POULTRY SLAUGHTERHOUSE WASTEWATER TREATMENT USING BIO-PHYSICO-PRETREATMENT SYSTEMS COUPLED WITH AN EXPANDED GRANULAR BED REACTOR

By

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DECLARATION

I, **Cebisa Thabo Mdladla**, declare that the contents of this thesis represent my own work, and that the thesis/dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Poultry slaughterhouse wastewater (PSW) is having a high organic matter content which contains constituents such as blood, undigested food, meat debri and feathers, colloidal particles as well as soluble proteins. This type of wastewater in turn is high in fats, oils and grease (FOG), chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS) and volatile fatty acids (VFA) from slaughtering and facility cleaning activities which when released untreated end-up in drinking water sources. The poultry industry is therefore mandated through stringent environmental rules to treat the wastewater to acceptable contaminant levels and to reduce the amount of wastewater that is released into natural water sources. As such, a variety of treatment processes are used by the poultry industry to reduce the discharge of untreated wastewater into the environment. These processes include physical, chemical and biological treatment processes.

Biological treatment processes are nontoxic, and produce extracellular, biopolymeric substances secreted by algae, yeast, and bacteria in the processes for numerous purposes. Due to these properties and the lack of secondary pollution, biological remediation has been identified as an alternative to chemical and physical treatment options. This research was aimed to determine whether pretreating the PSW with a commercially produced product, i.e., Eco-Flush^{TM} which is a biodelipidation agent, would result in the reduction of COD, FOG and TSS and therefore allow optimal treatment of the PSW using an Expanded Granular Sludge Bed (EGSB) reactor, an anaerobic bioreactor selected for this study.

A volume (250 L) of the PSW was collected from a poultry slaughterhouse using sterile 25L polypropylene bottles and stored at 4˚C. The raw PSW was analyzed for FOG, COD, and TSS prior to the addition of the PSW to the pretreatment tank. The PSW was pretreated by mixing 20 mL Eco-Flush^{TM} mixed into 20L of raw PSW. The mixture was aerated for 24 h then allowed to settle for a further 24 h to allow the Eco-Flush^{TM} time to properly hydrolyze FOG and flocculate-coagulate proteins including TSS within the PSW and reduce the level of dissolved oxygen (DO) in the PSW prior to it being supplied to the EGSB reactor operated at 37 ˚C. The pretreated PSW was then filtered to remove feathers, pieces of meat and the flocculated organic matter was skimmed off such that clogging will be minimized in the EGSB bioreactor. The EGSB reactor containing a slurry of activated sludge, milk as a substrate and PSW was also allowed to acclimatize for 3 days, for bacterial growth to be at an exponential phase, prior to feeding the EGSB reactor with pretreated PSW from the pretreatment tank. The EGSB reactor was initially fed PSW for 16 h a day for one month to allow the activated sludge to adapt to the new feed and for proper optimization of the plant's operational parameters. The system was then run continuously, for 7 days a week, over a period of four months, with 2L samples being collected three times a week namely Monday, Wednesday and Friday from the pretreated PSW and the effluent from the EGSB bioreactor. The PSW's FOG, COD and TSS content was determined to assess the effectiveness of the pre-treatment process, EGSB bioreactor anaerobic treatment to observe the remedial action of the combined pre-treatment-EGSB system. The average removal in the pretreatment tank for COD, FOG and TSS was 43%, 66% and 59%, respectively. The EGSB recorded upper limits of 76% COD removal, upper limits of 96% were recorded for TSS and FOG removal peaked at 97% with an average of 66%. An increased treatment efficacy was noted for the combined PSW treatment system, whereby the COD, FOG and TSS removal averaged 76%, 88% and 87%, respectively. The process developed is intended for micro, small and medium poultry slaughterhouses.

Keywords: anaerobic digestion; bio-delipidation; expanded granular sludge-bed bioreactor (EGSB); poultry slaughterhouse wastewater (PSW)

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“I can do all things through Christ who strengthens me.”

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- **Cape Peninsula University of Technology**, for the financial support towards this research.

DEDICATION

This thesis is dedicated to my late mother

Mrs. Thembisa Mdladla

Rest in eternal peace Tyopho, Nokwindla Xhamela, Ncancashe,
Magwebulikhula, Malambedlile, sikhukhukazi ezikhusela amantshontsho
waso noxa sesikwelemimoya.

Memories of the love you gave, and your teachings shall forever remain

Love Your Son.

RESEARH OUTPUTS

This thesis has been published in part in the Department of Higher Education and Training (DHET) accredited article(s) and conference proceeding(s) cited below:

Published Conference Proceedings:

1. **Mdladla, C.T.**, Meyo, H., Basitere, M. & Ntwampe, S.K.O. 2019. "Biological Pre-treatment of Poultry Slaughterhouse Wastewater." 16th SOUTH AFRICA Int'l Conference on, Chemical, Biological & Environmental Sciences (ACBES-19) Nov. 18-19, 2019 Johannesburg (S.A.) https://doi.org/10.17758/EARES8.EAP1119148.

Published Extended Abstract

2. **Mdladla, C.T.**, Meyo, H., Basitere, M. & Ntwampe, S.K.O. 2021. "The Aerobic Pretreatment of Poultry Slaughterhouse Wastewater Coupled with Remediation in an Expanded Granular Sludge Bed Reactor." 12th Eastern European Young Water Professionals Conference. 31 March -02 April 2021, Riga, Latvia, http://iwa-ywp.eu/.

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3. **Mdladla, C.T.**, Dyosile, P.A., Njoya, M., Basitere, M., Ntwampe, S.K.O., and Kaskote, E. 2021. Poultry Slaughterhouse Wastewater Remediation using a Bio-delipidation Pre-treatment Unit Coupled with an Expanded Granular Sludge Bed Reactor. Manuscript ID: processes-1403152 (Accepted 15 September 2021).

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THESIS LAYOUT

This research project was conducted in laboratory 1.27, Department of Chemical Engineering, Cape Peninsula University of Technology, Bellville Campus.

Thesis breakdown:

Chapter One; This chapter reviews the background of the study, list research questions, aims and objectives, hypothesis and highlights the significance of the study.

Chapter Two; This chapter consists of the literature review of the study reviewing the different biological pretreatment techniques currently in use and how they can be adopted to assist in the remediation of poultry slaughterhouse wastewater. This chapter was published and presented in the 16th SOUTH AFRICA Int'l Conference on Agricultural, Chemical, Biological & Environmental Sciences (ACBES-19). Nov 18-19, 2019 Johannesburg (S.A.).

Chapter Three; This chapter contains the research methodology and experimental results. It describes the materials and equipment, experimental set-up and operations, sampling, operating conditions of the system as well as data analysis. This chapter also discusses results.

Chapter Four; Conclusions and recommendations are listed.

References; Bibliography consulted for the success of this study. The end of each of the chapters have their own references listed.

Appendices; Additional data not reported within the body of the thesis.

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ABBREVIATIONS

Abbreviation	
AD	Anaerobic Digestion
BOD	Biological Oxygen Demand
CCT	City of Cape Town
COD	Chemical Oxygen Demand
CPUT	Cape Peninsula University of Technology
EGSB	Expanded Granular Sludge-Bed Bioreactor
FOG	Fats, Oils and Grease
HRT	Hydraulic Retention Time
OLR	Organic Loading Rate
PSW	Poultry Slaughterhouse Wastewater
SA	South Africa
SAB	South African Breweries
TSS	Total Suspended Solids
UASB	Up-flow Anaerobic Sludge Bed

GLOSSARY

Activated Sludge:	Microbial culture responsible for organic matter assimilation.
Aerobic Digestion:	A processing where organic matter is converted into harmless by-products in the presence of oxygen.
Anaerobic Digestion:	A process where organic matter is converted into harmless by-products in the absence of oxygen.
Biogas:	Gas release from degradation of organic matter in the absence of oxygen.
Biological Oxygen Demand:	Required oxygen for biodegradation of organic matter in wastewater.
Bioremediation:	The breakdown of organic matter by microorganisms.
Chemical Oxygen Demand:	Required oxygen for the oxidation of organic matter to occur.
Eco-Flush^{TM}:	A commercial product containing naturally occurring bacteria harvested from the soil, is activated by water and breaks down hydrocarbons.
Hydraulic Retention Time:	Time with which wastewater is kept in a reactor for treatment.
Hydrolysis:	The breaking down of organic matter into soluble compounds.
Organic Loading Rate:	Feeding rate of organic matter.
Organic Matter	Water oxygen consuming matter which consists of proteins, fats and carbohydrates including other pollutants.
Slaughterhouse Wastewater	High strength wastewater generated from slaughtering processes.

