobilized, through which the wastewater is pumped either in an up-flow or downflow configuration. This reactor uses a packing material at the top and/or bottom to fix the biomass in place. This packing material is intended to support biofilm formation and not to act as a physical barrier for the solids contained within the DWW. This is a very similar configuration to that of an anaerobic filter; however, there are no filtration chambers present within the APBR configuration. An issue with this technology is that it can be prone to clogging due to the accumulation of biomass. This issue can be bypassed by allowing biomass to flow out of the reactor with the effluent stream, necessitating further downstream treatment. This solution also raises another issue of low biomass retention within the system. A remedy to this would be to use granulated sludge within the reactor body. The benefits of using granulated sludge would be higher shock resistance, higher toxicity resistance, and higher retention within the reactor. Packed bed

reactors were highly effective at reducing COD, BOD₅, and volatile suspended solids content of up to 96%, 93%, and 90%, respectively (Janet Joshiba et al., 2019).

2.2.2.3 Up-Flow Anaerobic Sludge Blanket Reactors

Up-flow anaerobic sludge blanket reactors (UASBRs) are some of the most used systems for the treatment of DWW. These types of reactors are fed wastewater through the bottom of a reactor column which is packed with anaerobic sludge granules that have been somewhat fixed in place. The treated effluent is then extracted from the top end of the reactor, with the methane being siphoned off through a vent at the top of the reactor. A schematic representation of an UASBR can be seen in Figure 5. UASB reactors are highly suited for treating wastewater streams which have a high COD content of up to 40,000 mg/L (Buntner, Sánchez & Garrido, 2013), making them an ideal choice for treating DWW. They have been observed to achieve a COD reduction of up to 96.3% with a hydraulic retention time (HRT) of 3 h (Passeggi, López & Borzacconi, 2012). A common issue experienced with ASB reactors is the accumulation of organic material in the sludge blanket, possibly due to the difficulties in the breakdown of the FOG of anaerobic bacteria. This problem can be resolved by implementing a hydrolysis stage before the reactor or by increasing the HRT of the reactor (Passeggi, López & Borzacconi, 2012).

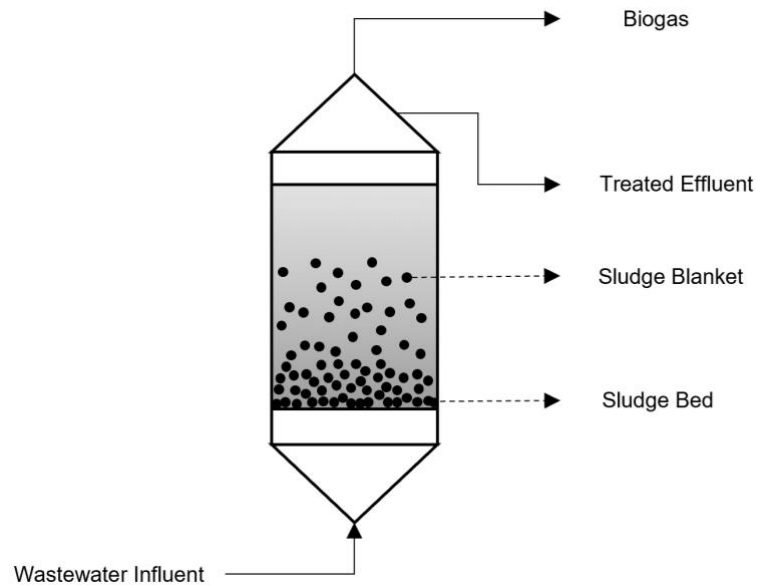


Figure 2-5 Schematic diagram of an up-flow Anaerobic Sludge Blanket Reactor (UASBR) (Goli et al., 2019).

2.2.3 Aerobic–Anaerobic Systems

Aerobic–anaerobic systems have been widely investigated for various wastewaters with high organic contents. The combination of these two technologies allows for a more complete remediation of DWW as each stage focuses on different pollutants within the wastewater. The aerobic stage effectively reduces the ammonia, phosphorous, H_2S , and BOD_5 content, whereas the anaerobic stage reduces the COD and nitrate content of the water (Zhao et al., 2020). In theory, it should be possible to achieve removal yields from a two-stage system similar to the yields that can be achieved by the individual processes. This is due to the different pollutants that are being processed by each individual system. In a practical study of an anaerobic filter combined with an aerobic one, an SBR was able to achieve a COD removal of 90% from a high-strength dairy stream with an influent COD content of approximately 8000 g/L (Omil et al., 2003). Another study achieved a COD removal of 95%, in a full-scale plant, using an up-flow anaerobic sludge

bracket reactor followed by aerobic denitrification (Britz, van Schalkwyk & Hung, 2004). These systems are highly versatile as the selection of reactor configurations is not limited to those found in the literature and can be evaluated on a case-to-case basis. This ultimately depends on the strength of wastewater being treated and the discharge standards to be met. One of the main benefits of using aerobic–anaerobic systems is that a more complete remediation of the wastewater can be achieved. This means that the treated wastewater has the potential to be used for a larger variety of applications when compared to standalone aerobic or anaerobic systems.

2.3 The Effects of Experimental Parameters on the Performance of Biological Treatment Systems

Certain shortfalls that occur with the biological systems outlined above can be resolved by manipulating various physical parameters. However, this is not a straightforward task when dealing with microbial colonies as they have a complex makeup and can sometimes express unwanted behaviour if taken out of their ideal conditions. Conversely, microbial systems can be highly adaptable to sustained changes in their environments and can therefore be manipulated to perform in a more favourable way through the changes in physical parameters. The following parameters are considered as critical as they can have significant effects on the performance of a biological system without inherently changing the feed characteristics.

2.3.1 pH Adjustment

There are three main groups of microorganisms which are found in AD: acidogenic (acid producing), acetogenic (acetate-producing), and methanogenic (methane-producing). Each of these has a pH range which it prefers. Acidogens have an

optimal pH range between 5.2 and 6.5; acetogens from 6.6 to 7.6; and methanogens from 7.5 to 8.5 (Demirer & Chen, 2004). The pH of the system should be carefully controlled because an imbalance, either too high or too low, can lead to an overproduction or accumulation of certain compounds, which can then result in digester failure (Bella & Rao, 2021). It is important to select an appropriate pH based on the specific need of the system. However, the reactions which occur during the remediation of DWW produce volatile fatty acids (VFAs) from the breakdown of FOGs, which alter the pH as the process occurs. This necessitates the need for constant pH adjustment throughout the process in order to remain at an optimal pH whilst VFAs are being produced.

2.3.2 *Dissolved O₂ Levels*

Aerobic digestion is enhanced by higher levels of dissolved O₂; it therefore follows that anaerobic digestion will be enhanced by the removal of dissolved O₂ from the wastewater. One straightforward approach to eliminate dissolved oxygen is to pass another gas through the wastewater. Some potential deoxygenating agents for anaerobic digestion are nitrogen or biogas. Biogas is a convenient option as an amount of CO₂ and methane is produced by anaerobic digestion. However, the use of biogas poses an environmental issue due to methane being a greenhouse gas. Nitrogen gas is a suitable gas to use as it is non-reactive and does not pose any inherent environmental risks (Bella & Rao, 2021). Another method of deoxygenation is to increase the temperature of the wastewater. The heating of the water decreases the solubility factor between oxygen and water and allows for the dissolved gas to escape. Pressure is another factor which has an inversely proportional effect on the solubility factor between oxygen and water, which is described by Henry's Law. In other words, the lower the pressure of the system, the less dissolved oxygen will be present within the wastewater. However, both changing the temperature and the pressure within a system can be energy-

intensive and costly to run on a large scale. The use of deoxygenating agents is not energy-intensive; however, it may increase the operating costs as large quantities of gas may be needed when implemented in a real-world scenario.

2.3.3 *Hydraulic Retention Time*

Hydraulic retention time (HRT) refers to the average time that a liquid substrate will spend within a reactor. HRT is largely dependent on the type of reactor being used and the organic load of the wastewater being treated. A small HRT can sometimes result in high biomass washout, whereas a long HRT can require large reactor volumes (Bella & Rao, 2021). The growth of the various groups of microorganisms used in AD are favoured by different HRTs. Methanogens prefer a longer HRT period as opposed to acidogenic bacteria, which prefer low HRTs. The determination of optimal hydraulic retention time (HRT) is a complex decision that requires the consideration of both the process and the desired outcomes, and it should be evaluated on a case-by-case basis. As previously mentioned, increasing the HRT improved the ability of an ASB reactor to better metabolize FOGs, which is the prevailing shortfall of anaerobic systems. A higher HRT usually results in greater reactor performance in terms of removal efficiency and has been observed to do so for an SBR with dairy wastewater influent COD levels between 10,000 mg/L and 20,000 mg/L (Tocchi et al., 2012). This system was shown to increase the removal efficiencies of all major components of interest in the wastewater for longer HRT periods. However, there is a point of diminishing returns for a system where increasing the HRT will yield either minimal increases in removal efficiency or efficiencies that have exceeded the levels which are required for the system. HRT is an easily manipulated variable, which can produce significant results when optimised according to the needs of the system.

2.3.4 Aeration and Agitation

An important factor in aerobic treatments is the aeration which is introduced into the system. It is the driving force behind the reactions which aerobic digestion is utilized for, namely, the oxidation of ammonia and the breakdown of complex organic compounds. Therefore, an optimal aeration regime is essential for the performance of any treatment method used. At lower aeration levels, it was observed that there was a significant decrease in COD removal efficiency and an even more drastic decrease in the efficiency of ammonia removal (Tocchi et al., 2012). This indicates that the available oxygen is being used to oxidise the carbon substrates in the wastewater as opposed to the ammonia. The most likely explanation is that ammonia requires a larger amount of O₂ when compared to the organic components. The same study also observed that there was a decrease in biodiversity at lower aeration levels, likely due to the difficulty of certain species of microbes to adapt to prolonged anoxic conditions (Dubber & Gray, 2011). This could result in a decrease in the removal of certain components as the microbes best suited for their breakdown would no longer be present in the colonies. Higher levels of aeration were seen to improve the removal of both ammonia and COD, with the highest levels showing the greatest performance. Aeration has the additional effect of dislodging a certain percentage of dissolved gases, which is also the principle behind using nitrogen gas to reduce the DO content in the wastewater.

Agitation or physical mixing within an aerobic bioreactor has been seen to increase both cell growth and enzymatic activity. This is due to the agitation facilitating the efficient dispersion of macromolecules within the medium (Feng et al., 2003). High agitation can negatively affect both the growth and enzymatic activity of the microbes due to the shearing effect (Shioya et al., 1999). This can prevent larger colonies of biomass from forming due to the lack of fluid stability. Similar to

aeration, agitation has the benefit of allowing some of the dissolved gas, which is produced by the treatment process, to escape from the water.

2.3.5 *Temperature*

The temperature plays a critical role in the reactions involved in wastewater remediation. Different temperature ranges can have both favourable and unfavourable effects on the performance of a bioreactor. High temperatures can cause the denaturation of proteins within the microbes, causing them to lose their enzymatic activity and die. Low temperatures can cause the microbes to become dormant, inactive, or die. It is, therefore, essential that the temperatures within a bioreactor are carefully controlled and are not allowed to go beyond the range within which the microbes are able to thrive. However, choosing an optimal temperature for which to run a system at depends on the specific microorganisms used within it. Most anaerobic digesters used for wastewater treatment typically operate at mesophilic conditions, between 35 °C and 37 °C. Temperature needs to be carefully controlled as large fluctuations can be detrimental for these processes (Goff, Hill & Ferrer, n.d.).

An important effect which temperature has on DWW has to do with the physical properties of the fats found within the wastewater. The main lipids found in milk are known as triglycerides, with the main fatty acids being long-chain fatty acids, accounting for around 67%, and with the remainder being short-chain fatty acids. This results in milk fats having a melting point temperature of 37 °C (Goff, Hill & Ferrer, n.d.), which is significant as it means that the fatty solids in DWW can be easily removed from the wastewater through physical methods, namely, reducing the temperature of the wastewater followed by passing the water through a strainer to remove the larger solid particles.

2.4 Accessory Treatment Options

Another route for overcoming the shortfalls of certain biological systems is the addition of either pre-treatment or post-treatment systems to compensate for the weaknesses in the main treatment process. This can sometimes be easier than manipulating system parameters to obtain a better performance, as the accessory processes can be specifically tailored for the main system and can be implemented without too much trouble. The downside is that this is usually a more costly option. The following are processes which can be integrated into biological systems smoothly, as they do not utilize harsh chemicals or change the feed characteristics in a way that would interfere with the main biological system. A summary of the below accessory treatment options can be seen in Table 3.

2.4.1 *Hydrolysis*

Anaerobic systems generally have difficulty in degrading FOGs and ammonia, as these are oxidation reactions which occur. High concentrations of FOGs can contribute to clogging within the reactor as they are poorly broken down through AD. An effective solution to these problems is a hydrolysis stage before the anaerobic digester. An up-flow ASB reactor (UASB) was observed to be able to effectively treat wastewaters with high FOG contents which had been hydrolysed before treatment. It was noted that when fed unhydrolyzed wastewater, the UASB showed unstable COD removal and a tendency to accumulate some of the fats within the sludge (Leal et al., 2006). This suggests that the inclusion of a separate hydrolysis stage can mitigate the risk of clogging within the reactor. Additionally, studies show that DWW that has been hydrolysed increases the production of methane when compared to unhydrolyzed DWW (Pascale et al., 2019). This can be attributed to the oxidation degradation of the complex organic molecules into more easily digested compounds.

One study, using an up-flow anaerobic sludge blanket reactor, observed that the inclusion of a hydrolysis pre-treatment stage showed small improvements in the COD removal of the reactor, with an average of 90% COD removal compared to the 82% which was observed using unhydrolyzed wastewater. However, it was also seen that utilizing high-strength unhydrolyzed wastewater (1000 mg_{FOG}/L) caused FOG build-up in the reactor and resulted in sludge with 25% higher FOG content than that observed from the reactor utilizing hydrolysed wastewater (Leal et al., 2006). Additionally, the FOG content of the effluent stream from the reactor using the high-strength (1000 mg_{FOG}/L) hydrolysed wastewater was observed to be between 100 and 200 mg_{FOG}/L. This is significantly lower than the FOG content of the unhydrolyzed effluent stream, which was observed to be in the region of 700 mg_{FOG}/L (Leal et al., 2006). Another study showed a COD removal of 90% and a biogas production of 4710 mL using pre-hydrolysed wastewater, compared with a COD removal of 66% and biogas production of 2330 mL when using unhydrolyzed wastewater of the same initial FOG content (Mobarak-Qamsari et al., 2012). These studies highlight the weakness of single-stage anaerobic digestion when it comes to processing high FOG-content wastewater. The addition of an enzymatic pre-hydrolysis treatment stage has been seen to improve the efficiency of single-stage anaerobic digestors in processing high-strength DWW by increasing both the COD and FOG removal efficiency which results in a more complete remediation of the wastewater. An important effect of reducing the FOG content before anaerobic digestion is that the risk of clogging is reduced within the system. This is an essential factor when considering the real-world operation of a continuous process as it results in a more stable system with less unwanted stoppages.

2.4.2 Coagulation

Coagulation/Flocculation are currently the most commonly used processes for the removal of suspended and dissolved solids, colloids, and organic components in

industrial wastewater (Mateus et al., 2017). There are two types of coagulants commonly used in wastewater treatment, namely, inorganic and organic coagulants. Inorganic coagulants are usually metal-based salts, usually containing aluminium or iron. The use of Alum ($X'Al(SO_4)_2 \cdot 12H_2O$, where X is a metal ion such as potassium or sodium) as a coagulant in the treatment of DWW was observed to reduce the turbidity of the water by 95% and reduce COD by 68% (Loloei et al., 2014). This was further enhanced by the addition of polymeric coagulants, resulting in a reduction in COD by 85%. However, inorganic coagulants produce large amounts of metal-rich floc, which must be further treated before it is disposed of. They can also alter the pH of the water requiring pH control and corrosion-resistant equipment. When trying to integrate inorganic coagulants with biological systems, it is important to evaluate whether the microbial colonies will be able to function unhindered.

Organic coagulants can either be polymeric or natural coagulants. Polymeric coagulants produce longer polymer chains without any metals or hydroxides and produce smaller volumes of floc. They also do not impact the pH of the water. They also produce low-density floc, which does not always settle well. Natural coagulants are being investigated widely and there have been many studies which show their effectiveness in the treatment of wastewater. An extract made from the bark of *Guazuma ulmifolia* was used as a coagulant for DWW (3037 mg_{COD}/L & 1283 mg_{BOD₅}/L) and was observed to remove 95.8% of the turbidity, 76% of the COD and 81.2% of the BOD₅ (Muniz, Borges & Silva, 2020). Natural coagulants' low cost and eco-friendliness make them a suitable alternative to the more commonly used synthetic coagulants. Another benefit of natural coagulants is that they are easier to integrate with biological systems as they do not have an impact on the pH of the resulting effluent and are not introducing any potentially harmful substances into the system.