CHAPTER ONE

INTRODUCTION

1. CHAPTER ONE: INTRODUCTION

1.1. Background into poultry slaughterhouse wastewater

South Africa recently experienced water shortages which have resulted in most agricultural sectors exploring alternative means to reduce water wastage [1]. The poultry slaughterhouse industry is one of the highest consumers of potable water which results in a generation of high volumes of wastewater. It is estimated that 26.5 litres of portable water are required to process one live bird and a greater percentage of the consumption is attributed to evisceration and sanitation of equipment [2], [3]. As a result, poultry processing wastewater has high concentrations of biological oxygen demand (BOD) and chemical oxygen demand (COD) which can end up in drinking reservoirs [4]. Due to this increasing usage of water, high concentration of pollutants escape or are released into streams and rivers [5]. Due to stringent regulatory environmental rules, water supply insecurities and the eminent water scarcity in South Africa, an intensive treatment process prior to wastewater being discharged is needed by poultry product producing industries to mitigate against these current water shortages [1].

A significant volume of highly polluted wastewater is generated by poultry slaughterhouses during the slaughtering stage and periodic washing of residual particles, which results in significant variations in the biodegradable organic matter concentration in it. Therefore, an efficient treatment process should be implemented by poultry slaughterhouses to treat the wastewater before it is discharged into receiving water bodies and to subsequently prevent severe environmental pollution [6]. Several treatment methods have been reported for PSW over the past few decades. Biological (aerobic and anaerobic) treatment methods have been traditionally used for slaughterhouse wastewater treatment. The ability of different bacterial communities to either be beneficial or detrimental to industrial process, needs to be better understood as they are purported to replace chemical or physical processes [7]. Some of these processes include aerobic and anaerobic processes.

Anaerobic bacteria are responsible for the production of methane gas from sewage sludge, they facilitate the decomposition of macromolecular organic matter into simpler compounds, therefore, they play an important role in the wastewater treatment processes [8]. Anaerobic treatment processes are often impaired because of accumulation of FOG and TSS which in turn leads to the reduction in the methanogenic activity and biomass washout.

Aerobic treatment has been used to pretreat PSW [9]. This treatment process has high energy consumption for aeration and generates a large amount of sludge, moreover, as the pretreated water is transferred into the bioreactor, the aerobic bacteria which initially had access to oxygen in the pretreatment tank is unable to continue replicating in oxygen depleted bioreactors and subsequently die.

The treatment disposal of poultry slaughterhouse wastewater is both a public health and an economic necessity [10]. Effective wastewater treatment will thus benefit the poultry industry processing plants by reducing potable water demand and the volume of wastewater generated for disposal [3]. However, depending on the degree of treatment required, poultry processors have a variety of options including the use of chemical, physical and biological treatment systems. Each system type possesses unique treatment advantages and operational difficulties [5]. For the purpose of this study, the focus was on the use of biological pretreatment systems to reduce TSS and accumulation of FOGs prior to treatment of PSW in the ESGB reactor. This study also seeks to illustrate that a pretreatment step is required for efficient digestion of biodegradable organic matter PSW.

1.2. Research problem

The accumulation of FOG within the EGSB reactor tends to result in sludge washout. There is therefore a need for pretreatment to reduce the FOG before the anaerobic digestion process to effectively treat PSW. This study evaluated the effectiveness of a bio-physico-pretreatment system dosed with a commercial FOG hydrolysing agent (Ecoflush^{TM}) coupled with an EGSB reactor in treating PSW.

1.3. Hypothesis

Ecoflush^{TM} can successfully hydrolyze FOGs, COD and TSS in PSW. Therefore, using Ecoflush^{TM} as a pretreatment agent may improve the biological degradation of fatty material in the PSW, and accelerate processing of PSW while providing a seamless environment for the remediation of PSW by activated sludge in the EGSB bioreactor. There is, therefore, a need to investigate the biodegradability of organic matter in PSW with a bio-physico-pretreatment-anearobic bioreactor system.

1.4. Aims and objectives

This study's aim was to investigate the use of bio-physico-pretreatment in reducing the organic load in PSW for the proper treatment of water in an EGSB reactor.

To achieve this aim, the following objectives must be met:

- Evaluate the effectiveness of Ecoflush^{TM} supported pretreatment unit in reducing FOG, COD and TSS in PSW in a pretreatment unit,
- To investigate the performance of the EGSB reactor by quantifying effluent quality parameters, i.e., FOG, COD and TSS,
- To compare the overall effectiveness of the combined treatment system for PSW, i.e., Ecoflush^{TM} supported pretreatment unit coupled with anaerobic digestion using an EGSB reactor

1.5. Significance of the study

This study provides information on how effective the Ecoflush^{TM} supported pretreatment unit is at pretreating PSW, and as to how pretreated PSW affects the EGSB reactor system performance, as well as to note any improvements in COD, FOG and TSS removal when using the combined Ecoflush^{TM} supported pretreatment unit-EGSB reactor system for PSW removal.

1.6. Delineation of the research

This study did not focus on the following:

- Biogas production
- Parameters other than COD, FOG and TSS and
- Scale up of the reactor system.

1.7. References

[1] M. Basitere, Y. Williams, M. S. Sheldon, S. K. O. Ntwampe, and D. de Jager, “Performance of an expanded granular sludge bed (EGSB) reactor coupled with anoxic and aerobic bioreactors for treating poultry slaughterhouse wastewater,” vol. 11, no. 1, pp. 86–92, 2016, doi: 10.2166/wpt.2016.013.

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CHAPTER TWO

LITERATURE REVIEW

This chapter was published as a conference proceeding in 2019 as:

Mdladla, C., Meyo, H., Basitere, M. & Ntwampe, S.K.O. (2019). Biological Pre -treatment of Poultry Slaughterhouse Wastewater. **16th SOUTH AFRICA Int'l Conference on Agricultural, Chemical, Biological & Environmental Sciences (ACBES-19)** Nov. 18-19, 2019 Johannesburg (S.A.). https://doi.org/10.17758/EARES8.EAP1119148.

2. CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The agricultural sector, particularly the poultry industry is exploring alternative means to reduce water wastage due to recent water shortages experienced in South Africa [1]. The poultry slaughterhouse industry has been noted to be one of the highest contributors to the consumption of potable water, which results in the generation of high volumes of wastewater. Processing of one live bird requires an estimated average of about 26.5 litres of potable water and the greater percentage of the consumption is attributed to evisceration and sanitation of equipment [2], [3].

The organic load contribution comes from different materials such as fat, oil and grease (FOG), lard, blood, undigested food, loose meat, paunch, colloidal particles suspended materials and soluble proteins [4]. As a result, poultry (bird) processing wastewater has high concentrations of biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), fats, oils and grease (FOGs) and cleaning activities which filters into drinking reservoirs [5]. The treatment process needs to be intensified by poultry product producing industries prior to water being discharged to avert the excessive usage of water, reduce the high concentration of wastewater pollutants escaping into streams and rivers [6], to comply with the stringent regulatory environmental rules, relieve the water supply insecurities and the eminent water scarcity in South Africa [1].

The treatment disposal of poultry slaughterhouse wastewater (PSW) is both a public health and an economic necessity [7]. The poultry industry will benefit from the effective wastewater treatment by being able to reduce potable water demand and minimize the quantity of wastewater generated for disposal [3]. Three distinct treatment systems have been employed by the poultry industry depending on the treatment process required. These options include physical, chemical and biological treatment systems and each system type possess unique treatment advantages and operational difficulties [6].

This review focuses on the use of biological systems to reduce sludge formation and accumulation of FOGs prior to treatment of PSW in the Expanded Granular Sludge Bed (EGSB) reactor.

2.2 Preferred poultry slaughterhouse wastewater treatment process

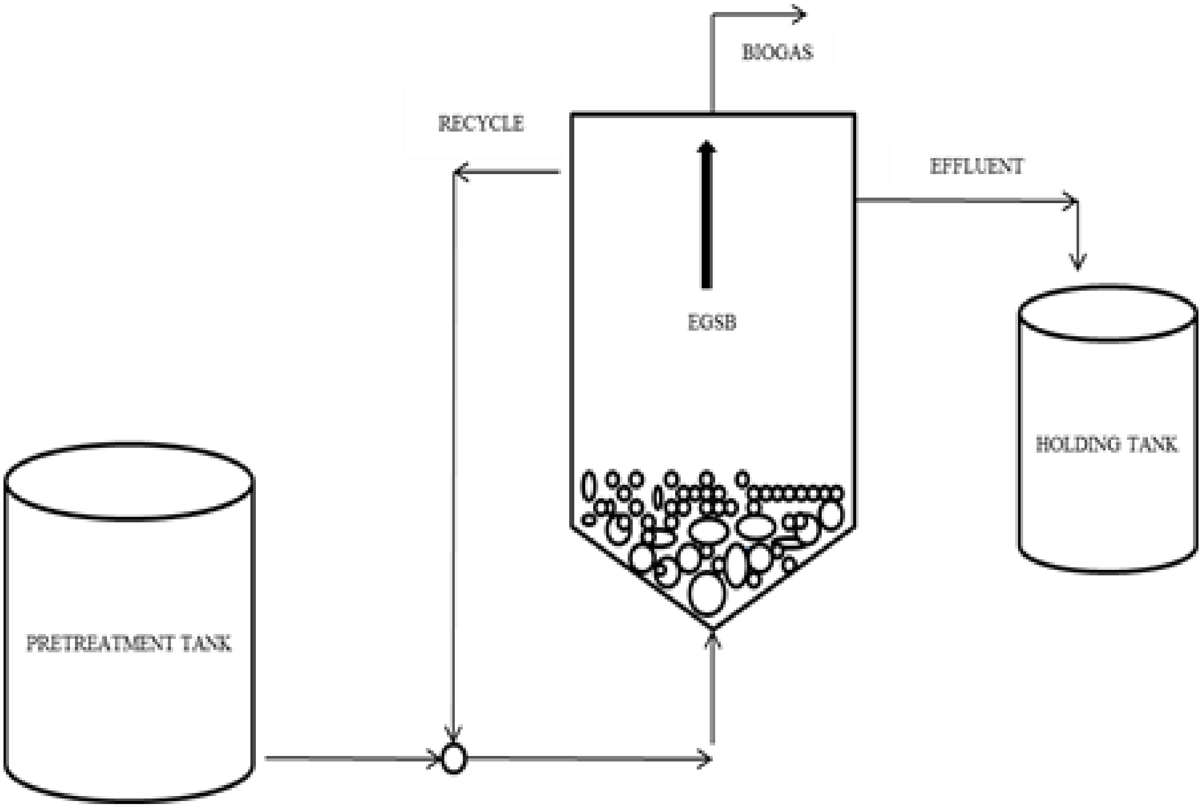

Figure 2:A A schematic diagram of the PSW treatment process

The bioreactor system consists of a pretreatment tank for the degradation of organic soluble which would otherwise cause clogging up of the EGSB reactor. The EGSB reactor is also fitted with a recycling step to help prevent sludge washout and clogging up of the EGSB reactor. The effluent is collected in a holding tank and further testing for BOD, COD, TSS and FOGs will be conducted from the effluent. The used of the EGSB was determined to be effective as reported elsewhere [26].

2.3 Pretreatment using biological processes

A significant volume of highly polluted wastewater is generated by poultry slaughterhouses during the slaughtering stage and periodic washing of residual particles, which results in significant variations in the biodegradable organic matter concentration. Therefore, an efficient treatment process should be carried by poultry slaughterhouses to treat the wastewater before it is discharged into receiving water bodies and to subsequently prevent severe environmental pollution [4]. Several treatment methods have been reported for PSW over the past few decades. Biological (aerobic and anaerobic) treatment methods have been traditionally used for slaughterhouse wastewater treatment. The ability of different bacterial strains to produce a variety of by-products which are either beneficial or detrimental has enhanced the need to better understand the impact of harnessing of biological processes instead of chemical or physical processes [8]. Anaerobic bacteria are responsible for the fermentation of methane gas from sewage sludge, they facilitate the decomposition of macromolecular organic matter into simpler compounds, and therefore, they play an important role in the wastewater treatment processes [9]. Anaerobic treatment processes are often impaired because of accumulation of FOGs and SS which in turn lead to reduction in the methanogenic activity and biomass washout.

Aerobic treatment has been used to pretreat PSW in processes such as the Dissolved Air Floatation (DAF) pretreatment tank [10]. This treatment process requires high energy consumption for aeration and generates large amounts of sludge, moreover, as the pretreated water is transferred into the bioreactor the aerobic bacteria which initially had access to oxygen in the pretreatment tank is unable to continue replicating in oxygen-depleted bioreactors and subsequently die. This review seeks to illustrate that a pretreatment step is required for efficient digestion of biodegradable organic matter. Bacterial cultures with bioremedial activity and can survive in both aerobic as well as anaerobic conditions are most suitable for pretreatment of PSW as they remain active under both conditions and would further ensure that the remediation that occurs in the pretreatment tank continues to occur in the bioreactor which is oxygen depleted.

2.4 Flocculation in PSW pretreatment

Flocculation has been commonly used for the gradual accumulation of colloids, cells and suspended solids in the treatment of drinking water, fermentation processes, production of food and treatment of wastewater [11]. There are distinct groups in which flocculants are classified into, namely: 1) organic synthetic flocculants such as polyacrylamide derivatives, 2) inorganic synthetic flocculants such as polyaluminium chloride and 3) naturally occurring flocculants such as chitosan. Organic and inorganic synthetic flocculants are widely used in industrial fields for their cost efficiency and cost-effectiveness, but their use may also result in some environmental and health issues [12]. Health issues caused by these flocculants may include Alzheimer’s disease [13], which is caused by Aluminum salts, as well as the formation of neurotoxic and carcinogenic acrylamide monomers that are harmful to humans and the environment [14]. This has therefore sparked renewed interest in a less toxic method of flocculation namely naturally occurring bioflocculants for the precipitation of organic matter.

Bioflocculants are non-toxic and biodegradable extracellular biopolymeric substances secreted by algae, yeast, and bacteria [15]. They rely on the difference in composition and properties of polysaccharides and proteins which lead to differences in the charge of bioflocculants [16]. In general, bioflocculants cause the aggregation of particles and cells by bridging and charge neutralization [15]. Bioflocculant composition consists of macromolecular substances such as protein and polysaccharide-protein [5], [17] and is dependent on the type of bioflocculants producing microorganisms (BPMs) [18]. Due to these properties and the lack of secondary pollution of their degradative intermediates [15], bioflocculants have been identified as a possible alternative to flocculation which requires the use of chemicals including ferric chloride, polyaluminium chloride and polyacrylamide [19]. Table 2:A provides a list of bioflocculant producing microorganisms, their preferred energy source as well as their mode of action.

Table 2:A Depicts the different types of bioflocculant producing microorganisms, their preferred energy source, and their mode of action.

Bioflocculants	**Energy source**	**Mode of action**
Gyrodinimimpudicum KG03	Acidic heteropolysaccharide	Galactose and uronic acid production
Nannocystis species Nu-2	Glycoprotein	Bleaching acid red and direct emerald blue
Rhodococcuserythropolis	Proteins	Enzymatic digestion

2.5 Pretreatment with biosurfactants

The importance of surfactants in household and industrial applications is undeniable and they have been used to confer excellent detergency, emulsifying, foaming and dispersing traits [20]. Surfactants are amphipathic molecules that have both hydrophobic and hydrophilic moieties that partition preferentially at the interphases such as liquid/liquid, gas/liquid or solid/liquid. Surfactants also have different degrees of polarity and hydrogen bonding, as such surfactants are mostly chemically synthesized and petroleum-based [21].

The environmental implications of using surfactants such as toxicity, biodegradability, ecological acceptance and affordability encouraged the search trend towards using environmentally friendly technologies [22]. Biosurfactants have therefore gained much attention because they exhibit environmental ecological advantages. Biosurfactants produced by microorganisms in the environment assist in the uptake of hydrocarbons as a carbon source. This is done by either the microorganism changing its cell surface so that the contaminant can be absorbed or by making available the hydrocarbon by releasing biosurfactants into the environment. Because of their hydrocarbon dissolving agents, biosurfactants were identified as potential replacements for synthetic surfactants in food, oil and pharmaceutical industries [23]. Table 2:B below gives a comparison of the advantages and disadvantages of using chemical and biological surfactants.