2.4.3 Membrane Filtration

Membrane filtration methods such as microfiltration are effective at significantly reducing the TSS within the wastewater. However, it has little effect on the TN, COD, and BOD₅ content and is commonly used as a pre-treatment step in a wastewater treatment process. Reverse osmosis (RO) is a viable option for dairy wastewater treatment and has been observed to reduce the TN and TOC by 94% and 84%, respectively (Bortoluzzi et al., 2017). Nanofiltration has been observed to be effective at reducing COD and TSS levels but does not remove all ions of interest, such as phosphates and nitrates, from the wastewater stream [(Andrade et al., 2014). There have been some applications of nanofiltration and RO being used in conjunction with a bioreactor, which was observed to greatly improve the overall ion removal in addition to enhancing the reduction in COD and TSS. There are issues associated with RO and membrane filtration, which affect the long-term uses of this type of system. RO is an extremely energy-intensive process and is sometimes impractical to operate at the specifications required (Zhao et al., 2021). The replacement of this technology can be costly in the event of physical damage or fouling. In addition, this process produces a highly concentrated retentate which needs to be disposed of. This is a major concern as the disposal of the concentrate that is formed is more problematic than that of the wastewater itself. However, this downside can be somewhat mitigated when used in conjunction with a biological treatment system, as the water to be processed will contain already reduced amounts of TN, COD, and TS. This application can be observed in an MBR.

Table 2.3 Summary of Accessory Treatment Options

Process	Examples	Merits & Demerits	Reference
Hydrolysis	<ul style="list-style-type: none"> Formic Acid <i>P. aeruginosa</i> extract 	<ul style="list-style-type: none"> Able to effectively break down FOGs Enhances methane production Able to improve efficiency of anaerobic systems Reduces risk of clogging in anaerobic systems 	(Li & Zhang, 2002; Mobarak-Qamsari et al., 2012; Pascale et al., 2019; Bella & Rao, 2021; Khorasani, Khodaparasti & Tavakoli, 2021)
Coagulation	<ul style="list-style-type: none"> Alum Ferric chloride Polyaluminium chloride <i>Acacia mearnsii</i> tannin extract + polyaluminium chloride <i>Guazuma ulmifolia</i> bark Okra Passion fruit seeds 	<ul style="list-style-type: none"> The selection of coagulant needs to be thorough to ensure that there are no negative effects on the microorganisms in the system May require pH control Natural coagulants are relatively low cost Natural coagulants are usually safe to use in conjunction with biological systems 	(Mobarak-Qamsari et al., 2012; Loloie et al., 2014; Mateus et al., 2017; Dela Justina et al., 2018)
Membrane Filtration	<ul style="list-style-type: none"> Nanofiltration Reverse Osmosis 	<ul style="list-style-type: none"> Energy-intensive Prone to membrane fouling Produces high concentrated effluent Able to be easily integrated into biological systems Costly to replace 	(Andrade et al., 2014; Bortoluzzi et al., 2017; Muniz, Borges & Silva, 2020)

2.5 The Way Forward and Conclusions

The treatment of DWW is an essential part of an ever-expanding dairy industry, with advancements in these processes not only affecting this industry but all industries processing high-strength organic wastewater. The potential to reclaim water that would otherwise be discarded is vital in South Africa due to the water shortages experienced by a large portion of the population. Much of the waste produced from the processing of dairy products has the potential to be remediated using biological methods. Biological treatment methods have been proven to be highly effective in the remediation of dairy wastewater from all sources and strengths. Apart from the versatility of these systems, they are also more environmentally friendly than many chemical-based processes and physical treatment methods.

The two categories of biological treatment, aerobic and anaerobic, each have their own advantages and disadvantages when it comes to the bioremediation of DWW. Aerobic treatment is effectively used to reduce the BOD₅, the phosphorous and nitrogen content as well as the odour of the DWW. Aerobic processes are additionally more cost-effective than anaerobic ones for the removal of fats from DWW. On the other hand, aerobic treatments suffer from high energy utilization due to the aeration required. Anaerobic treatments are primarily aimed at reducing the organic content of wastewater and are suited for the remediation of wastewater with high organic loads. Additionally, these systems also have the potential of methane production from organic-rich wastewater. Anaerobic systems are, however, less effective at removing the FOGs, ammonia, nitrates, and phosphorous and may require further downstream treatment or a suitable pre-treatment method to meet discharge standards. Biological processes have several factors to which they are sensitive, such as pH, temperature, hydraulic retention time, agitation, and the dissolved O₂ levels in the wastewater.

The optimization of these parameters has not been fully explored when investigated simultaneously to establish whether any synergistic effects exist or not. Some further aspects which need to be considered for the real-life implementation of these technologies are highlighted below.

- The effects that various parameters have on the efficiency of aerobic and anaerobic digestion must be examined. It is important to examine and understand the effects that changes in multiple parameters will have collectively, and possible synergistic effects between these parameters, as this will aid in developing better control strategies for optimal DWW treatment.
- The optimal operating conditions for each of these parameters based on the discharge standards to be met and the varying characteristics of different DWWs must be determined.
- Future work must consider developing a more complete understanding of the reactions taking place in both aerobic and anaerobic digestion to be able to better optimize each process individually and together. This would be a big step in understanding the synergies between the two processes when used in a multi-stage system for the improved efficacy of DWW treatment processes.
- Considerations must be made into optimizing pre-treatments that can be used in conjunction with a biological treatment system, and to evaluate the effects that this will have on the remediation efficiency of the process. This will be primarily aimed at treating the wastewater and not at the production of biogas or methane.
- The viability of accessory treatment options must be made a key consideration and evaluated for the purposes of overcoming the various shortfalls of certain biological processes.

3 PROJECT OUTLINE

3.1 Aims and Objectives

The primary aim of this project is to evaluate the performance of the lab-scale plant in treating Domestic Wastewater (DWW), focusing on the removal of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Fat, Oil, and Grease (FOG), as well as specific compounds of interest. Concurrently, the project aims to optimize each stage of the treatment process tailored for DWW. Additionally, the study seeks to determine the optimal configuration and operating conditions of the system to achieve the best performance in line with city discharge standards. Therefore, in order to achieve the aims of this study, the following specific objectives have been set out:

- Analyse the physical and chemical properties of the DWW before and after remediation.
- Optimize each process step of the multi-integrated lab scale plant to maximize the performance of each individual step and the overall system.
- Assess the effectiveness of the multi-integrated lab scale plant to bioremediate DWW.
- Identify any drawbacks of the system and recommend any potential improvements.
- Evaluate the effectiveness of okra as a coagulant for DWW.
- Identify the component of okra which has the best coagulation properties.
- Determine an ideal dosage for okra for the purpose of coagulation.

3.2 Key Questions

1. What are the characteristics of DWW?
2. What is the role of enzymes on the pre-treatment of DWW?
3. What would be the optimal dosage of enzymes in the pre-treatment stage?
4. What effects will the pre-treatment stage have on the efficiency of the EGSB?
5. What are the parameters affecting the performance of the EGSB reactor?

6. How effect will Okra be as a coagulant for DWW?
7. What component of the Okra will be the most effective when used as a coagulant?

3.3 Hypothesis

The efficacy of the multi-integrated lab-scale plant in treating DWW will mirror its success in treating wastewater with high FOGs, COD, and BOD. Additionally, the specified dosage of Enzymes in the pre-treatment stage will optimize treatment effectiveness. Moreover, increasing the aeration rate during the pre-treatment stage will enhance unit performance through improved mixing, given its aerobic nature. Lower dissolved oxygen levels will favourably impact the performance of the EGSB due to its anaerobic process. Furthermore, the use of okra as a coagulant will yield significant decreases in TS and COD. Both the flesh and seeds of okra will be able to be effectively used as a coagulant.

4 MATERIALS & METHODS

4.1 Pre-Treatment & EGSB Operation Methodology

4.1.1 Collection of DWW

Samples of DWW were collected from a dairy processing plant in Cape Town, Western Cape, South Africa. These samples were obtained during the cleaning processes on the plant, when the vessels were being washed and sanitized. Sampling times were restricted to times when the wastewater was particularly opaque and were ended when the wastewater stream was seen to be turning translucent. Prior to sampling, the 25L containers that were used, were washed thoroughly with tap water and soap, were sanitized with a 70% alcohol solution, and were left to dry. These samples were stored at 4 °C, and when required for experimental use, were allowed to rise to room temperature (21 °C).

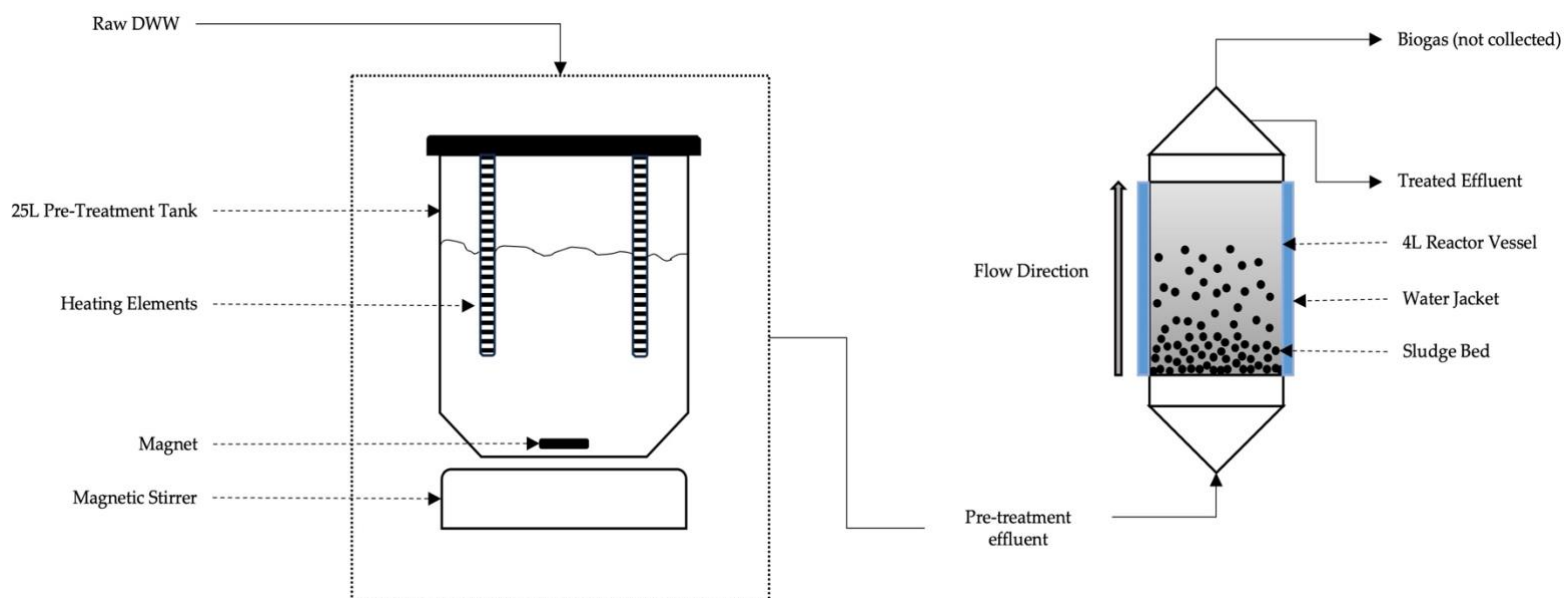


Figure 4-1: Schematic diagram of the pre-treatment and EGSBR system used to conduct this study

4.1.2 Equipment

The experimental setup involved several key pieces of equipment, including a 4-liter reactor vessel equipped with a water jacket to facilitate temperature control. Additionally, four peristaltic pumps were employed to manage fluid flow within the system, ensuring precise and controlled conditions. To further support

temperature regulation and stability, a water bath was utilized as part of the experimental apparatus. For a pre-treatment tank, a 20L bucket was used, with heating elements fixed to the lid. For agitation, a mechanical stirrer was used. Figure 4-1 illustrates the system equipment, and the flow of materials within it.

4.1.3 EGSB Reactor

The EGSB reactor is an up-flow reactor column being used as the primary method for organic matter removal in this treatment system. The column contains ceramic marble packing in the underdrain for the purpose of sludge retainment. A recycle stream is included in this column to regulate the upward flow within the reactor. This reactor will be operated between 35 and 37 °C, controlled by a water bath and a heat exchanger. The inoculation period of the EGSB will be 72 hours, at a stagnant state without DWW addition. Thereafter the reactor will be run continuously at anaerobic conditions for the entire period of the study. The microbial activity within this reactor was observed over the course of its operation and an optimal residence time was chosen based of performance metrics.

4.1.4 Pre-treatment Tank

The pre-treatment tank is the unit where the raw DWW will be dosed with the bioremediation agent. The purpose of the bioremediation agent in this stage is primarily for the hydrolysis of the FOGs in the DWW stream. Enzymes also oxidises the ammonia (NH_3) into NO_3^- and NO_2^- . There is the additional effect of reducing the population for H_2S -producing bacteria, which in turn diminishes the odour of the wastewater stream (Meyo et al., 2021). The pre-treatment stage will operate as a batch process, at room temperature, for a 24-hour period while being aerated. Once this time has elapsed the aeration will be stopped to allow for flocculation to occur. The wastewater will then be screened, to remove the flocs and a portion of the solid particles, and then emptied into a holding tank for the EGSB reactor.

4.1.5 Morma Description

Morma, an industrial biological agent, represents a breakthrough in the treatment of dairy processing wastewater (DWW). Comprising a blend of anaerobic bacteria, Morma lies dormant until it encounters optimal conditions, notably DWW. The manufacturers recommend an optimal operating temperature of 38°C to ensuring maximum efficacy. Initially deployed as a drain cleaner within the dairy processing plant, where the DWW was collected from, Morma's potential extends beyond mere unclogging. Its introduction was rooted in the intention of mitigating fats, oils, and grease (FOG) as well as total solids (TS) in DWW. This application is particularly significant due to the persistent challenges faced by traditional anaerobic digestion methods, such as the Expanded Granular Sludge Bed (EGSB) reactor used in this study, which is prone to clogging and biomass washout, exacerbated by excessive FOG content. Thus, Morma emerges as a promising solution, poised to revolutionize wastewater management in industrial settings.

4.1.6 Inoculation

A 4-liter cylindrical jacketed reactor was employed for this experimental run. The jacket, filled with water, facilitated precise temperature control within the reactor. To initiate the inoculation process, 300 grams of porous stones were introduced as packing material. Subsequently, 600 mL of sludge from a brewery and a Morma-DWW mixture, dosed to 30mL/ L DWW, were added to the reactor. The Morma-DWW mixture continued to be added until the reactor was nearly full, allowing for a slight buffer to accommodate potential increases in volume. The sealed reactor was left undisturbed for 72 hrs, with the temperature maintained at 36 °C to promote optimal biomass growth.

4.1.7 Pre-treatment

The experimental procedure was initiated with the anaerobic pre-treatment of 20 litres of Dairy Wastewater (DWW) per batch, incorporating a designated anaerobic

pre-treatment process. Following this, Morma was dosed into the pre-treated DWW at a concentration of 10 mL per litre. To ensure uniform distribution and effectiveness of the treatment, a mixer, along with a mixing stone, was employed for thorough agitation until a homogenous mixture was achieved. Throughout the experimental process, a constant temperature of 38°C was maintained using two fish tank heating elements under anaerobic conditions.

To assess the impact of Pre-treatment (PT) on the anaerobic digester, variations in Hydraulic Retention Time (HRT) were implemented during the 100 days of operation. Initially set at 36 hrs, the HRT was reduced to 24 hrs after the first 40 days of operation. Subsequently, a further reduction to 12 hrs occurred after an additional 30 days, maintaining this configuration for the remaining 30 days of the study period. This systematic adjustment allowed for a comprehensive examination of the PT effects on the anaerobic digestion process over the course of the operational timeframe.

4.1.8 Operation

The pre-treated Dairy Wastewater (DWW) underwent initial processing before being introduced into a continuously agitated 25L feed tank. Subsequently, the treated DWW was pumped into the reactor at a steady flow rate of 0.15L/hr, with the reactor operating under anaerobic conditions and maintaining a constant Hydraulic Retention Time (HRT) throughout the study. To enhance efficiency, a recycle stream was extracted from the top of the reactor and combined with the pre-treated feed before being reintroduced into the reactor. The final product stream, obtained from the top of the reactor, served as a designated sampling point for assessing the quality of the reactor product.

4.1.9 Analyses

The experiment included a thorough assessment of the system's performance by analyzing key indicators. Chemical Oxygen Demand (COD) measurements were conducted to evaluate the system's efficiency in removing organic load, while Total Suspended Solids (TSS) analyses provided insights into the system's capability to reduce solid particle concentrations. External analysis of Fats, Oils, and Grease (FOG) highlighted the system's effectiveness in dealing with high FOG wastewater.

4.2 Coagulation Study Methodology

4.2.1 Equipment

The experimental setup involved several key pieces of equipment, including 5L glass reactor vessels. These were agitated using mechanical stirrers, and heated using heating elements attached to rubber stoppers which were fixed to the vessel opening. The rubber stoppers were used to ensure an anaerobic environment within the reactor. Figure 4-2 illustrates the equipment used to conduct this study.