Table 2:B A comparison between chemical and biological surfactants.

	Advantages	**Disadvantages**
Chemical	Cost-effective, High flocculating activity	Health issues
Biological	Environmentally friendly, biodegradable, free risk of secondary pollution, non-toxic and harmless to humans, animals and environment	High production costs and high dosage requirements

Biosurfactants can be used in a range of industrial applications including, crude oil drilling, lubricants, bioremediation of pollutants, health care, enhanced oil recovery and food processing [24]. Most biosurfactants are complex molecules comprising of different structures including glycolipids, polysaccharides-protein complex, lipopeptides, phospholipids and fatty acids [25] and they are classified based on their chemical composition, their mode of action and the microorganisms that produce them [23]. Table 2:C list microorganisms which have been identified to produce biosurfactants as well as their lipo-structures or groups.

Table 2:C Biosurfactant producing microorganisms [23].

Microorganism	**Biosurfactant lipo-structures or group**
Pseudomonas aeruginosa	Rhamnolipids
Acinetobacter calcoacerticus	Lipopolysaccharides (biodispersant)
Bacillus subtilise	Lipopetides and lipoproteins (surfactin)
Bacillus licheniformis	Lipopeptides (lichenysin)
Mycobacterium species	Trehalolipids
Nocardia species	Trehalolipids
Tsukamurella species	Di and oligosaccharide lipids

Biosurfactant producing microorganisms are mainly isolated from sites that are or were contaminated with wastewater, contaminated soils, petroleum hydrocarbons and effluents. They can grow on substrates considered to be potentially noxious for other non-biosurfactant-producing microorganisms. Biosurfactants play a physiologic role in increasing bioavailability of hydrophobic molecules, which are involved in cellular signaling and differentiation processes, which facilitate the consumption of carbon sources present in the poultry wastewater [23].

2.6 Conclusion and Recommendations

Biosurfactants are of particular interest due to their ability to decrease surface tension in waste as well as their ability to degrade hydrocarbons. PSW contains high concentrations of FOGs which are high in hydrocarbons and thus provide a consistent supply of carbon source for biosurfactant producing bacteria. The use of bioflocculants for coagulation of organic waste through the formation of flocs and biosurfactants to reduce surface tension and hydrolyze hydrocarbons within the pretreatment tank can be employed symbiotically to help reduce the formation of sludge, prevent the sedimentation of the formed flocs and thus allowing the filtration of dissolved organic solubles from the pretreatment tank to the bioreactor. However, no studies have been conducted to investigate if bioflocculant and biosurfactant producing bacteria can be used symbiotically as a pre-treatment option for anaerobic bioreactors. This review probes for the use as a combined biological pretreatment option and to quantitatively determine its performance in reducing the accumulation of organic load in poultry slaughterhouse wastewater prior to treatment in a bioreactor.

2.7 Summary

The formation of sludge from the accumulation of organic load such as fats, oils and grease (FOG), lard, blood, undigested food loose meat, paunch, colloidal particles, suspended materials and soluble proteins within the bioreactor hinders the treatment of poultry slaughterhouse wastewater (PSW). Bio-physico-pretreatment of PSW which includes coupling biological remediation with the physical treatment in bioreactor has the potential to reduce the accumulation of organic matter within the bioreactor. Bioflocculants and biosurfactants are biodegradable, nontoxic, extracellular, biopolymeric substances secreted by algae, yeast, and bacteria. Due to these properties and the lack of secondary pollution of their degradative intermediates, biological remediation has been identified as an alternative to chemical and physical treatment options. This review aims to assess current pretreatment options and to identify further developments which could help reduce the amount of time spent in the pretreatment stage.

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CHAPTER THREE

METHODOLOGY AND RESULTS

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3. CHAPTER THREE: METHODOLOGY AND RESULTS

Poultry Slaughterhouse Wastewater Remediation using a Bio-delipidation Pre-treatment Unit coupled with an Expanded Granular Sludge Bed Reactor

3.1 Introduction

Poultry slaughterhouse wastewater (PSW) contains a high concentration of organic matter and other pollutants such as fats, oils and grease (FOG), colloidal particles as well as soluble proteins from slaughtering and cleaning activities from the poultry slaughterhouses. If the PSW is discharged into surface water sources, the nutrient rich, and high organic matter it contains could result in pollution, eutrophication and deoxygenation of water bodies it is discharged into [1], [2]. The poultry industry is therefore mandated to reduce wastewater contaminant levels, as per Department of Water Affairs and the City of Cape Town 2014 Bylaws of South Africa [3], i.e. to reduce contaminants to specified levels prior to its release into receiving water bodies [4]. Over the years, several treatment processes have been employed by the poultry industry to meet the regulatory PSW discharge standards set by regulatory bodies. These treatment processes include biological, physical, and chemical treatment processes, with anaerobic digestion (AD) being the primary treatment technology of choice.

AD facilitates the reduction of solids as the sludge can act as a biofilter, provides effective pathogen destruction, reduces odor potential, and can also provide an energy source in the form of biogas. Furthermore, AD is also a predominant organic matter removal process and does conserve energy in comparison to aerobic digestion [1]. AD has been noted to be suitable for effectively treating high strength industrial wastewater while providing energy generation, low sludge output, and when stabilized, provides an effluent with consistent concentration of monitored parameters when compared with aerobic and physicochemical methods. The first stage of degradation of organic matter is its solubilization and hydrolysis of complex polymeric organic carbon structures in the wastewater being treated [5], [6]. Hydrolysis has been noted as one of the rates limiting steps in wastewater treatment resulting in the slow degradation rates by the sludge in an AD [7]. One of the main causes of slow sludge hydrolysis is the low biodegradability potential of the constituents in the sludge [8]. It is therefore important to improve sludge hydrolysis potential and by pre-treating wastewater before treating it in an AD. The introduction of hydrolytic bacteria and their constituents in the pre-treatment step, i.e., microorganisms or biomolecules which can convert carbohydrates even partially, hydrolyze FOG including sugars, can improve sludge performance for the treatment of wastewaters such as PSW [9].

Pre-treatment refers to the treatment of wastewater to enhance the availability of substrates to microorganism in subsequent processes; thereby, improving the removal of organics and enhancing the decomposition of any other pollutants [10] Pre-treatment provides several advantages resulting in an improved AD system. These include, decreasing the viscosity of sludge which permits greater organic loading rates for the AD. An increase in non-hydrolyzed constituents and solids concentration in the wastewater feed including their accumulation within an AD system either culminates in small digester volume capacity or reduces hydraulic retention times of the digester [11]. Another advantage of pre-treating wastewater is that it increases the amount of released soluble substrate significantly enhancing volatile fatty acids (VFAs) generation for improved treatment and biogas production [12]. Overall, pre-treatment methods have also achieved significant results in the lysis or disintegration of solids in wastewater, resulting in enhanced biogas production [7]. Table 3:A consist of a list of pretreatment methods currently in use for the reduction of FOG, biological oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids in PSW. Most of these methods are physico-chemical methods.

Table 3:A List of pre-treatment methods used for the removal of COD, BOD and FOG.

Pre-treatment methods	Purpose	Efficacy	References
Dissolved air floatation (DAF)	Uses liquid-solid separation by air introduction for floatation,	75% removal for FOG, BOD and TSS,	[13] & [14]
Coagulation-flocculation and sedimentation	Destabilizes colloidal particles to form flocs and sediments dense particles	Achieves up to 80% BOD, COD and TSS removal	[15]
Membrane processes (microfiltration, ultrafiltration, nanofiltration and reverse osmosis)	Removes macromolecules, organic matter, pathogens, colloidal particles	Achieves up to 90% removal efficiency but requires further processing for nutrient removal	[16]
Electrocoagulation	Uses electric current for the removal of organics, heavy metals and pathogens	Up to 80, 81 and 85% removal for BOD, TSS and COD, respectively	[17], [18]

Compared to physico-chemical methods, biological pre-treatment are a preferred option due to their non-toxicity as they use biodegradable, extracellular biopolymeric substances secreted by algae, yeast, and bacteria. Due to this, reduced secondary pollution is significantly reduced. Overall, biological constituent supported remediation technology has been identified as an eco-friendly alternative to chemical and physical treatment options, even for pre-treatment of wastewater. Additionally, biological constituents have also been used to mimic the functions of chemicals, even for flocculation systems whereby extracellular polymeric substances (EPS) in high concentrations of proteins have been observed to impart charged amino groups resulting in stronger electrostatic interactions to support a desired function [7].

Previous studies have focused on identifying a bacterial culture from PSW which has the natural ability to dissolve FOG and facilitate a total chemical oxygen (tCOD) removal [19–20]. There is little focus on currently commercially available products (many of which are readily available, albeit with different qualities) with these capabilities, which would invariantly reduce the need to find suitable organisms, optimize culture conditions to obtain the desired traits of the final product and develop new production systems to manufacture the desired product with an appropriate quality. This disincentivizes micro, small and medium poultry slaughterhouses to implement effective PSW treatment technology, as this requires additional capital investment. Therefore, in this study, a cheap commercially available product, i.e., Eco-Flush™, consisting of a bacterial enzyme blend used in the remediation of hydrocarbon-contaminated soil and which facilitates the decomposition of various forms of organic waste, was used. It catalyzes the decomposition of numerous types of waste and has the ability to provide a flocculation-hydrolysis function. The constituents include glaucids and essential amino acids, which can stimulate organisms in wastewater being pre-treated such that the proliferation of other bacterial species in the wastewater is supported, thus producing other enzymes capable of breaking down hydrocarbons in organic matter and providing a mixture of soluble fatty acids. This can lower tCOD, BOD, FOG, and foul odors and alleviate most challenges encountered in operating grease traps [21]. Using such a biological agent in a pre-treatment unit, prior to an AD system, could significantly improve the performance of any combined pre-treatment-AD system for effective PSW treatment. However, the choice of an appropriate AD system, which treats the wastewater post pre-treatment, is of paramount importance.

Studies by Bustillo-Lecompte and Mehrvar [22] revealed that the anaerobic process was economically more attractive for PSW treatment because it had low energy requirements and achieved a low sludge production. However, further treatment methods were required to fulfil wastewater discharge standards and reduce sludge washout and the accumulation of FOG within the AD, which resulted in the design of an Expanded Granular Sludge-Bed bioreactor (EGSB) as the preferred AD system for PSW treatment. However, Bustillo-Lecompte and Mehrvar [22], Kaskote et al. [23] and Njoya et al*.* [24] all recommended that a pre-treatment step would successfully facilitate the remediation of FOG, which resulted in sludge washout and the clogging of the EGSB. Therefore, this research aims to identify whether pre-treating PSW with a commercially available biological product containing essential constituents for the biological modification of colloidal particles, including tCOD and FOG removal, even in small quantities, followed by an EGSB, could result in the optimal treatment of PSW suitable for micro, small and medium poultry slaughterhouses. Furthermore, this study evaluated how this pre-treatment process, combined with an EGSB, could improve the overall efficiency of PSW treatment at a high throughput, small plant footprint, and low cost.

3.2 Materials and Methods

3.2.1 Poultry slaughterhouse wastewater collection and pre-treatment process set-up

PSW was collected from a local poultry abattoir situated in the Western Cape province of South Africa and stored at 4 °C over the course of the experiment to minimize acidification. EcoFlush™, a viscous brown liquid, containing with delipidating properties, was procured in a 20L bottle from Mavu Biotechnologies (Pty) Ltd (South Africa), was used in a 25L aerated pre-treat unit whereby the PSW was also fed. In the pre-treatment system, Ecoflush^{TM} was mixed fed-batch wise, i.e., by aeration, at an Ecoflush^{TM}-PSW ratio of 20mL/20L PSW with the mixture having a hydraulic retention time of 48h. Subsequently, a 25L post pre-treatment holding tank was used to settle the pre-treated sample and to reduce the dissolved oxygen levels in the PSW prior to it being fed to the EGSB, as illustrated in Figure 3:A.

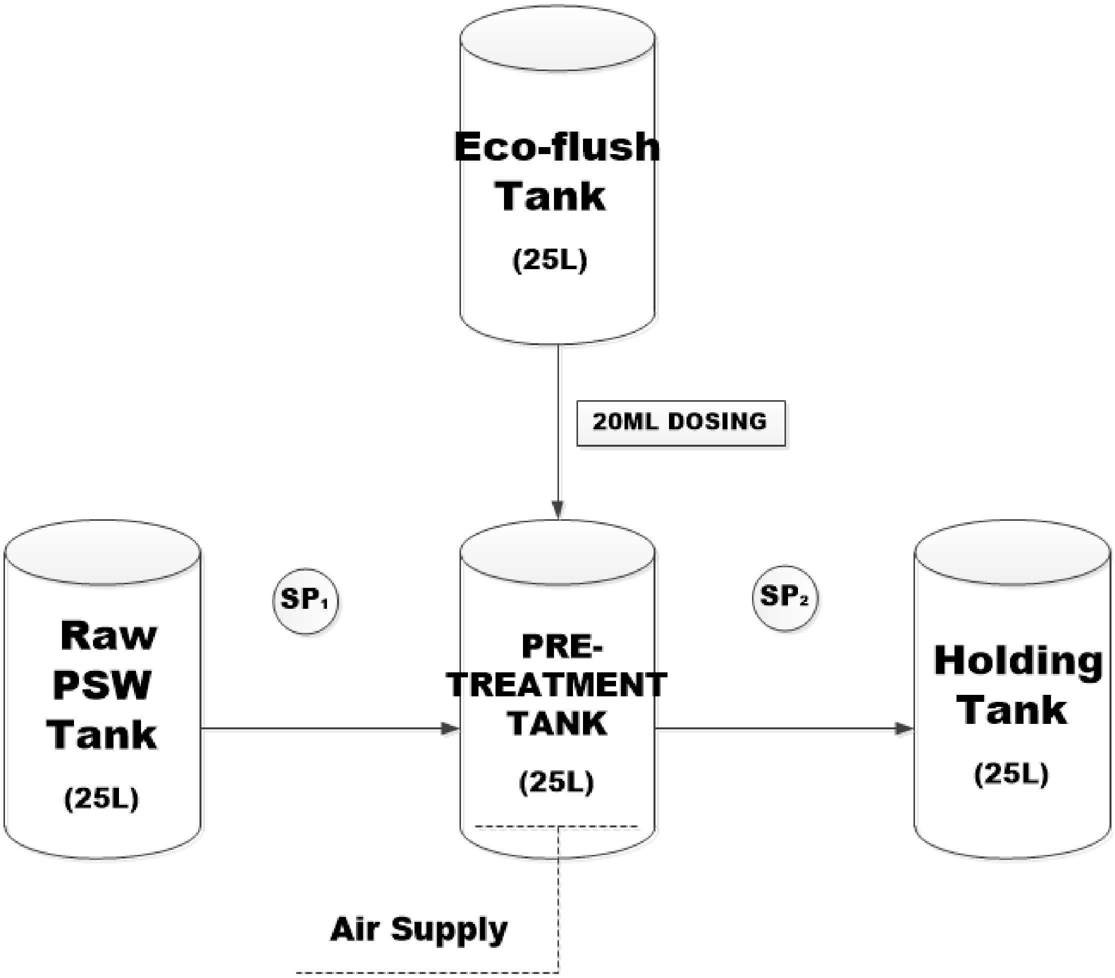


Figure 3:A Pre-treatment process schematic diagram

3.2.2 Operating conditions and sample preparation

To ensure that there was enough dissolved oxygen for the optimum proliferation of aerobic bacteria in the Ecoflush^{TM} PSW mixture, the mixture was aerated for 24h to promote flocculation-coagulation of colloidal particles, and in particular FOG. Furthermore, the flocs were removed using a 75um Madison test sieve while the cake attached to the inside walls of the pre-treatment tank were physically skimmed out, prior to the PSW transfer into a sealed holding tank with a purge port whereby it was held for an additional 24h under low stirring conditions using a magnetic stirrer to attain a homogeneous mixture, to allow further biological activity within the stored PSW and to reduce dissolved oxygen levels prior to the PSW being continuously suppled into the EGSB.

3.2.3 EGSB operation

The EGSB consisted of a 2L sized interior at which pumice stones were used as an underdrain to prevent granular sludge washout and feed (PSW) channeling at the feed port, and to improve the distribution of the PSW to the anaerobic biomass. Surrounding the outer casing of the EGSB, temperature regulated water to maintain the reactor at a steady 37°C was used for optimum operation. The system is a modification of an Up-flow Anaerobic Sludge-Bed reactor with a recycle, as illustrated in Figure 3:B, to prevent the accumulation of a FOG induced sludge cake forming within the bioreactor, resulting in blockage. This lab-scale plant pre-treatment-EGSB unit was designed and manufactured under Malutsa (Pty) Ltd., Western Cape, Wellington Industrial Park, SA.