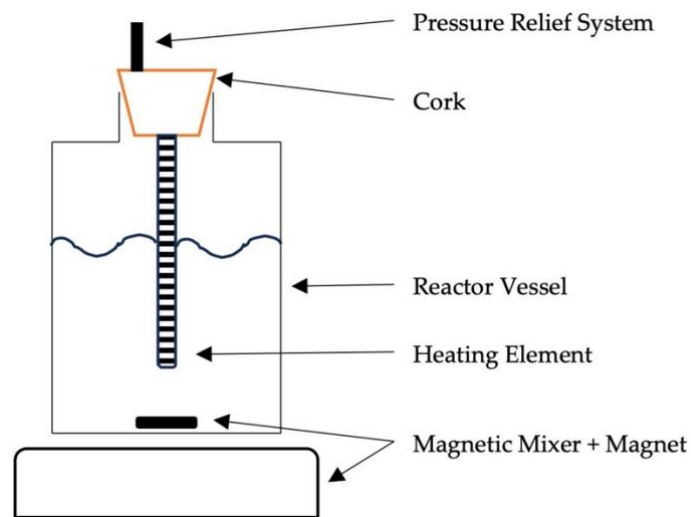


Figure 4-2: Schematic diagram of the equipment used in this study

4.2.2 Okra

Okra (*Abelmoschus esculentus*) thrives in warmer, subtropical regions. In South Africa, okra typically costs between R30 to R50 per kilogram, depending on the season and region. The use of okra in this investigation is to determine its suitability as its use as a coagulant for DWW treatment. It was chosen for this study for both its biodegradability and its low cost.

4.2.3 Analysis of DWW

500mL of each container were taken and mixed, with the resulting mixture being used in the analysis of the Raw DWW. This ensured that each sample was representative of the entire batch that was collected on that specific day. The COD and TSS were tested according to standard lab procedures.

4.2.4 Batch testing protocol

The okra underwent an initial separation into three distinct batches: the first comprised solely of okra seeds, the second consisted exclusively of the flesh, and the third was a combination of both components. Subsequently, these batches underwent a drying process at 105 °C for 12 hours, followed by manual crushing. The crushed okra was then introduced in varying doses (2.5g, 5g, 7.5g, 10g) into 2 liters of Dairy Wastewater (DWW) and agitated for 1 hour to ensure comprehensive contact between the okra and the wastewater. After this agitation period, a 30ml/L dosage of Morma was added, and the mixture was allowed to undergo anaerobic digestion for 24 hours at 30 °C. Each of these tests were done in triplicate to ensure data reliability. All the relevant graphs, along with their error bars have been included under Appendix C.

5 OPERATION OF THE TWO STAGE ANAEROBIC DWW TREATMENT SYSTEM: EGSBR & PRE-TREATMENT

Part of this section will be submitted for publication, detailed as follows:

An evaluation of a two-stage anaerobic DWW treatment system consisting of an expanded granular sludge bed reactor (EGSBR) paired with an anaerobic pre-treatment stage.

(Ramsuroop, J., Moodley, R., Ayinde, W.B., Basitere, M., & Manono, M.S.)

5.1 Introduction

Anaerobic treatment processes play a pivotal role in addressing the complexities of Dairy Wastewater (DWW) by primarily targeting the reduction of its organic content, particularly when dealing with high organic loads (Zhao et al., 2020). These systems demonstrate efficiency in converting nitrites and nitrates into nitrogen gas. Despite their effectiveness, anaerobic treatments exhibit limitations in removing Fats, Oils, and Grease (FOGs), ammonia, and phosphorous. To meet stringent discharge standards, supplementary downstream treatments or appropriate pre-treatment methods may be required. Addressing the challenge of breaking down complex polymeric compounds within DWW, such as FOGs, often necessitates a hydrolysis or oxidation step, transforming them into smaller, soluble molecules like short-chain fatty acids and alcohols (AS & NN, 2001). The catalytic process of hydrolysis, facilitated by the addition of acids or enzymes in the presence of water, becomes particularly relevant in anaerobic processes, where lipid content tends to be a limiting factor for optimal performance (Hanrahan, 2012)(Pascale et al., 2019). Furthermore, the potential for methane production from organic-rich wastewater enhances the versatility of anaerobic treatment methods. Notable applications include anaerobic filters, sludge blanket reactors, and packed bed digesters, making anaerobic processes a key player in the comprehensive treatment of Dairy Wastewater.

To address limitations in certain biological systems, an alternative approach involves incorporating either pre-treatment or post-treatment systems to compensate for inherent weaknesses in the primary treatment process. This method proves advantageous as it offers a streamlined solution without the need for extensive adjustments to system parameters. Moreover, these auxiliary processes can be tailored to complement the main treatment system seamlessly and implemented with relative ease. However, it is important to note that this option is often associated with higher costs. Anaerobic systems face challenges in

effectively degrading Fats, Oils, and Grease (FOGs) and ammonia due to the oxidation reactions involved. High FOG concentrations can lead to reactor clogging as they resist breakdown during anaerobic digestion (AD). A solution to these issues is implementing a hydrolysis stage before the anaerobic digester. Research indicates that an up-flow Anaerobic Sludge Blanket (UASB) reactor effectively treats high FOG-content wastewaters following hydrolysis. When fed unhydrolyzed wastewater, the UASB exhibited unstable Chemical Oxygen Demand (COD) removal and fat accumulation in the sludge, emphasizing the need for a separate hydrolysis stage to mitigate clogging risks (Leal et al., 2006). Hydrolyzed Dairy Wastewater (DWW) has been shown to enhance methane production compared to unhydrolyzed DWW, attributed to the oxidation degradation of complex organic molecules. Studies using an up-flow anaerobic sludge blanket reactor underscored improved COD removal (90% with hydrolysis, 82% without) and reduced FOG content in the effluent stream (Leal et al., 2006). The inclusion of an enzymatic pre-hydrolysis treatment stage enhances the efficiency of single-stage anaerobic digesters, ensuring more thorough remediation of high-strength DWW by increasing both COD and FOG removal efficiency and reducing the risk of clogging in continuous processes (Mobarak-Qamsari et al., 2012).

The primary objectives of this study are twofold. Firstly, it aims to assess the efficiency and performance of an up-flow anaerobic digestion reactor in the treatment of Dairy Wastewater (DWW). This involves a comprehensive examination of the reactor's capability to mitigate organic content and address the specific challenges associated with DWW, such as Fats, Oils, and Grease (FOGs). Secondly, the study seeks to investigate the impact of varying Hydraulic Retention Times (HRTs) during the pre-treatment stage on the subsequent operation of the anaerobic digestion reactor. By systematically altering the HRTs, the study aims to elucidate the correlation between pre-treatment conditions and the overall efficacy

of the anaerobic digestion process, providing valuable insights for optimizing the treatment of Dairy Wastewater.

5.2 Objectives

- To analyse the physical and chemical properties of the DWW before and after remediation
- To optimize each process step of the multi-integrated lab scale plant to maximizing the performance of each individual step and the overall system
- To assess the effectiveness of the multi-integrated lab scale plant to bioremediate DWW
- To identify any drawbacks of the system and to recommend any potential improvements

5.3 Materials and Methods

5.3.1 *Equipment*

The experimental setup involved several key pieces of equipment, including a 4-liter reactor vessel equipped with a water jacket to facilitate temperature control. Additionally, four peristaltic pumps were employed to manage fluid flow within the system, ensuring precise and controlled conditions. To further support temperature regulation and stability, a water bath was utilized as part of the experimental apparatus. For a pre-treatment tank, a 20L bucket was used, with heating elements fixed to the lid. For agitation, a mechanical stirrer was used.

5.3.2 *Inoculation*

A 4-liter cylindrical jacketed reactor was employed for this experimental run. The jacket, filled with water, facilitated precise temperature control within the reactor. To initiate the inoculation process, 300 grams of porous stones were introduced as packing material. Subsequently, 600 mL of sludge from a brewery and a Morma-DWW mixture, dosed to 30mL/ L DWW, were added to the reactor. The Morma-DWW mixture continued to be added until the reactor was nearly full, allowing

for a slight buffer to accommodate potential increases in volume. The sealed reactor was left undisturbed for 72 hrs, with the temperature maintained at 36 °C to promote optimal biomass growth.

5.3.3 Pre-treatment

The experimental procedure was initiated with the anaerobic pre-treatment of 20 litres of Dairy Wastewater (DWW) per batch, incorporating a designated anaerobic pre-treatment process. Following this, Morma was dosed into the pre-treated DWW at a concentration of 10 mL per litre. To ensure uniform distribution and effectiveness of the treatment, a mixer, along with a mixing stone, was employed for thorough agitation until a homogenous mixture was achieved. Throughout the experimental process, a constant temperature of 38°C was maintained using two fish tank heating elements under anaerobic conditions.

To assess the impact of Pre-treatment (PT) on the anaerobic digester, variations in Hydraulic Retention Time (HRT) were implemented during the 100 days of operation. Initially set at 36 hrs, the HRT was reduced to 24 hrs after the first 40 days of operation. Subsequently, a further reduction to 12 hrs occurred after an additional 30 days, maintaining this configuration for the remaining 30 days of the study period. This systematic adjustment allowed for a comprehensive examination of the PT effects on the anaerobic digestion process over the course of the operational timeframe.

5.3.4 Operation

The pre-treated Dairy Wastewater (DWW) underwent initial processing before being introduced into a continuously agitated 25L feed tank. Subsequently, the treated DWW was pumped into the reactor at a steady flow rate of 0.15L/hr, with the reactor operating under anaerobic conditions and maintaining a constant Hydraulic Retention Time (HRT) throughout the study. To enhance efficiency, a

recycle stream was extracted from the top of the reactor and combined with the pre-treated feed before being reintroduced into the reactor. The final product stream, obtained from the top of the reactor, served as a designated sampling point for assessing the quality of the reactor product.

5.3.5 Analyses

The experiment included a thorough assessment of the system's performance by analyzing key indicators. Chemical Oxygen Demand (COD) measurements were conducted to evaluate the system's efficiency in removing organic load, while Total Suspended Solids (TSS) analyses provided insights into the system's capability to reduce solid particle concentrations. External analysis of Fats, Oils, and Grease (FOG) highlighted the system's effectiveness in dealing with high FOG wastewater.

5.4 Problems faced

5.4.1 Clogging

A major problem faced in the operation of the EGSBR was the clogging which was experienced. This was caused by a combination of biomass washout and fat solidification. The issue of biomass washout was due to the stability of the biomass colony and the latching of it to the packing stones. The fat solidification occurred in the tubes surrounding the reactor, where there was a lack of heating. This caused major blockages in the system causing the formation of leaks and pump failure. The downtime taken to clean the tubes may have additionally contributed to the stability issues which were experienced. A possible solution to this would be to have a filtration stage before the EGSBR to remove a portion of solids before entering the reactor.

5.4.2 Leaks

Throughout the period of study, many leaks occurred with both of the system vessels. The process to fix these leaks sometimes necessitated that the vessel be emptied and the process be paused. This would have had an impact on the EGSBR stability, and on more than one occasion the reactor had to be reinoculated.

5.4.3 Power Outages

During the initial stages of this study, there were daily scheduled power outages (load shedding) which would cause the pumps, heating, and agitation to switch off for a period of 2hrs. This would occur up to 4 times per day, and was only rectified later in the period of study. However, there were numerous instances of the electricity tripping which would only be detected whenever the system was checked on. This would occur multiple times a week and were not remedied.

5.4.4 Pump and Heater failure

There was an instance of pump failure which was only observed a day later, however it was quickly repaired and did not have an impact on performance. The water bath which was used to heat the reactor jacket also failed close to the end of the study. This was remedied using aquarium heaters in the place of the heating element. This method seemed to solve the issue, however it was unsure if the temperature of the heating element was met with the replacements. There was no visible drop in performance after the replacement so it was discounted.

5.5 Results and Discussion

Weeks 1 to 5 were system operation with a pre-treatment HRT of 36 hr (stage 1). Weeks 6 to 10 had a pre-treatment HRT of 24 hr (stage 2), and finally weeks 11 to 14 had a pre-treatment HRT of 12hr (stage 3). The purpose of decreasing the HRT

over the study, whilst keeping the remaining system parameters constant, was to try and observe possible reactor acclimatization while decreasing the overall required process time.

5.5.1 COD Removal

In Figure 5-1, we observe the COD removal rates of the pre-treatment stage and the entire system. In the first week, there was an increase in COD from both the pre-treatment and up-flow reactor due to an operational error. The pre-treatment stage received an overdose of morpholine (50mL/LDWW), leading to higher biomass production. This error was corrected in subsequent weeks.

Additionally, a significant amount of biomass washout was noted from the Up-flow reactor, contributing to the COD increase. A minor washout occurred in week 2, potentially due to the microbial colony reaching an equilibrium. The reactor stabilized between weeks 3 to 5, achieving an overall system removal rate ranging from 60% (week 3) to 26% (week 5).

During stage 2, significant biomass washout occurred in weeks 8 and 10, which was resolved using a backwash cycle. Notably, weeks with higher raw DWW COD content achieved higher removal rates (e.g., weeks 7 and 9, with overall COD removal rates of 60% and 80%, respectively). This suggests the system is effective for treating wastewater with higher COD content.

In stage 3, the stability of the UF reactor was compromised due to instances of clogging and biomass washout. This was attributed to a decrease in pre-treatment Hydraulic Retention Time (HRT) to 12 hrs, insufficient for breaking down the solids fed into the reactor.

From the data, no discernible effect of pre-treatment HRT on COD removal efficiency is evident. Overall, this system falls short of traditional AD treatment of

DWW in terms of COD removal, which usually achieve a COD removal rate of 90% (Passeggi, López & Borzacconi, 2012). There are two possible reasons for this lack of performance. Firstly, large amounts of biomass washout was observed throughout the study. This would have contributed to the COD content within the product stream. Secondly was the stability of the bacterial colony within the reactor. This was due to the many cloggages, pauses in operation, and pauses in heating which occurred. These factors could have contributed to this consistent lack in performance.

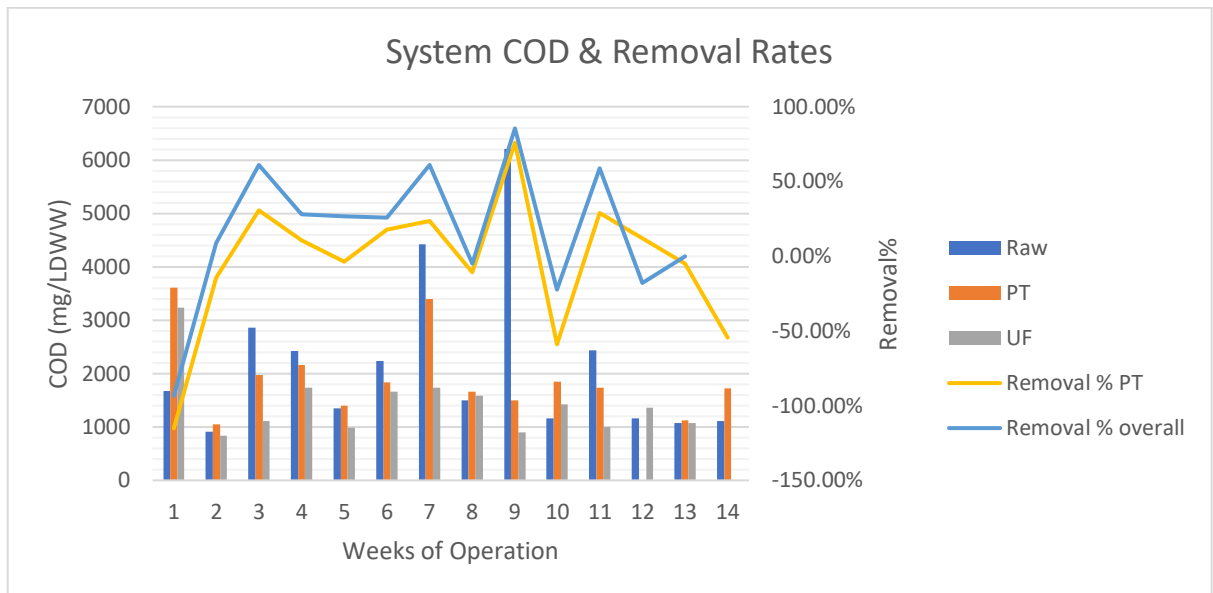


Figure 5-1: Removal rates of COD over the 14 weeks of operation, displaying both the pre-treatment and overall system removal rates

Based on Figure 5-2, it's evident that the pre-treatment Hydraulic Retention Time (HRT) doesn't significantly impact the COD removal efficiency of the reactor. The considerable variation in COD removal rates between the pre-treatment and the UF reactor indicates high reactor instability. The fluctuations in removal rates correspond to weeks where changes were made to the pre-treatment HRT or when

issues arose regarding biomass stability within the reactor. Notably, the decrease in efficiency from week 11 to week 13 highlights the inadequacy of the 12-hour HRT for pre-treatment.