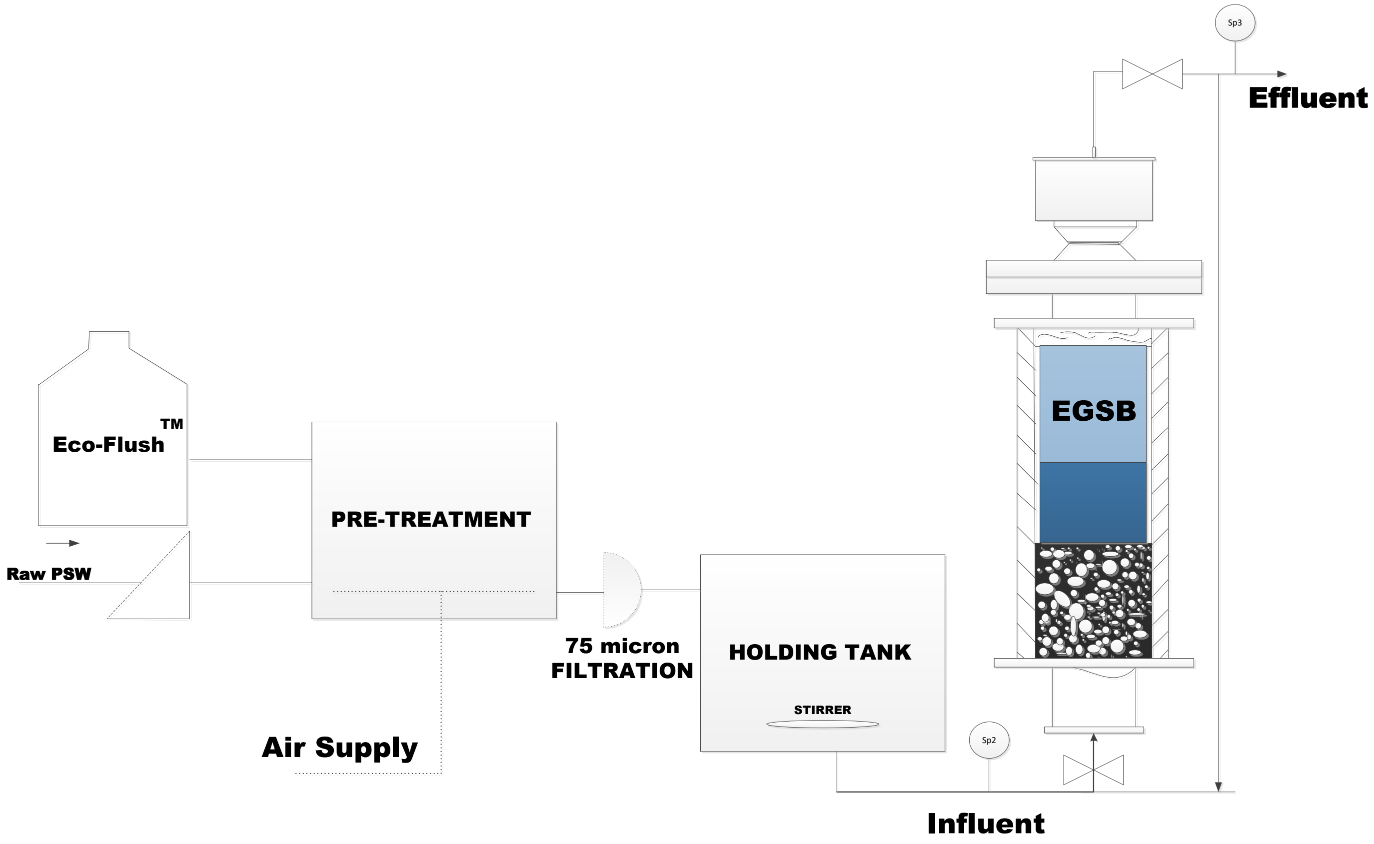


Figure 3:B The EGSB reactor treatment process

3.2.4 Conditions, sample preparation and analytical methods

The EGSB containing a mixture of activated sludge, a milk solution as a substrate and PSW was allowed contact time for acclimatization prior to feeding the EGSB with the pre-treated wastewater. The EGSB was initially fed with pre-treated PSW for 16h a day for two weeks to allow the activated sludge to adapt to the new feed and for proper optimization of the plant’s parameters. The system was then run continuously, over a period of `>100 days, 2L samples were collected every 48h from the pre-treated PSW and the effluent from the EGSB product port and the samples were taken to the City of Cape Town (CCT) for COD, FOG and TSS analyses. With reference to a representative sample taken prior to experimentation, a qualitative analysis was conducted by comparing the COD, TSS, as well as FOG levels, of the pre-treated PSW and the EGSB effluent. This assisted in identifying the efficacy of the Eco-Flush™ as a pre-treatment agent, the efficiency of the EGSB as well as the combined treatment efficacy of the combined pre-treated-EGSB system at treating PSW. Table 3:B represents the analytical methods used to measure the sample parameters. The data was analyzed using Python (programming language). Python libraries used to generate the figures included Matplotlib and Seaborn and each data point represents the average of the samples that were collected in triplicates.

Table 3:B Analytical methods used for measuring of the samples

Parameters	Methods
Total chemical oxygen demand (tCOD)	EPA Method 410.4
Fats, oils and grease (FOG)	EPA method 10056
Temperature	EPA method 9040C
Total suspended solids (TSS)	EPA method 160.2

3.3 Results and Discussion

3.3.1 PSW pre-treatment tank efficiency

Figure 3:C provides the variation of the concentrations of the tCOD, FOG, and TSS at the inlet and outlet of the pre-treatment process prior to anomaly detection and correction. Each parameter of the relevant distribution was evaluated using the inter-quartile range technique, which detected values that were far from the distribution range. The anomaly detection process was used to identify the outliers from their relevant distribution and to replace them by the median value of the corresponding distribution.

The tCOD influent and effluent revealed similar trends with the product consisting of far less tCOD when compared to the feed. There were peaks noted between day 40 and 80 for the tCOD product which could be attributed to higher tCOD content in the feed stream. This was further noted in the percentage removal of tCOD within that period, where the tCOD removal percentage was below 30%. The percentage tCOD removal peaked at 76% with an average of 43% in the pre-treatment tank. Research by Kundu et al. [25] observed that a higher percentage removal was achieved by increasing the aeration time, resulting in 77.7% COD removal. Similarly, TSS and FOG removal trends were observed, peaking after 40 days with the most successful removal being noted between day 80 and 100. The percentage of FOG removal was consistently above 50%, reaching a peak of 96% with a maintained average removal of 66%. These results are in line with the manufacturer's observations that Eco-flush™ has an active affinity for FOG and tCOD removal [21]. On the other hand, de Nardi et al. [14] noted a 91.1% peak removal of FOG in the dissolved air floatation (DAF) system; therefore, by comparison, the biological pre-treatment tank dosed with EcoFlush™ proved more effective than the DAF system.

The TSS fluctuated significantly at the beginning of the pre-treatment, resulting in its low removal (15%). However, the removal percentage increased towards the latter stages of the system operation. This may be due to more stabilized feed concentrations which resulted in a TSS removal of 59%. The studies by Dlangamandla et al. [26] on a bioflocculant-supported dissolved air floatation (Bio-DAF) system also achieved a low TSS removal of 56.5% in the initial stages of PSW treatment. However, the percentage removal improved to 91% once the Bio-DAF reached a steady state of operation. From this comparison, it can therefore be noted that pre-treating PSW with Eco-Flush™ is essential for the remediation of PSW. The treatment conditions do, however, need to be optimized to improve the efficacy of the EcoFlush™ supplemented pre-treatment tank regarding tCOD and TSS removal; although, its initial design intention was for FOG hydrolysis.