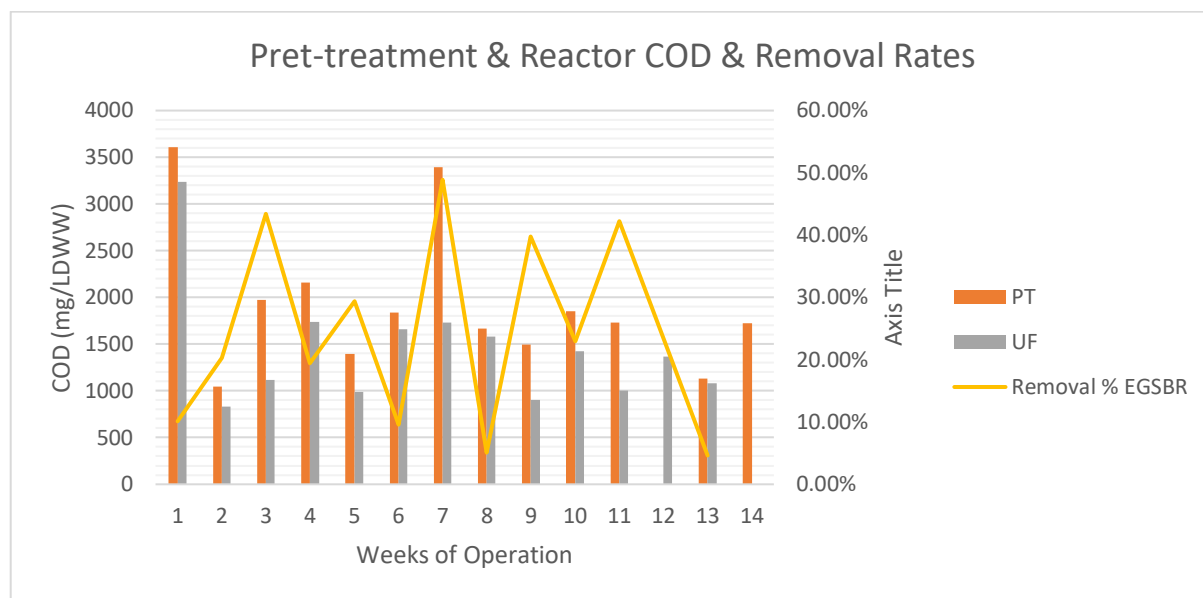


Figure 5-2: COD removal rates of the EGSBR to observe possible trends between pre-treatment HRT and reactor performance

Figure 5-3 depicts the system's response to various levels of Raw DWW COD throughout the study. A noticeable trend is observed: higher COD levels correspond to higher removal rates, whereas lower COD levels result in lower removal rates. Given that much of the DWW obtained had lower COD levels, the system's performance appears to be relatively low. Future investigations should focus on DWW with higher organic loads to better align with the typical use case of Up-flow anaerobic sludge blanket reactors, which are commonly employed for treating high-strength DWW with COD contents of up to 40,000 mg/LDWW (Buntner, Sánchez & Garrido, 2013). The negative removal rates that were observed are due to biomass washout from the EGSBR. This was a recurring issue with the system, which was observed on 5 occasions.

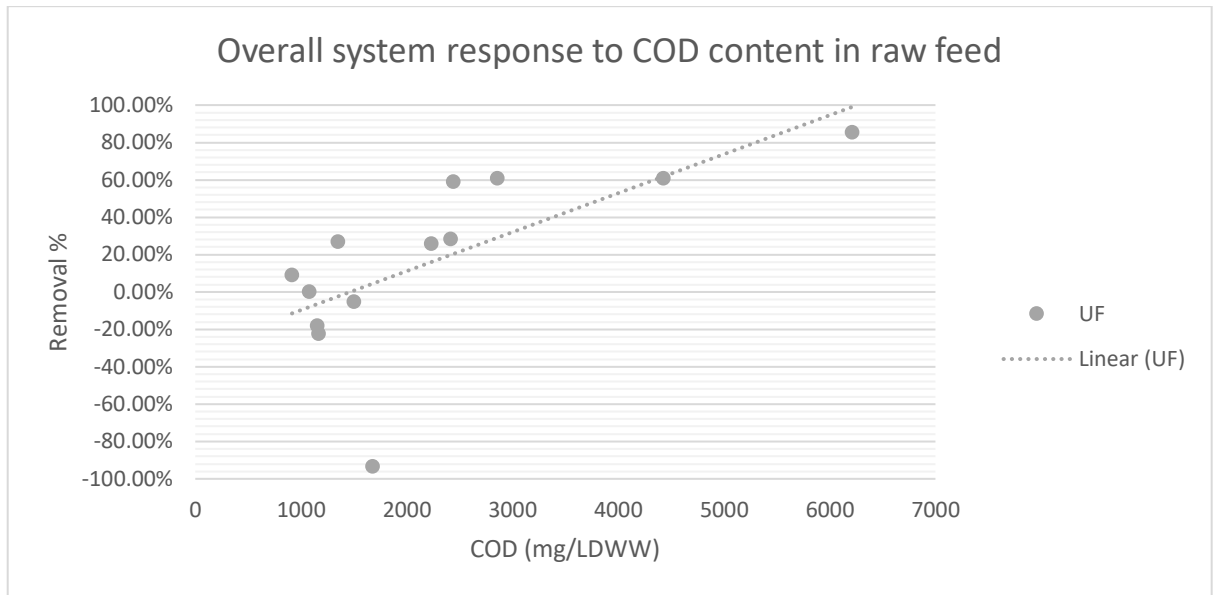


Figure 5-3: System COD removal rates related to the COD content in raw DWW feed

5.5.2 FOG Removal

Figure 5-4, the effects of pre-treatment on FOG content are observed to vary. Throughout each stage, there's no definitive impact of changes in Hydraulic Retention Time (HRT) on FOG removal. Both the PT and overall system performance were found to be highly inconsistent, which is expected given that typical digestion processes that reduce FOG content are aerobic, whereas both the PT and UF reactor are operated anaerobically. Consequently, the system's response to HRT changes is unpredictable.

Notably, weeks with FOG content exceeding 6000 mg/LDWW exhibited improved system performance. Conversely, weeks with systemic issues, such as week 8, showed lower FOG removal rates compared to unaffected weeks. The highest removal rate was observed in week 4, exceeding 90%. Week 10 also saw high FOG

removal rates, likely due to reactor clogging, which trapped solid fat and consequently increased FOG removal efficiency.

Comparing with previous studies using AD reactors, where FOG removal rates averaged around 47% (Omil et al., 2003), this study observed removal rates ranging from 27% to 94%, with an average of 66%. These results indicate the system's effectiveness in treating high FOG wastewater.

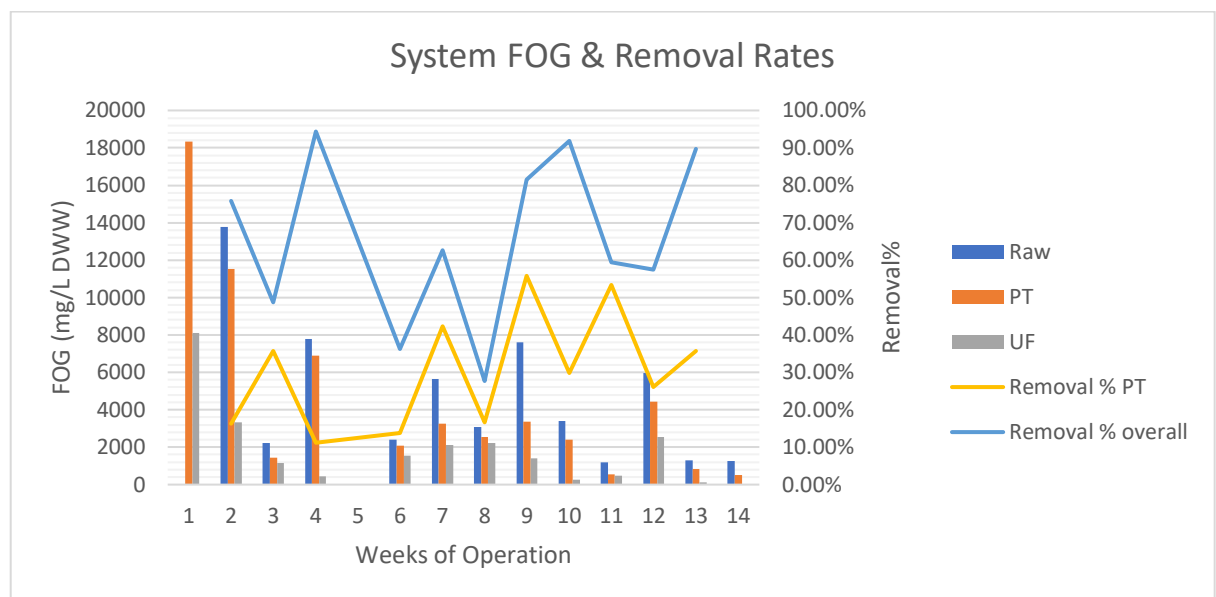


Figure 5-4: System FOG content and Removal Rates

Figure 5-5 shows the system response to the FOG content in the raw DWW. The system response is widely dispersed, which indicates that no correlation was observed. This should be further studied to determine if there is an effect on system performance due to the FOG content as the instability within the reactor may have caused a skew in some of the removal rates achieved.

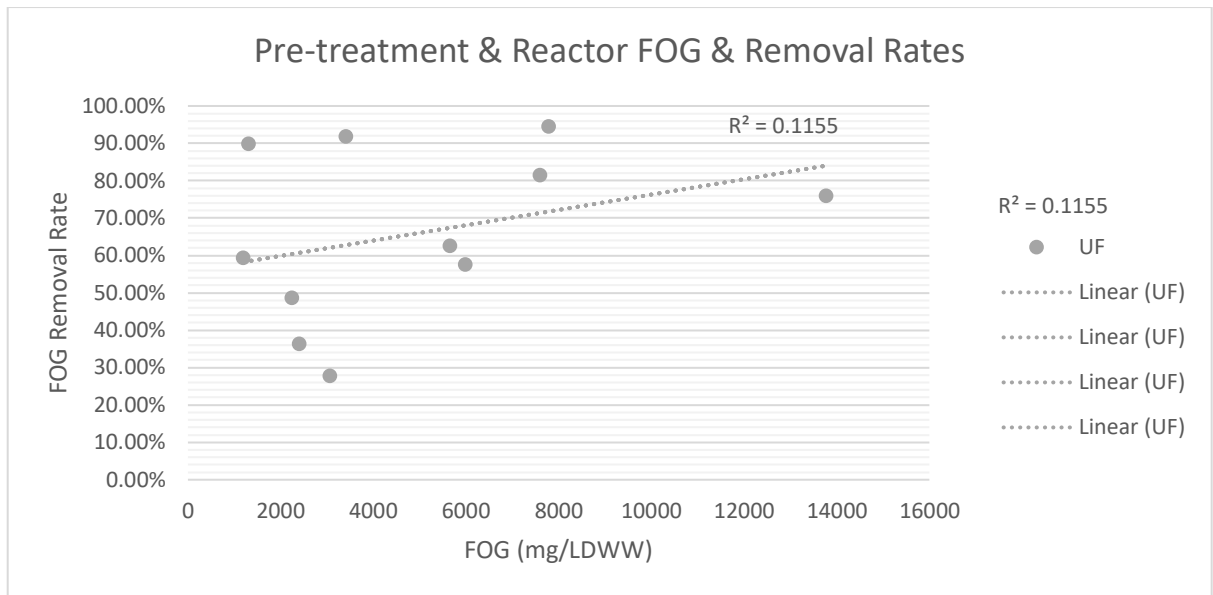


Figure 5-5: System Response to FOG Content in Raw DWW Feed

5.5.3 TSS removal

TSS removal rates served as indicators of reactor stability. As depicted in Figure 5-6, weeks with TSS increase from the UF reactor corresponded to instances of biomass washout. Specifically, weeks 1, 4, 8, and 10 revealed issues at the pre-treatment stage, where TSS elevation was noted from the PT stage. This highlights the need for further investigation into microbial activity in the PT stage to pinpoint the cause of TSS increase and, consequently, biomass washout.

Weeks 12 and 13 are particularly noteworthy, as the reduction of pre-treatment HRT to 12 hr led to decreased reactor stability. This suggests a possible link between microbial activity in the pre-treatment stage and reactor stability. Determining the optimal pre-treatment HRT is crucial, as shorter treatment times in full-scale operations allow for processing larger quantities of DWW.

Investigating microbial activity in the pre-treatment stage can provide insights into optimizing treatment processes for enhanced efficiency and scalability. The TSS results were those most heavily affected by the reactor instability, specifically biomass washout,

which occurred throughout the run. With the treatment system, which was used in this study, it would be beneficial to have a filtration or clarification stage after the reactor. This would solve the issue of biomass within the product stream. The filtered biomass could be used in animal feed or as part of a fertilizer.

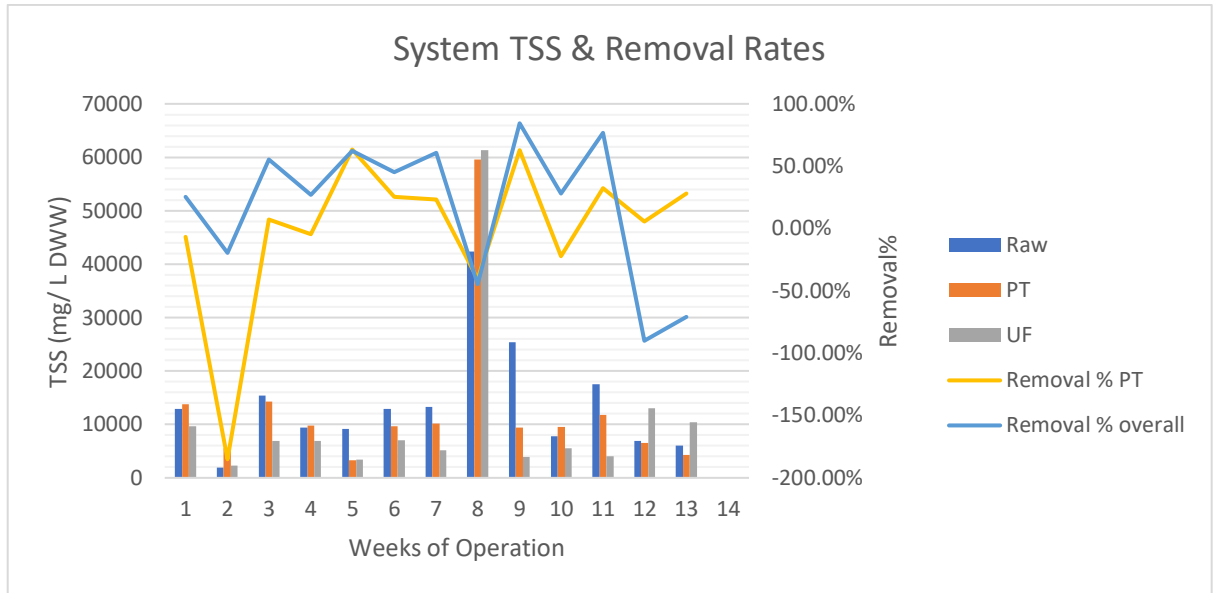


Figure 5-6: System TSS content and Removal Rates

5.6 Conclusions

In conclusion, our investigation into the performance of the wastewater treatment system has yielded significant insights and challenges. Initial stages of the experiment were marred by operational errors, notably the overuse of moroma in the pre-treatment stage, resulting in increased biomass production and subsequent elevation in chemical oxygen demand (COD). While subsequent corrections improved system stability, fluctuations persisted, particularly in the Up-Flow (UF) reactor.

The overall COD removal rates ranged from 60% in week 3 to 26% in week 5, falling short of traditional anaerobic digestion (AD) systems capable of achieving up to 90% COD removal. Noteworthy, the system demonstrated improved performance with higher raw COD levels, suggesting its potential for treating wastewater with elevated COD content.

During stage 2, biomass washout issues were mitigated with a backwash cycle, and weeks with higher raw COD levels exhibited increased removal rates. Stage 3 presented challenges in UF reactor stability due to clogging and biomass washout, attributed to a reduced pre-treatment hydraulic retention time (HRT) of 12 hours. However, the impact of pre-treatment HRT on COD removal efficiency remained inconclusive.

Analysis of fat, oil, and grease (FOG) removal revealed inconsistent system performance, with the highest removal rates occurring in weeks with FOG content exceeding 6000 mg/LDW. Our study demonstrated FOG removal rates ranging from 27% to 94%, averaging 66%, surpassing those of previous AD reactor studies. The observed correlation between FOG content and system performance warrants further investigation.

Total suspended solids (TSS) removal rates served as indicators of reactor stability issues, with weeks exhibiting increased TSS indicating biomass washout. Decreasing pre-treatment HRT to 12 hours in weeks 12 and 13 adversely affected reactor stability, highlighting the importance of optimizing pre-treatment parameters for efficient wastewater treatment.

In summary, our study underscores the system's efficacy in treating high-strength FOG wastewater while identifying challenges in maintaining stability, particularly in the UF

reactor. Further research is recommended to address observed inconsistencies, optimize pre-treatment parameters, and explore correlations between system performance and FOG content, ultimately enhancing the overall efficiency of the wastewater treatment system.

5.7 Recommendations

- The findings from the study provide valuable insights into the performance of the wastewater treatment system. Based on these results, several recommendations can be made to enhance the system's efficiency and address observed challenges:
- **Optimization of Pre-treatment Parameters:** Conduct further investigations into the microbial activity in the pre-treatment stage, particularly focusing on the impact of hydraulic retention time (HRT). Identifying the optimal HRT for pre-treatment is crucial for maintaining stability and reducing overall treatment time.
- **Operational Consistency:** Implement measures to ensure operational consistency, especially in the UASBR. Address issues such as biomass washout and clogging promptly to maintain stable system performance.
- **Raw Wastewater Quality:** Given the observed correlation between higher raw COD levels and improved removal rates, consider exploring wastewater with higher organic loads. This can be crucial for enhancing the overall performance of the system.
- **FOG Content Correlation:** Further investigate the correlation between FOG content and system performance. Understanding the impact of FOG levels on removal rates can help optimize the treatment process.
- **Microbial Activity in Pre-treatment:** Conduct a detailed study on microbial activity in the pre-treatment stage to identify the cause of increased TSS and biomass. This information is vital for addressing stability issues and optimizing the overall treatment process.

- **Extended Monitoring:** Extend the monitoring period to gather more data and identify long-term trends. This will provide a more comprehensive understanding of the system's performance and help in refining operational parameters.
- **Comparative Studies:** Conduct comparative studies with traditional anaerobic digestion (AD) systems to understand the strengths and weaknesses of the current system. This can guide improvements and innovations in the design and operation of the wastewater treatment system.

By addressing these recommendations, it is anticipated that the wastewater treatment system can overcome the challenges that were experienced, achieve greater stability, and enhance overall efficiency in treating high-strength dairy wastewater

5.8 References

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6 AN INVESTIGATION OF OKRA AS A COAGULANT PRECEEDING THE ANAEROBIC PRE-TREATMENT

Part of this section will be submitted for publication, detailed as follows:

An investigation into the use of okra as a biodegradable coagulant to be used as a pre-treatment stage in the anaerobic digestion of DWW.

(Ramsuroop, J., Moodley, R., Mngqeta, A., Ayinde, W.B., Basitere, M., & Manono, M.S.)

6.1 Introduction

Wastewater treatment systems are designed primarily to reduce organic content, especially when facing high organic loads, by effectively converting nitrites and nitrates into nitrogen gas (Zhao et al., 2020). Anaerobic treatment processes have been a staple in the treatment of dairy wastewater (DWW) for many years. However, limitations arise in the removal of Fats, Oils, and Grease (FOGs), ammonia, and phosphorous, necessitating additional downstream or pre-treatment methods for compliance with discharge standards (“Water Access in South Africa | Water for all”, n.d.). Addressing complex polymeric compounds like FOGs typically involves hydrolysis or oxidation steps to transform them into smaller, soluble molecules (AS & NN, 2001). While prone to clogging during Dairy Wastewater (DWW) treatment, the integration of pre-treatment systems, spanning chemical to physical methods, can alleviate these issues. Coagulation/Flocculation processes stand out as prevalent methods for eliminating suspended and dissolved solids, colloids, and organic constituents in industrial wastewater (Mateus et al., 2017). Two main types of coagulants find common use in wastewater treatment: inorganic and organic. Inorganic coagulants, often metal-based salts containing aluminium or iron, like Alum, significantly reduce DWW turbidity by 95% and COD by 68% (Loloei et al., 2014). Combining them with polymeric coagulants enhances COD reduction to 85%. However, the drawback lies in the generation of metal-rich floc, necessitating further treatment before disposal, pH alterations, and the requirement for corrosion-resistant equipment. The integration of inorganic coagulants with biological systems must consider potential hindrance to microbial colonies. On the other hand, organic coagulants, whether polymeric or natural, offer distinct advantages. Polymeric coagulants, generating metal-free longer polymer chains, produce smaller floc volumes without impacting water pH. Natural coagulants, like an extract from *Guazuma ulmifolia* bark, exhibit noteworthy effectiveness in DWW treatment, removing 95.8% of turbidity, 76% of

COD, and 81.2% of BOD5 (Muniz, Borges & Silva, 2020). Their low cost and eco-friendliness position natural coagulants as promising alternatives to commonly used synthetic counterparts. Furthermore, their compatibility with biological systems, without affecting effluent pH or introducing harmful substances, underscores their practical appeal.

The primary aim of this study is to investigate the use of okra as a coagulant. The use of coagulants have been widely investigated for their application in DWW treatment (Kaushal & Goyal, 2019). The use of Okra, paired with a pre-treatment digestion stage is of specific interest due to the challenges faced with the removal of solids using anaerobic digestors, without the addition of harsh chemicals. This is significant as the okra should not interfere with the operation of the digestors, whilst being an energy efficient method to reduce the solid content when compared with other physical methods such as filtration. The interaction between the okra and the pre-treatment agent used is also of interest as it would give insight to the robustness of the agent, and how it is able to handle changes in the medium is it fed.

6.2 Objectives of the study

- To evaluate the effectiveness of okra as a coagulant for DWW
- To identify the component of okra which has the best coagulation properties
- To find an ideal dosage for okra for the purpose of coagulation.

6.3 Materials and Methods

6.3.1 Equipment

The experimental setup involved several key pieces of equipment, including 5L glass reactor vessels. These were agitated using mechanical stirrers and heated using heating elements attached to rubber stoppers which were fixed to the vessel

opening. The rubber stoppers were used to ensure an anaerobic environment within the reactor.

6.3.2 Analysis of DWW

500mL of each container were taken and mixed, with the resulting mixture being used in the analysis of the Raw DWW. This ensured that each sample was representative of the entire batch that was collected on that specific day. The COD and TSS were tested according to standard lab procedures.

6.3.3 Batch testing protocol

The okra underwent an initial separation into three distinct batches: the first comprised solely of okra seeds, the second consisted exclusively of the flesh, and the third was a combination of both components. Subsequently, these batches underwent a drying process at 105 °C for 12 hours, followed by manual crushing. The crushed okra was then introduced in varying doses (2.5g, 5g, 7.5g, 10g) into 2 liters of Dairy Wastewater (DWW) and agitated for 1 hour to ensure comprehensive contact between the okra and the wastewater. After this agitation period, a 30ml/L dosage of Morma was added, and the mixture was allowed to undergo anaerobic digestion for 24 hours at 30 °C. Each of these tests were done in triplicate to ensure data reliability. All the relevant graphs, along with their error bars have been included under Appendix C.

6.4 Results and Discussion

In this study the two indicators of most relevance were TSS and COD. These were the metrics by which the effectiveness of the coagulant was determined.

6.4.1 TSS Removal

Figure 4-2 shows that the highest removal for TSS, being 32%, was achieved at a dose of 5g of the okra seeds with Morma. This is closely followed by the 5g dose of only seeds, with a removal rate of 28%. This indicates that the use of the seeds of okra result in significantly higher solid removal rates than with using the flesh or a combination of the two. Additionally, there seems to be a slight positive effect with the addition of morma at this dose. From figure 4-2, it can be observed that a 5g dosage resulted in the highest removal rates for each component of the okra, with the okra seeds being the only part of okra to show significant TSS removal rates. It is apparent that the flesh of okra is having an inhibitory effect on the removal of TSS, to the extent where it negates the positive properties exhibited by the okra seeds when separated from the flesh. A possible reason for the seeds displaying promising coagulant effects is that the seeds of okra contain oil and protein, whereas the pulp is mostly fibrous (SHARMA, 1993). The oils and proteins from the seeds should be further investigated to see if they are the reasons for the okra seeds effectiveness in removing TSS.

The negative removal rates observed when using the flesh of okra indicate that an increase of TSS was observed. This suggests that the flesh of okra is unsuitable for TSS removal, and in some cases will further increase the TSS when added. The highest removal rates, achieved using the okra seeds, are similar those observed in a similar study looking at the potential of okra to be a viable DWW treatment process (Kaushal & Goyal, 2019). This study achieved a TSS removal of 30%, which is extremely close to the TSS removal achieved in these experiments.

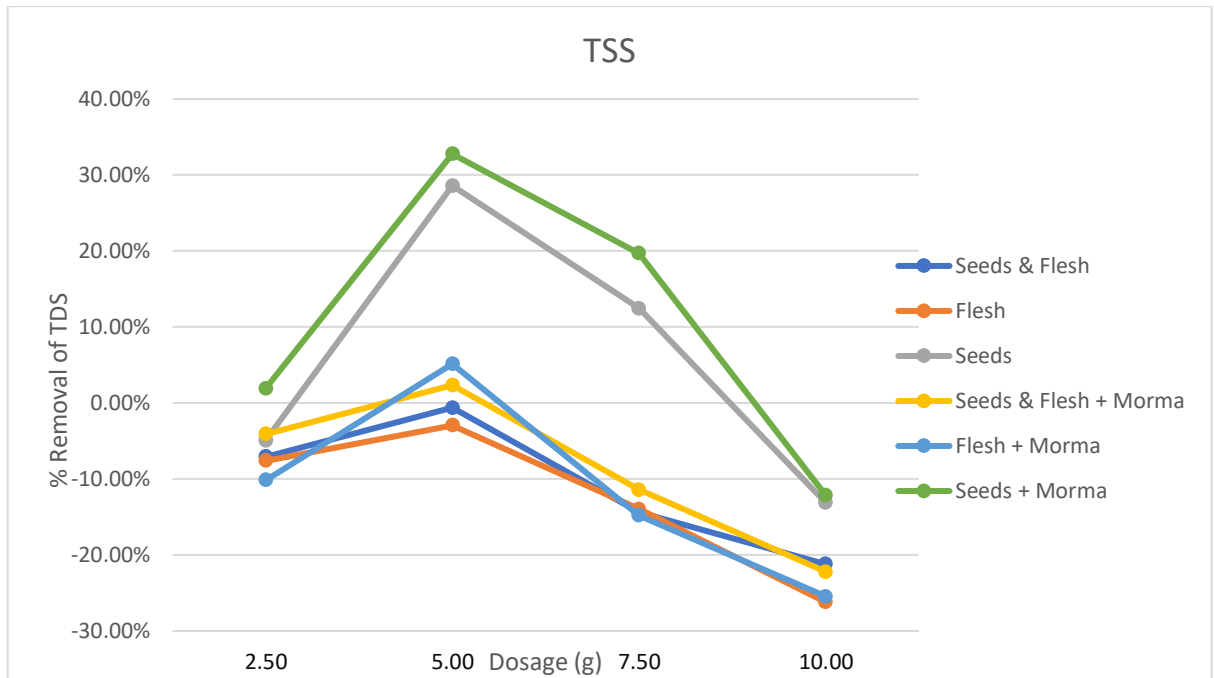


Figure 6-1: Removal rates of TSS at each dosage of okra.

6.4.2 COD Removal

Figure 4-3 illustrates the removal rates of COD found in this study. As seen in the figure, the highest removal rate was 31% at a dosage of 5g with only the seeds of Okra. Followed closely by the seeds + Morma, which achieved a removal of 25%. When comparing each component of okra with its reciprocal containing morma, it can be observed that the addition of morma decreases the removal rates. The negative removal rates are indicators of an increase in COD level. This suggests that the biomass produced by Morma is a contributor to an increase in COD. As Morma is intended to reduce the FOG content as its primary function, this is not seen as a serious issue. The removal rate of Okra seeds falls short of that observed in a similar study, where a decrease of 67% (Kaushal & Goyal, 2019) was observed. When looking at a traditional non-biodegradable coagulant such as alum, it

achieved a COD removal rate of 68% (Loloei et al., 2014). Figure 4-3 illustrates that increasing past a dose of 5g, the system experienced decreased COD removal rates for all the components of okra. And at the highest dosage of 10g, negative removal rates were observed for all tests, showing that the addition of Okra at increasing dosages, has an inverse effect on COD removal, and in some cases increases the COD levels.

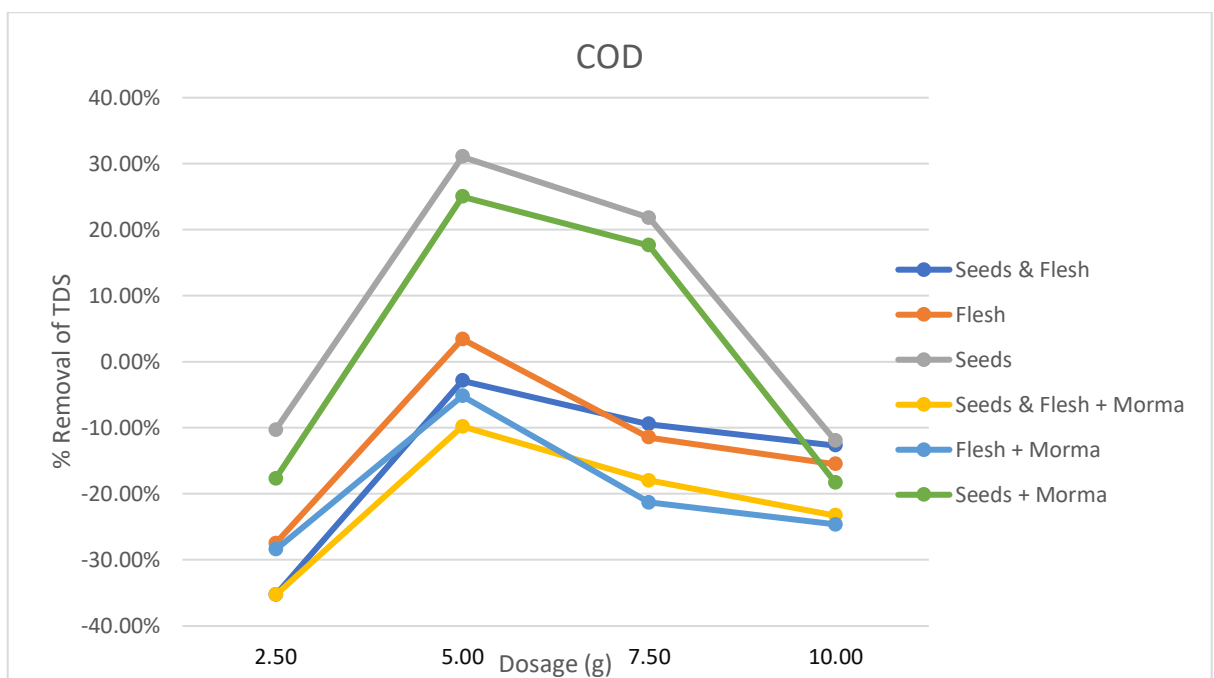


Figure 6-2: Removal rates of COD at each dosage of okra

6.5 Conclusions

In conclusion, the experimental results highlight the efficacy of okra, particularly its seeds, in the removal of Total Suspended Solids (TSS) and Chemical Oxygen Demand (COD) from Dairy Wastewater (DWW). The optimal removal of TSS, reaching 32%, is observed at a 5g dosage, specifically with okra seeds and Morma. This combination outperforms other doses, showcasing superior solid removal rates. However, escalating the okra dosage beyond 5g results in diminishing returns. The attained TSS removal rates closely align with a similar study (approximately 30%) on okra's potential as a DWW treatment process, confirming its promising effectiveness (Kaushal & Goyal, 2019). Regarding COD removal, the highest rate is 31% at a 5g dosage using only okra seeds, followed closely by seeds + Morma at 25%. Despite falling short of a similar study's COD reduction, okra's performance remains competitive, especially when compared to traditional non-biodegradable coagulants like alum (Loloei et al., 2014). Beyond the 5g dosage, the system experiences declining COD removal rates for all okra batches, and at the highest dosage of 10g, negative removal rates are observed, suggesting an inverse relationship between increasing okra dosage and COD removal. Overall, these results highlight the potential of okra, particularly its seeds, as a viable and eco-friendly option for the treatment of DWW.

6.6 Recommendations and Future Research

Based on the discussion provided, the following recommendations can be highlighted:

- **Consideration of Settling Stage:** The study suggests that the addition of okra seeds aids in the settling of solids, which could potentially enhance removal efficiency. Therefore, incorporating a clarification or settling stage in the treatment process may further improve solid removal rates, especially when utilizing okra-based treatments.
- **Evaluation of Biomass Contribution to COD Increase:** Although Morma contributes to an increase in COD removal, it is primarily intended for reducing fat, oil, and grease (FOG) content. Further investigation is recommended to understand the specific role of Morma-derived biomass in the treatment process and its overall impact on wastewater quality.
- **Comparison with Traditional Coagulants:** While okra-based treatments show promising results, it is essential to compare their performance with traditional non-biodegradable coagulants, such as alum. Understanding the relative effectiveness of okra-based treatments against conventional methods, especially regarding FOG removal, can guide decision-making in wastewater treatment selection.
- **Further Research for Enhanced Performance:** To improve COD removal rates and overall treatment efficiency, additional research is warranted to explore alternative okra-based formulations or treatment strategies. Investigating factors such as okra variety, processing methods, contact time, and interaction with other treatment additives could lead to optimized treatment protocols.

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7 OVERALL CONCLUSIONS

In conclusion, this investigation into the performance of the wastewater treatment system has provided valuable insights into its capabilities and limitations. Initial challenges that were encountered stemmed from operational errors, notably the overuse of Morma in the pre-treatment stage, leading to increased biomass production and elevated Chemical Oxygen Demand (COD). Despite subsequent corrections improving system stability, fluctuations persisted, particularly in the EGSBR. The overall COD removal rates ranged from 60% in week 3 to 26% in week 5, underscoring the system's potential but falling short of traditional anaerobic digestion (AD) systems capable of achieving up to 90% COD removal. However, the system demonstrated improved performance with higher raw COD levels, suggesting its efficacy in treating wastewater with elevated COD content.

Additionally, challenges were faced in EGSBR stability, attributed to clogging and biomass washout, which were exacerbated by a reduced pre-treatment hydraulic retention time (HRT) of 12 hours. Analysis of fat, oil, and grease (FOG) removal revealed inconsistent performance, with the highest removal rates occurring in weeks with FOG content exceeding 6000 mg/L DWW. Our study demonstrated FOG removal rates ranging from 27% to 94%, surpassing those of previous AD reactor studies. The observed correlation between FOG content and system performance warrants further investigation. Total Suspended Solids (TSS) removal rates served as indicators of reactor stability issues, with weeks exhibiting increased TSS indicating biomass washout. Decreasing pre-treatment HRT adversely affected reactor stability, highlighting the importance of optimizing pre-treatment parameters.