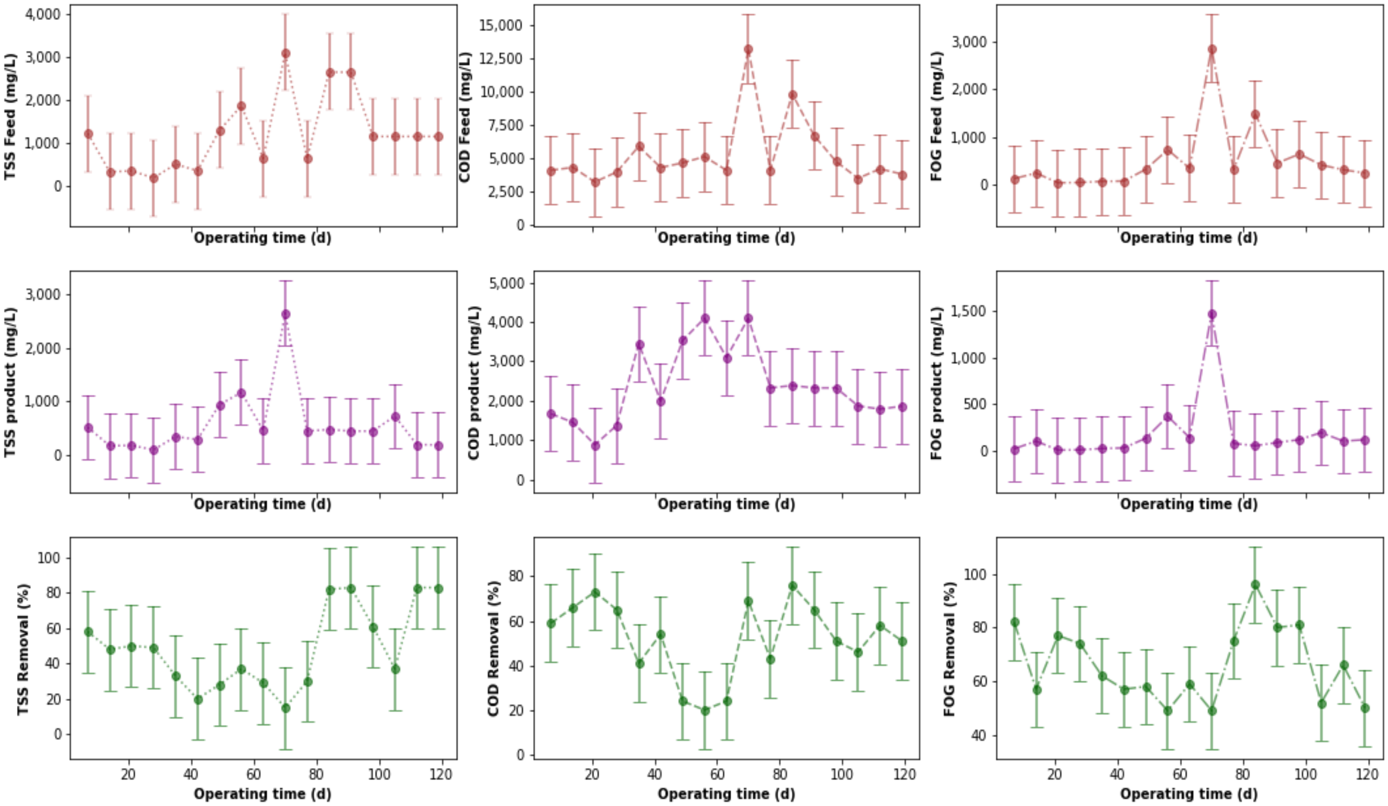
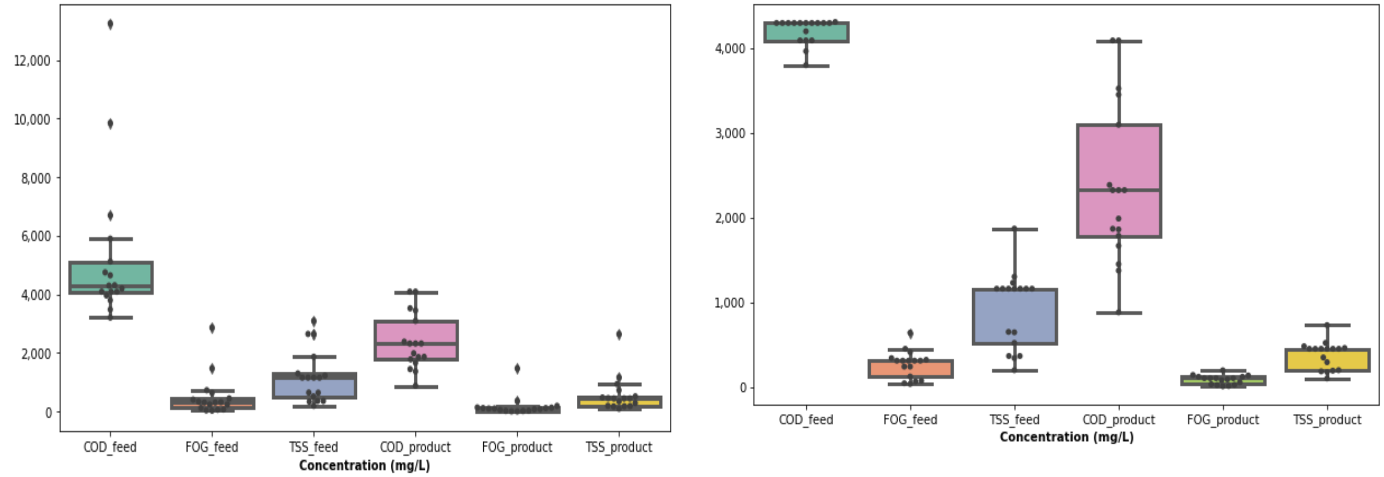


Figure 3:C Pre-treatment performance before anomaly detection and correction

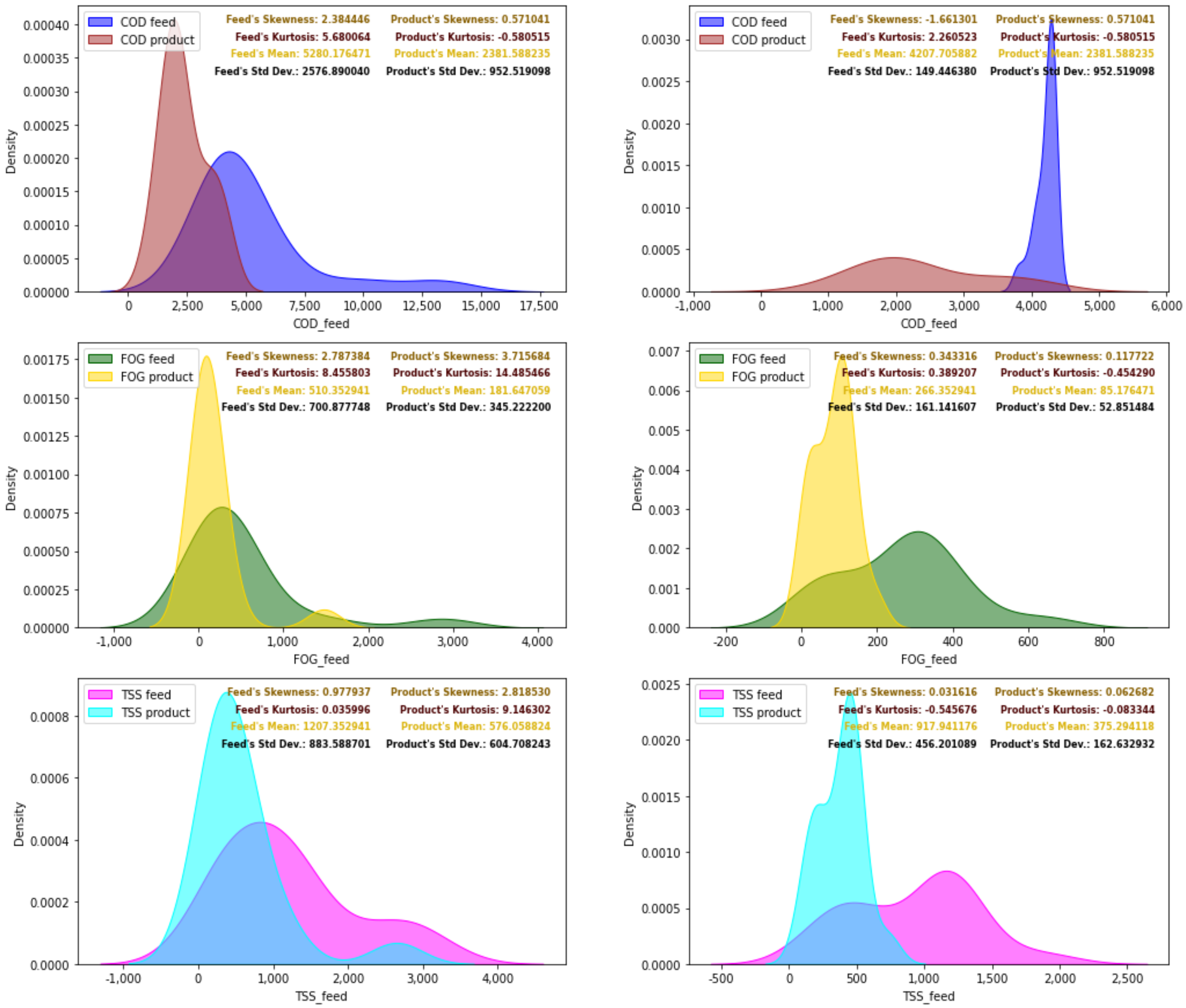
As boxplots enable the visual detection of outliers, as depicted in Figure 3:D:a, the elimination of such outliers and their replacement with median value for each distribution, can better describe the performance of the pre-treatment tank. After the replacement of these outliers by the median value of each distribution, the new distribution appears to better describe the performance of the pre-treatment tank, as depicted in Figure 3:D:b. The error bars represent the standard deviation of the data distribution of relevant parameters presented.