These challenges resonate with the findings from an adjacent study on the efficacy of okra, particularly its seeds, in the removal of TSS and COD from Dairy Wastewater (DWW). The optimal removal of TSS, reaching 32%, is observed at a 5g dosage, specifically with okra seeds + morma. The highest achieved COD

removal was at a 5g dose, of only the okra seeds. The study suggests that the seeds of okra has promising coagulant properties, where the flesh of okra displays adverse and inhibitory properties for both COD and TSS removal. Additionally it suggests that the optimal dose for okra as a coagulant would be at 5g into 2L of DWW, as it displayed the both the highest removal of COD and TSS.

The use of okra seeds showed promising results in reducing TSS and COD from DWW, offering an eco-friendly alternative to traditional non-biodegradable coagulants like alum. Integrating coagulant treatments alongside biological agents could enhance the removal of solids and FOGs, addressing the stability issues observed in the EGSBR and improving overall system performance.

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9 APPENDICES

9.1 Appendix A: Health & Safety Evaluation

Table 9.1 Risk assessment

Hazards	Risk evaluation			Controls
	Severity	Likelihood	Risk Level	
Thermal/chemical burns from spillage of chemicals	Moderate	Remote	Low	<ol style="list-style-type: none"> 1. Washing station nearby for rinsing of hands 2. Wearing appropriate PPE (lab coats, safety goggles, long pants & closed shoes) at all times
Spillage of sample	Low	Remote	Low	<ol style="list-style-type: none"> 1. Washing station nearby for rinsing of hands 2. Wearing appropriate PPE (lab coats, safety goggles, gloves, long pants & closed shoes) at all times 3. Cleaning supplies on hand to ensure sterilisation of workspace
Skin absorption of chemicals	Moderate	Remote	Moderate	<ol style="list-style-type: none"> 1. Washing station nearby for rinsing of hands 2. Wearing appropriate PPE (lab coats, safety goggles, long pants & closed shoes) at all times

Inhalation of toxic fumes	Moderate	Remote	Moderate	<ol style="list-style-type: none"> 1. Ensuring that the lab is well ventilated 2. Use of a fume hood when handling toxic chemicals
Skins cuts	Low	Occasional	Low	<ol style="list-style-type: none"> 1. Have first aid kits readily available in the labs 2. Have emergency contact numbers listed in the lab for extreme cases 3. Cover as much exposed skin as possible using PPE
Biological hazard	Low	Remote	Low	<ol style="list-style-type: none"> 1. Correct disposal of cultures/samples
COVID-19 (health hazard)	Moderate	Remote	Low	<ol style="list-style-type: none"> 1. Wearing masks at all times 2. Adhering to all COVID protocols

9.2 Appendix B: Project Timeline

Table 9.2 Gantt Chart



9.3 Appendix C: Coagulation Study Graphs With Error Bars Included

9.3.1 TDS

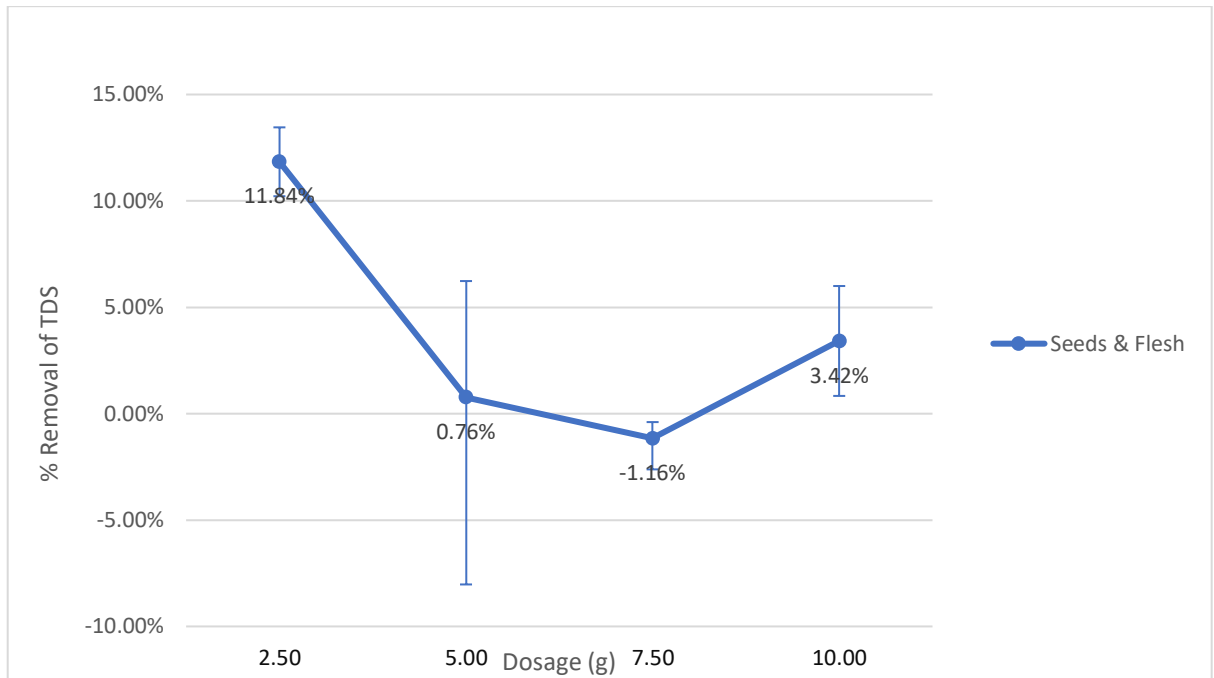


Figure 9-1: TDS Removal Rates (Seeds & Flesh)

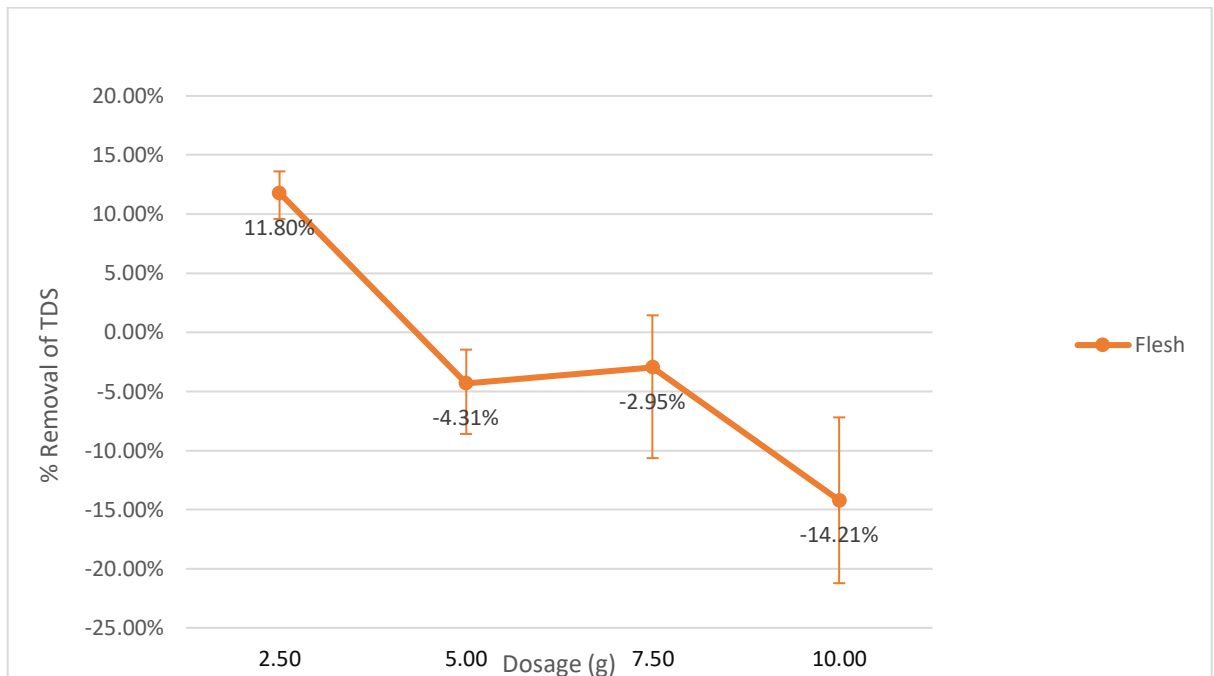


Figure 9-1: TDS Removal Rates (Flesh)

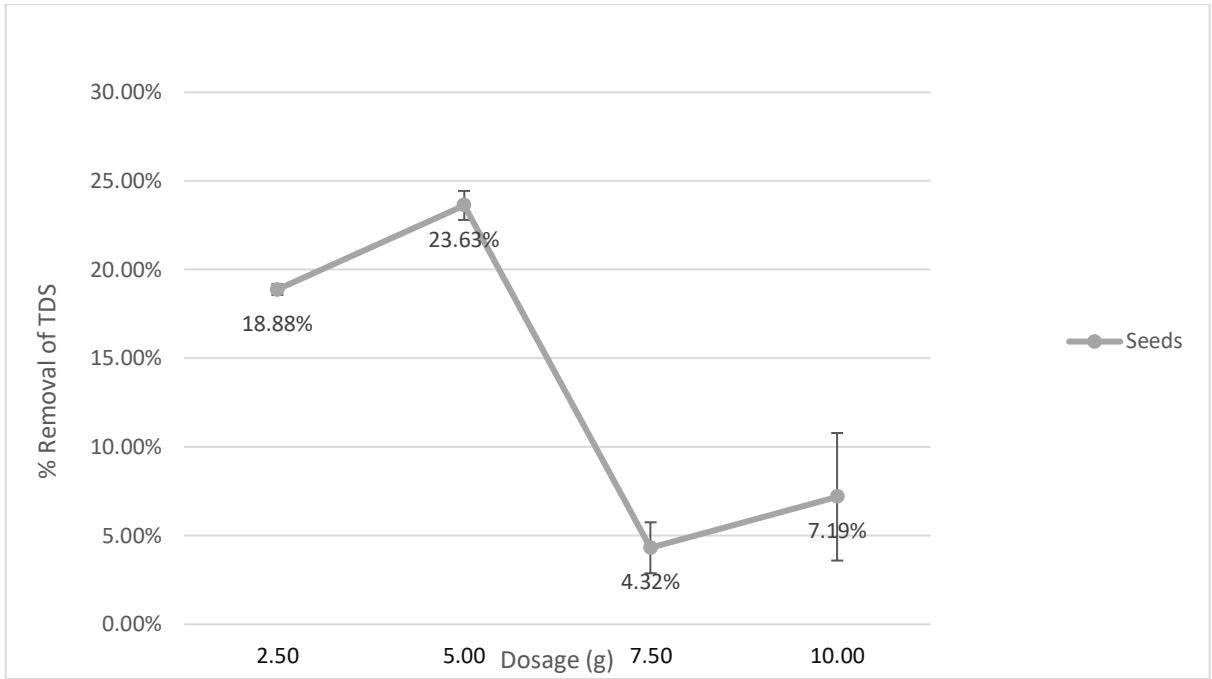


Figure 9-2: TDS Removal Rates (Seeds)

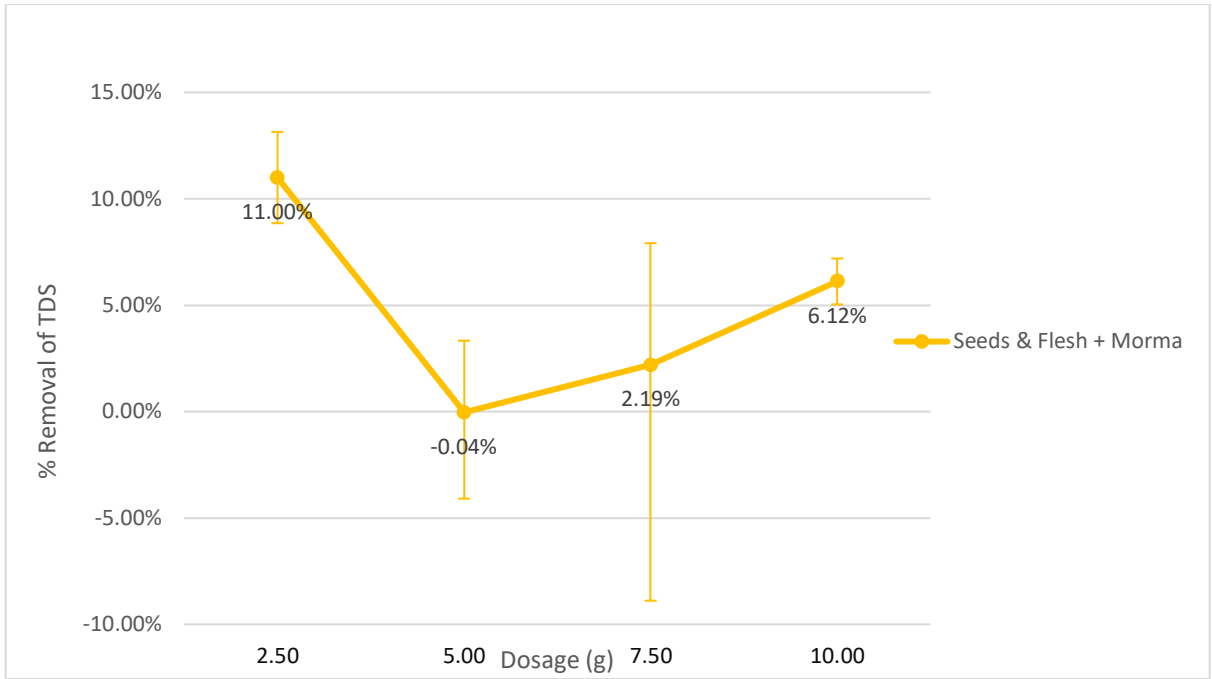


Figure 9-3: TDS Removal Rates (Seeds & Flesh + Morma)

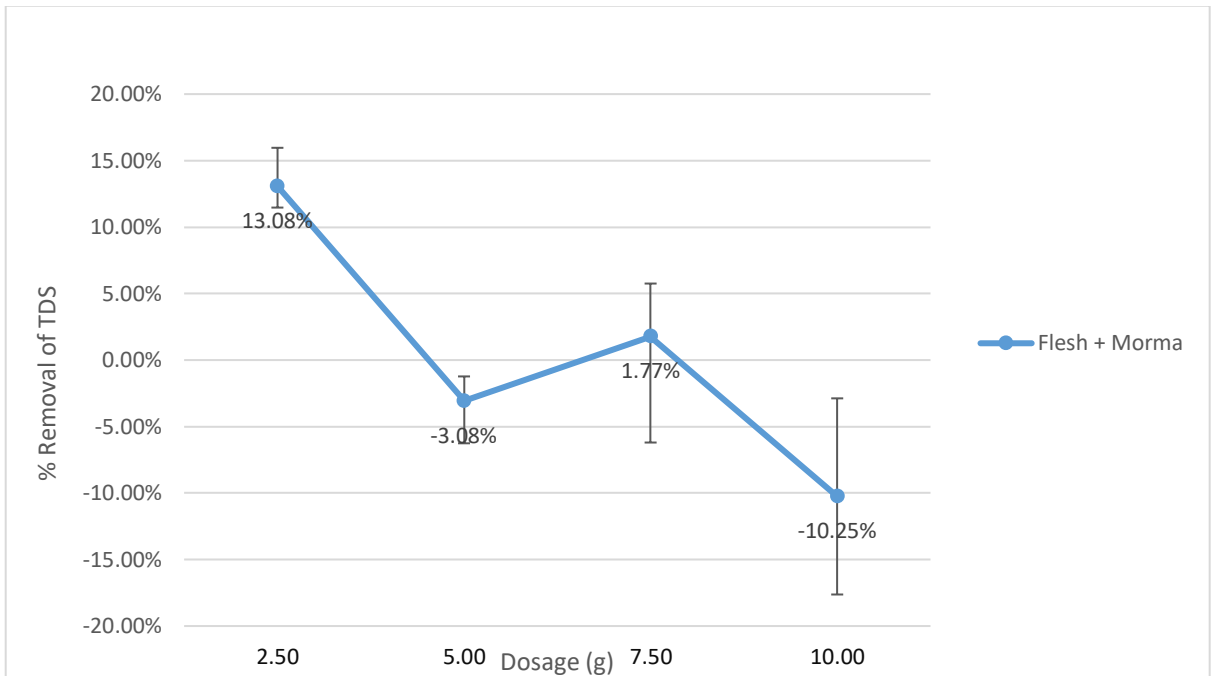


Figure 9-4: TDS Removal Rates (Flesh + Morma)

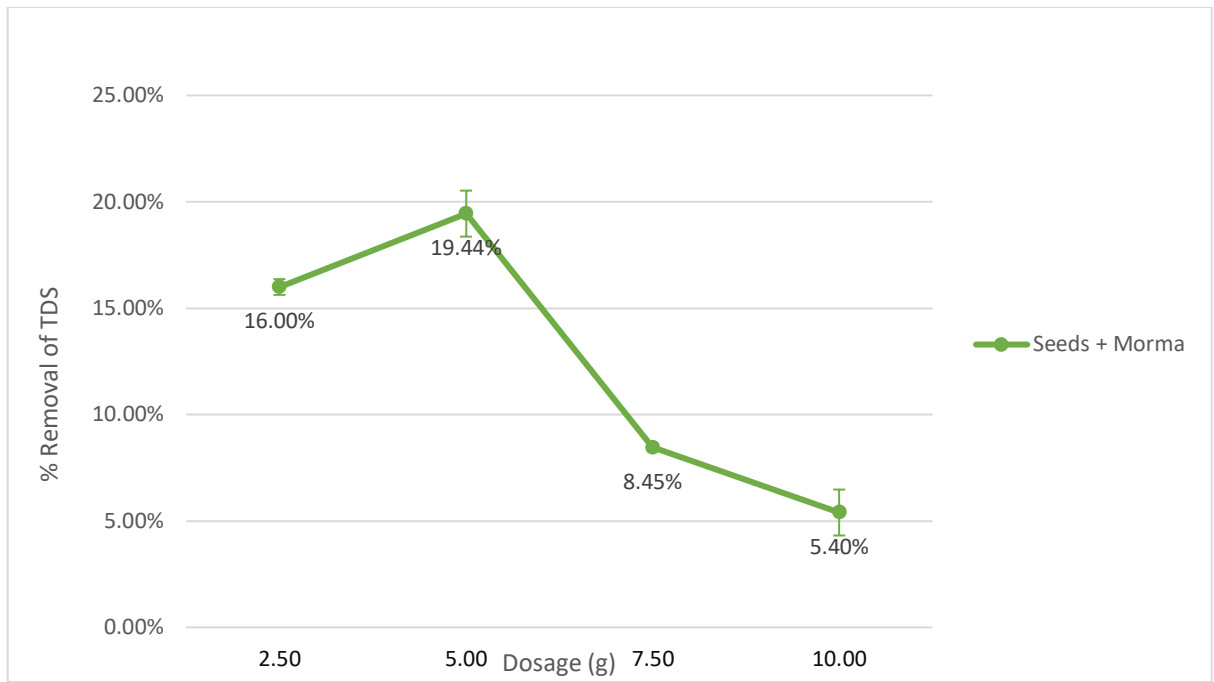


Figure 9-5: TDS Removal Rates (Seeds + Morma)

9.3.2 TSS

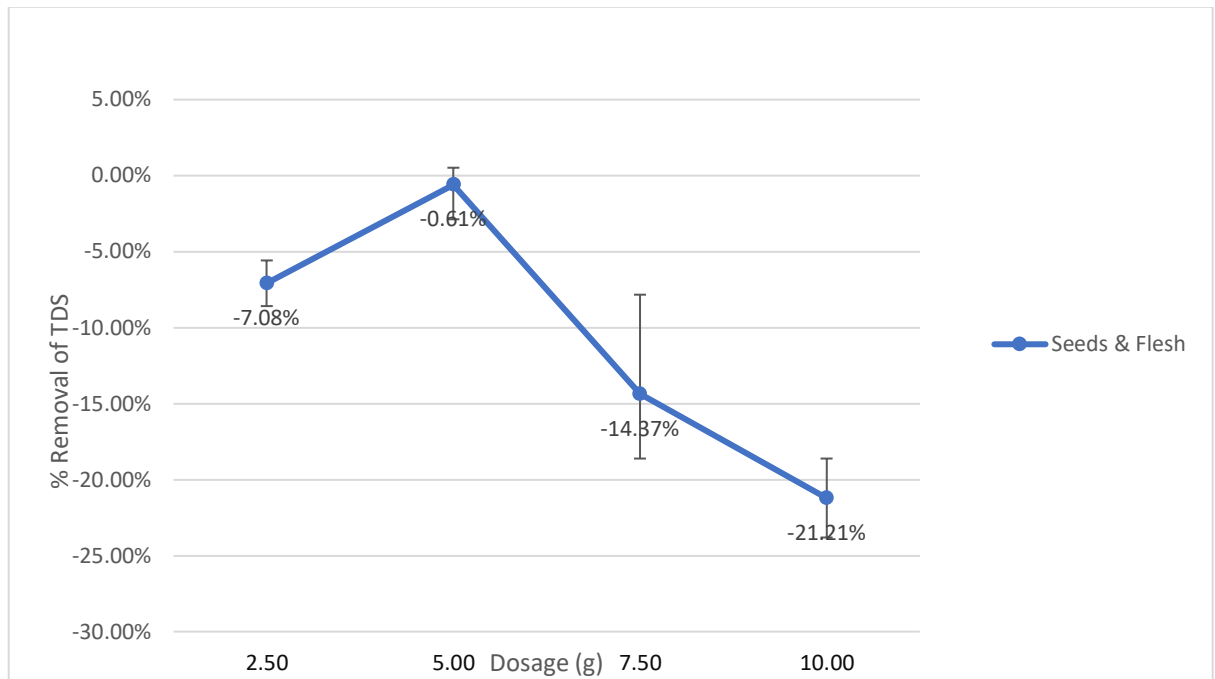


Figure 9-6: TSS Removal Rates (Flesh & Seeds)

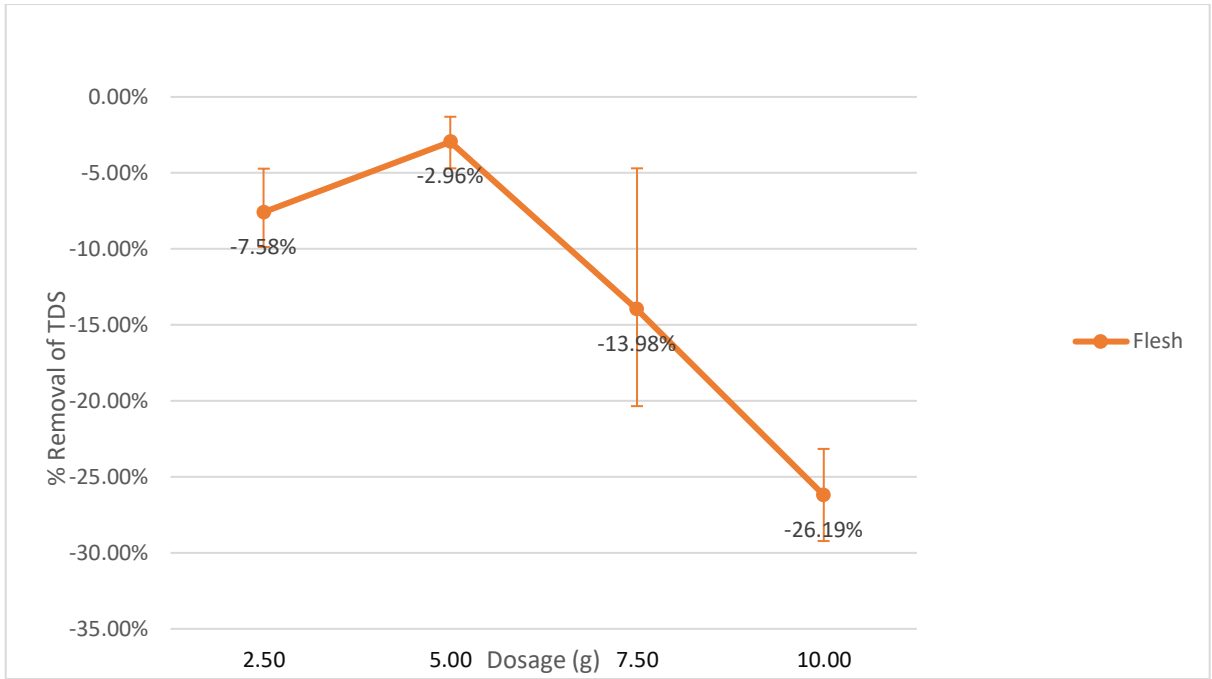


Figure 9-7: TSS Removal Rates (Flesh)

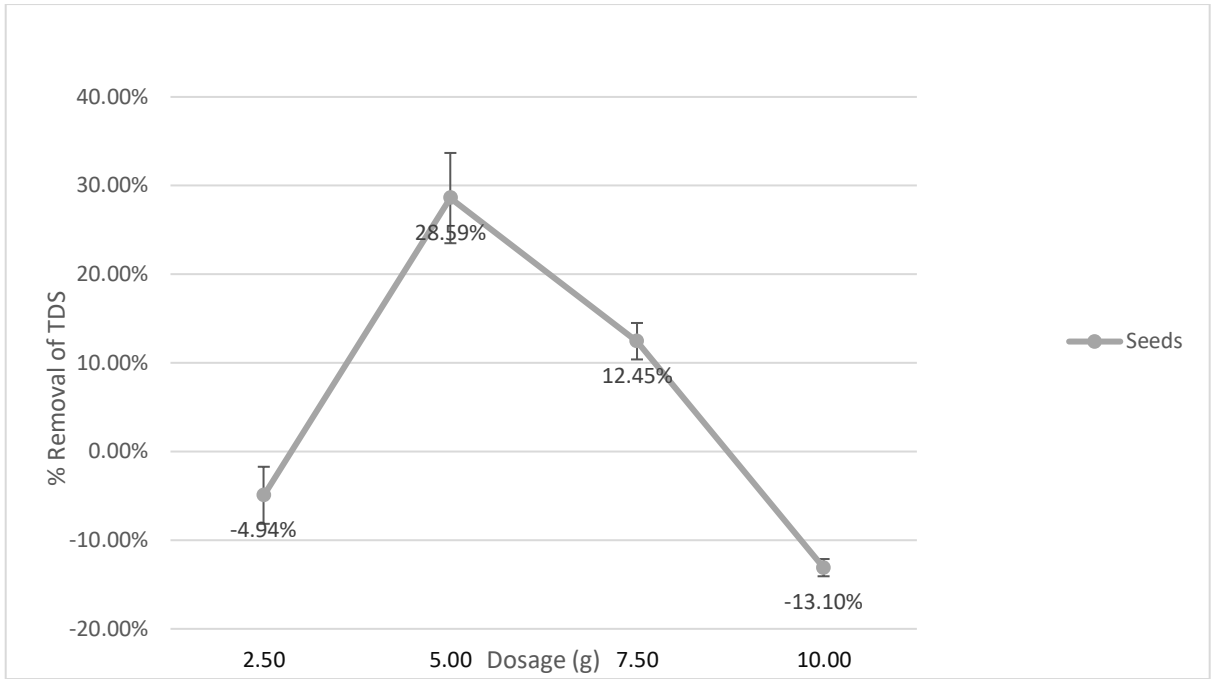


Figure 9-8: TSS Removal Rates (Seeds)

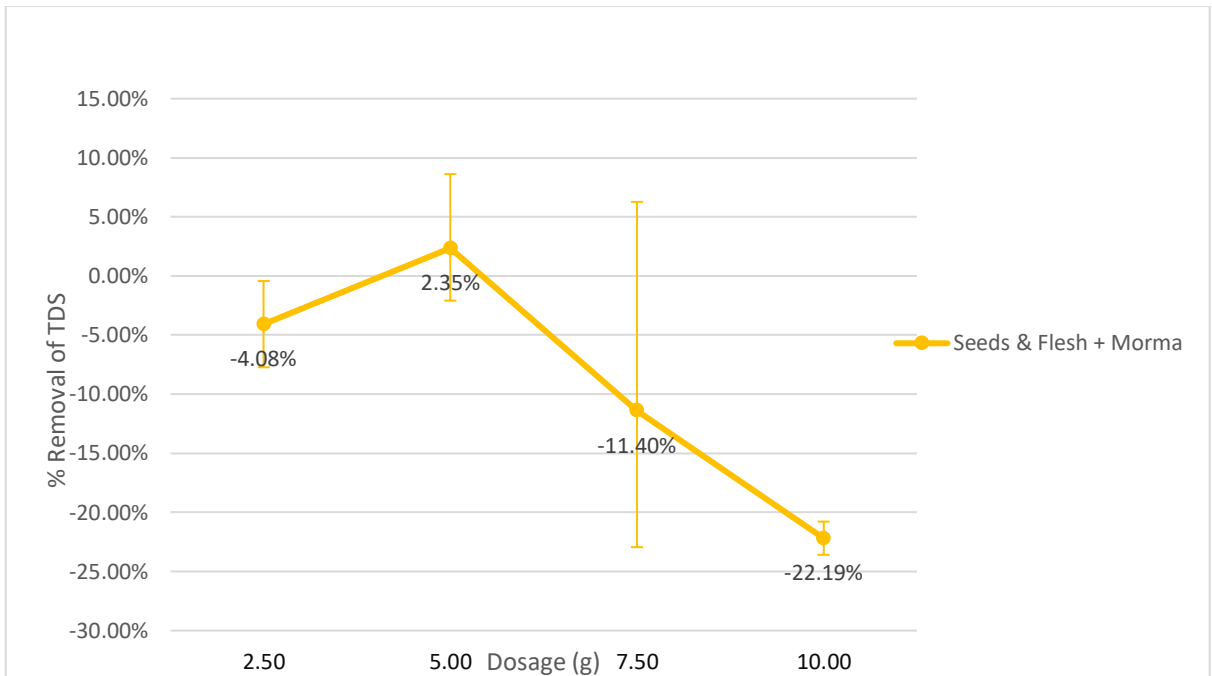


Figure 9-9: TSS Removal Rates (Seeds & Flesh + Morma)

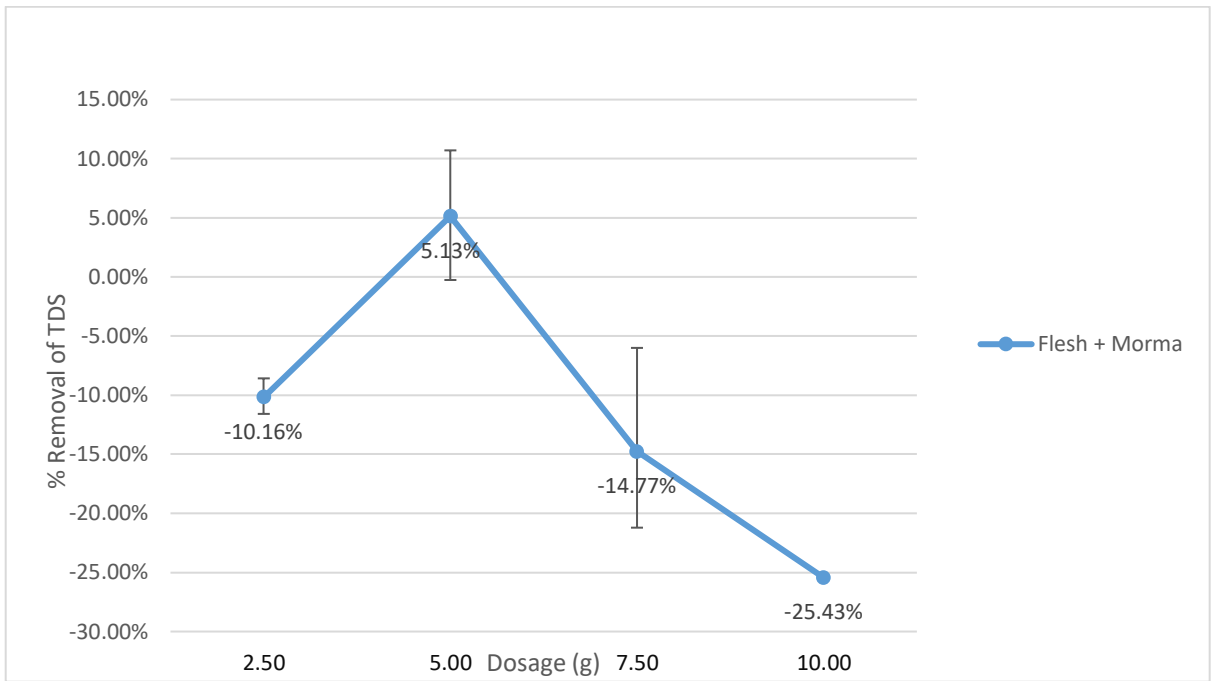


Figure 9-10: TSS Removal Rates (Flesh + Morma)

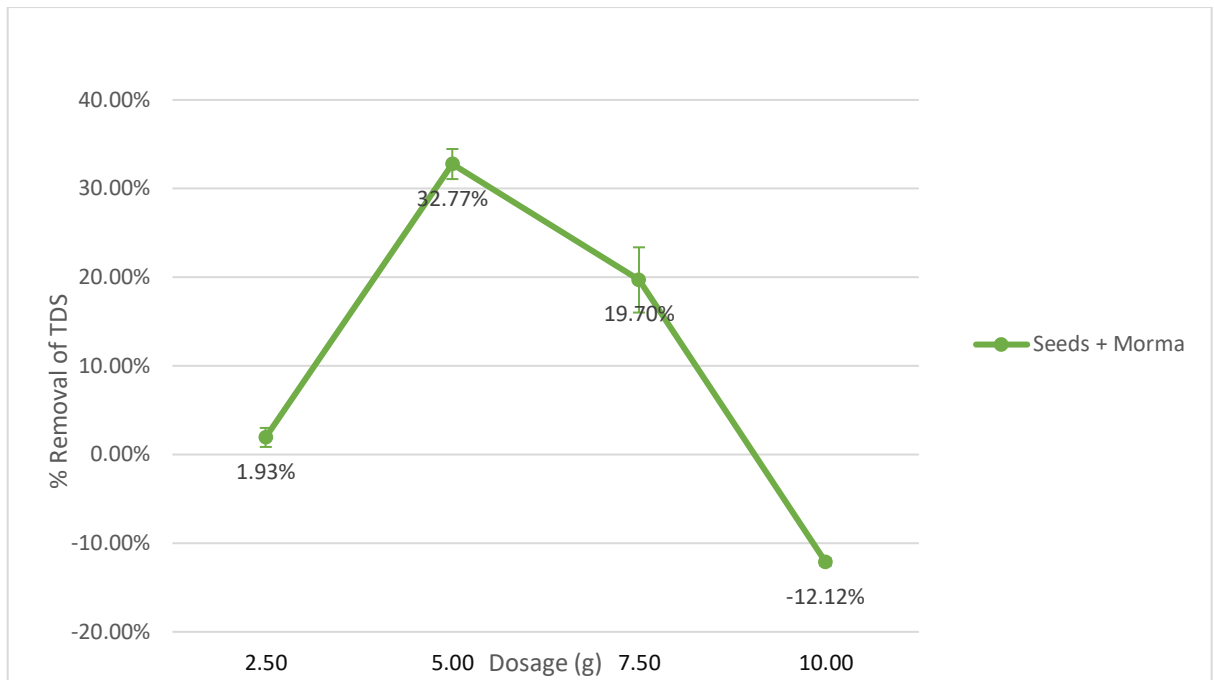


Figure 9-11: TSS Removal Rates (Seeds + Morma)

9.3.3 COD

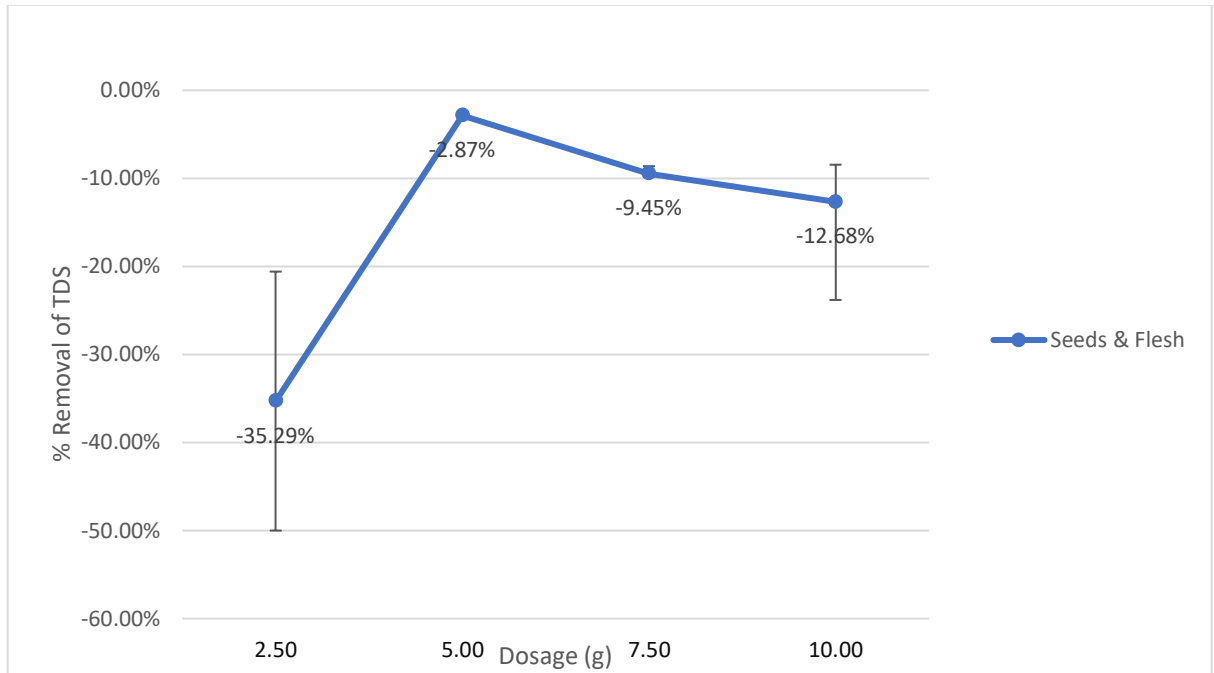


Figure 9-12: COD Removal Rates (Flesh & Seeds)

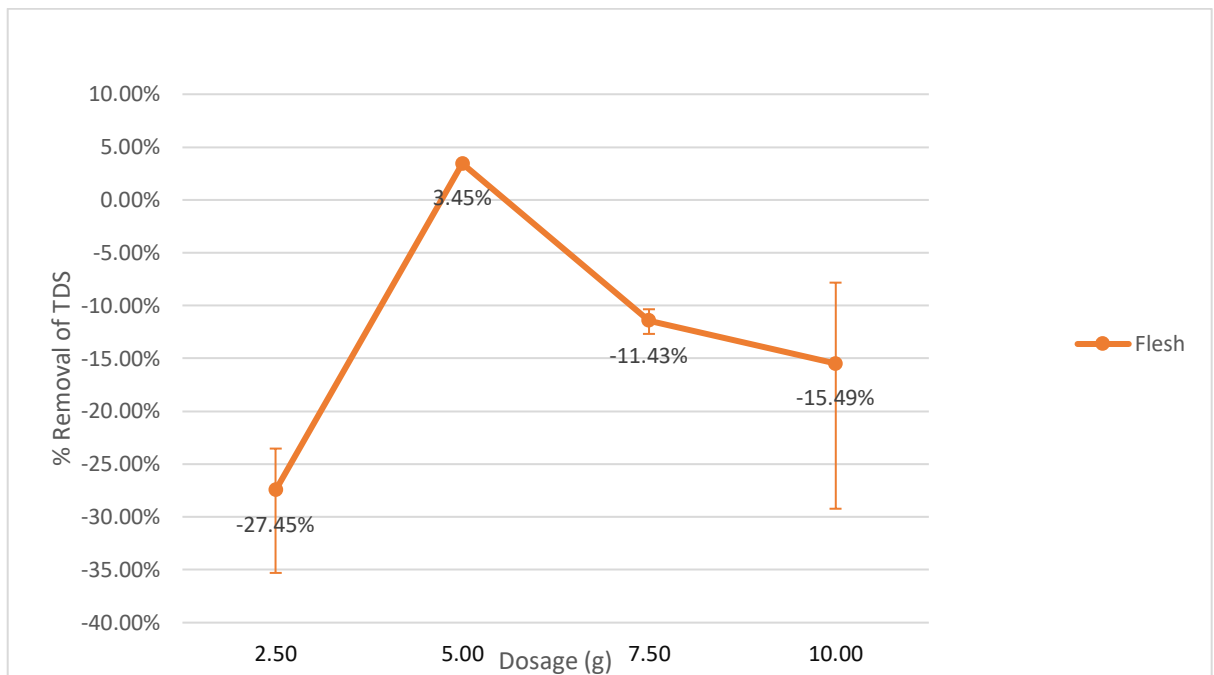


Figure 9-13: COD Removal Rates (Flesh)

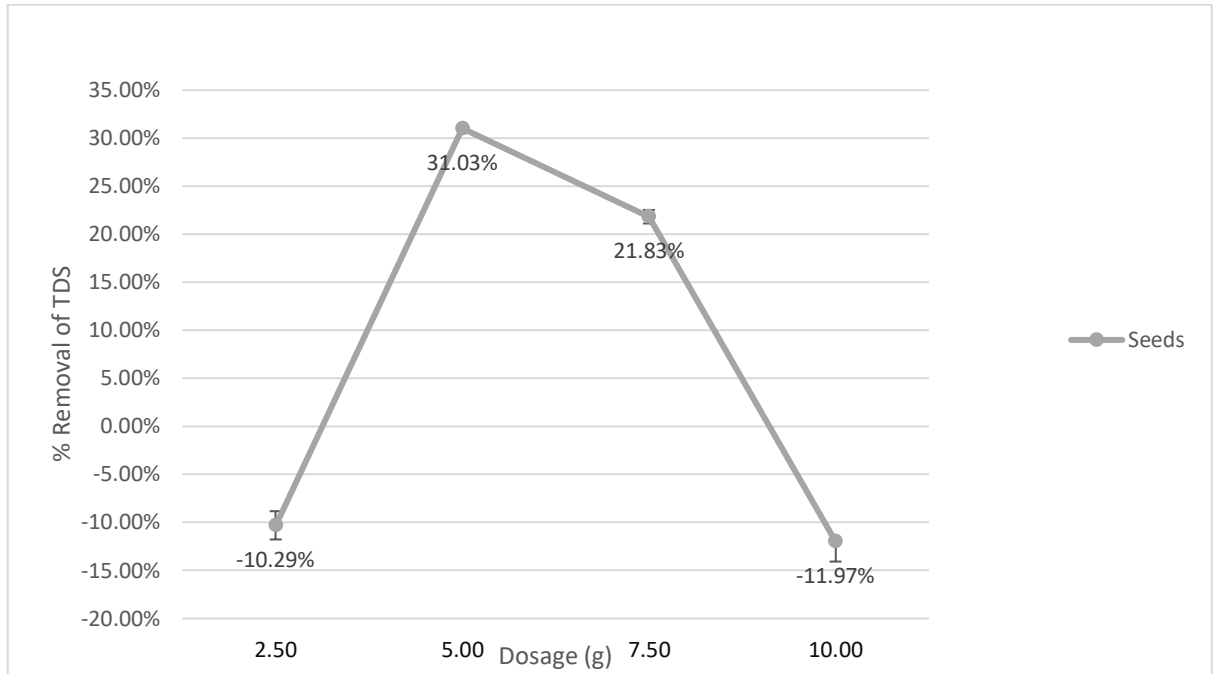


Figure 9-14: COD Removal Rates (Seeds)

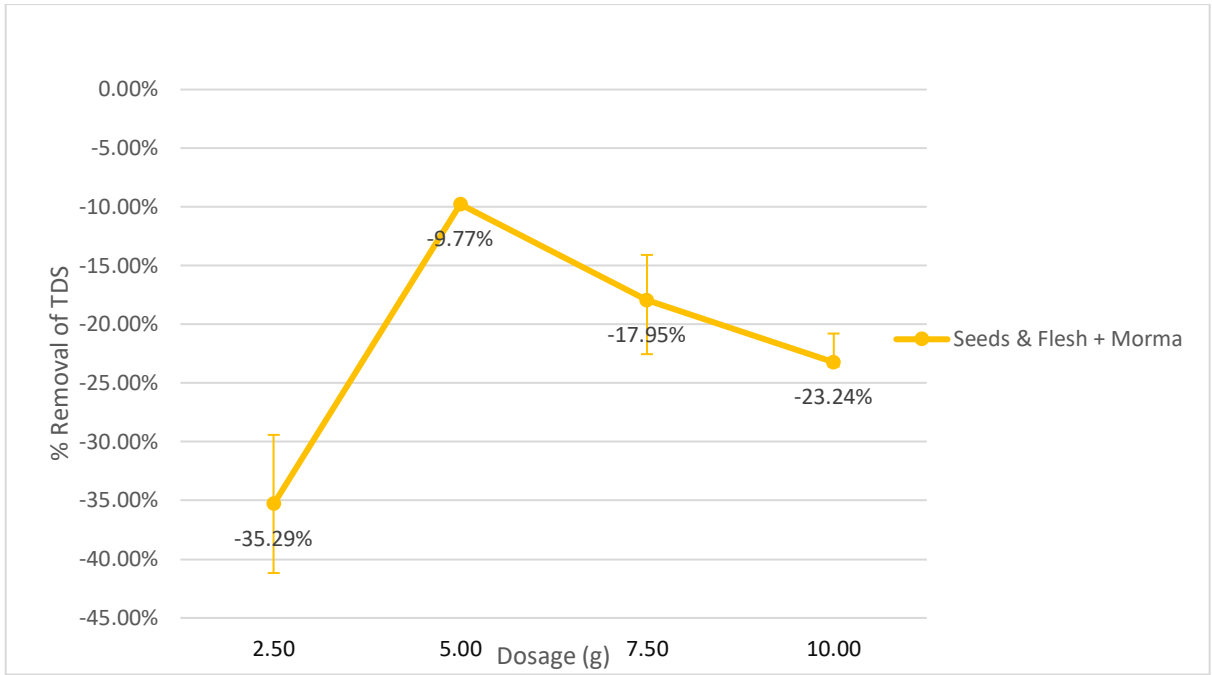


Figure 9-15: COD Removal Rates (Seeds & Flesh + Morma)

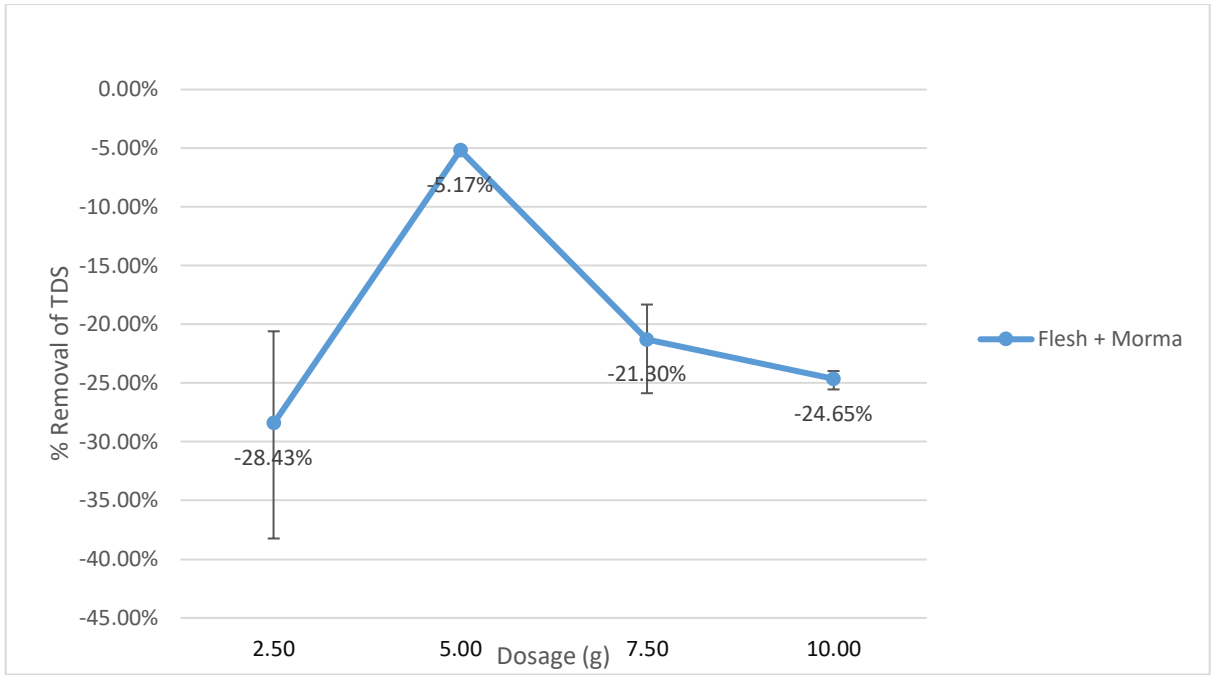


Figure 9-16: COD Removal Rates (Flesh + Morma)

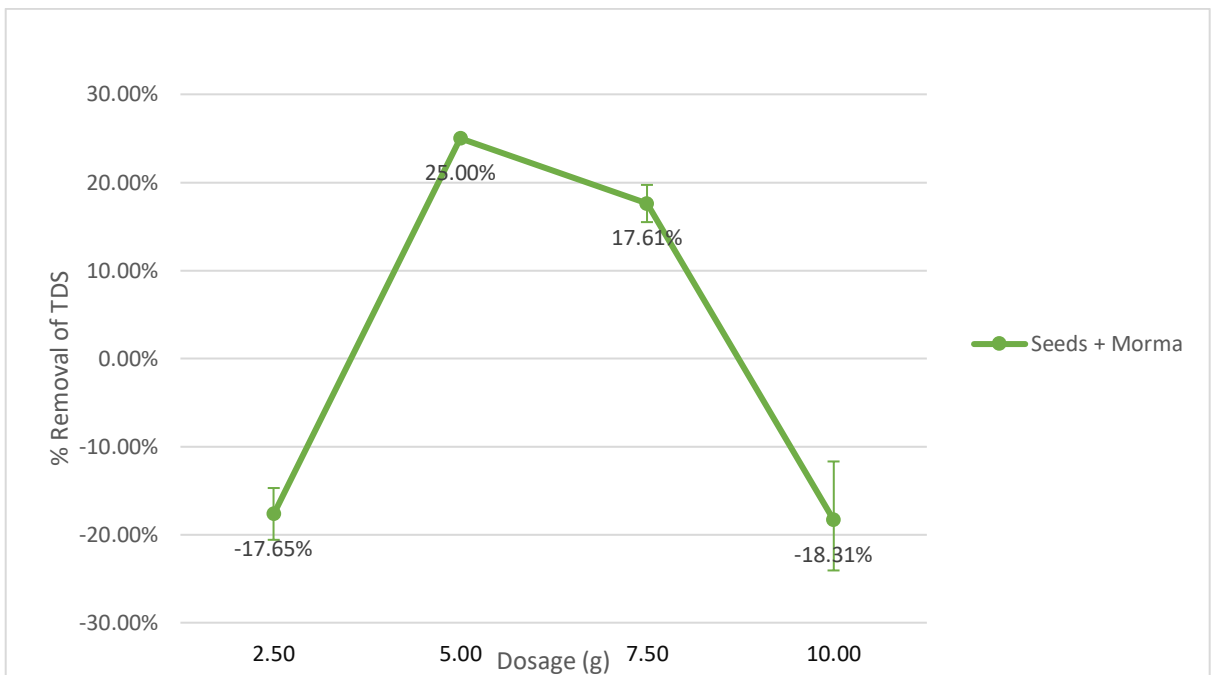


Figure 9-17: COD Removal Rates (Seeds & Flesh + Morma)