(a) (b)

Figure 3:D Boxplots of each pre-treatment parameter distribution before and after anomaly detection and correction. (a) Before anomaly detection and correction (b) After anomaly detection and correction.

Further analysis of the effects of the outliers' replacement is illustrated in Figure 3:E, from which a change in the value of the kurtosis, skewness, mean, and standard deviation of distribution including one of several outliers can be observed. One noticeable effect of the anomaly detection and correction is a distribution closer to normality with lower skewness and kurtosis values. Furthermore, this correction further dissociates the mode of each distribution.



(a) (b)

Figure 3:E Pre-treatment probability density functions before anomaly detection and correction. (a) Probability density functions before anomaly correction. (b) Probability density functions after anomaly detection

Figure 3:F depicts the variation of the concentration of the tCOD, FOG, and TSS at the inlet and the outlet of the pre-treatment process, as well as the variation of the removal efficiencies of the listed water quality assessment parameters, after each distribution anomaly detection and correction. Although, the replacement of outliers by the median value of respective distribution indicated a slight alteration of the performance of the pre-treatment stage. It was noticed that this processing stage yielded good results, particularly for the FOG.

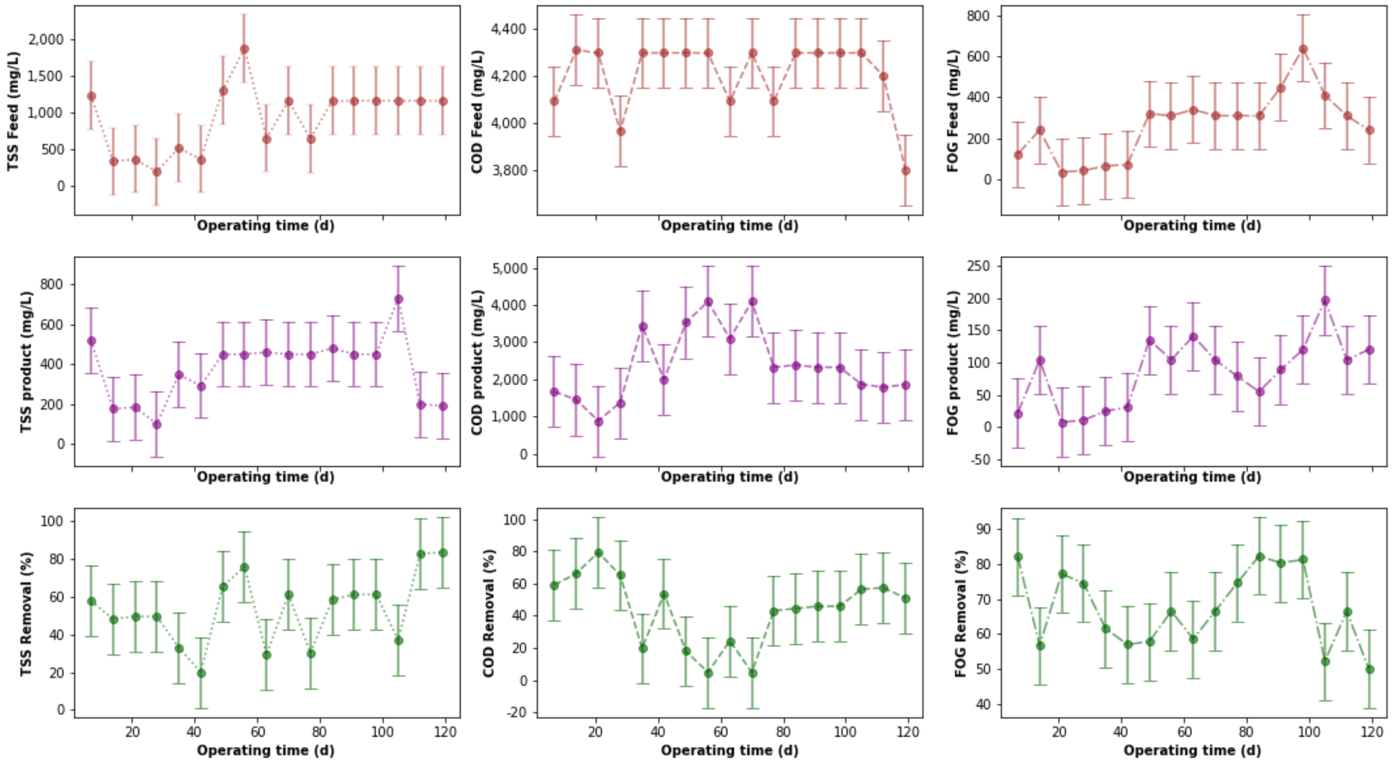


Figure 3:F Pre-treatment performance after anomaly detection and correction

Figure 3:G provides the correlation matrix between the pre-treatment stage tCOD, FOG, and TSS removal efficiencies, where *r* is the Pearson correlation coefficient and *p* is the p-value to validate or reject a null-hypothesis.

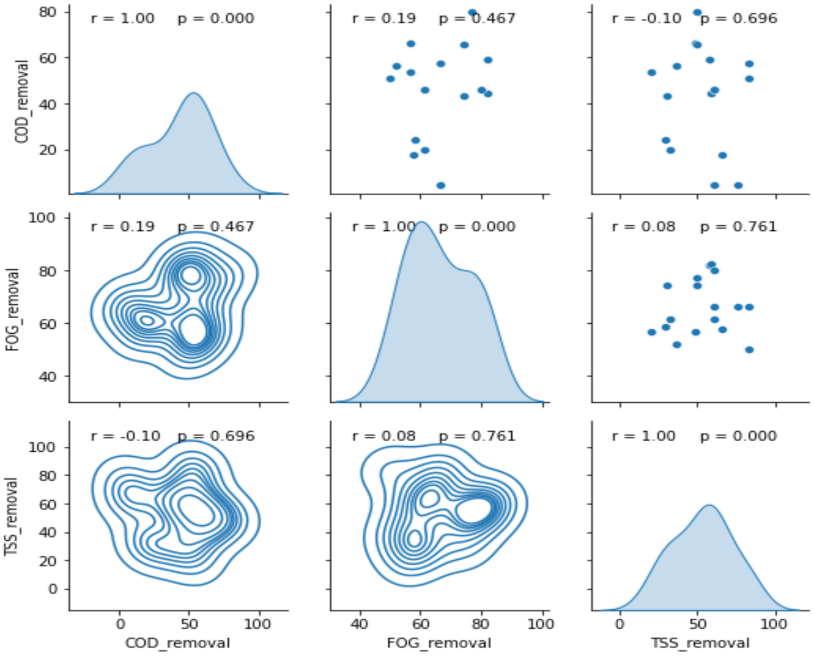


Figure 3:G Correlation matrix between the pre-treatment removal efficeincies

Typically, a p-value less or equal to 0.05 shows that an observation is statistically significant. In this case, there is no significant correlation between the removal efficiencies for the parameters evaluated.

3.3.2 EGSB PSW treatment efficiency

Figure 3:H provides the fluctuations of the PSW water quality parameters for the prior and post treatment by the EGSB. Furthermore, the same figure also depicts the fluctuations of the removal efficiencies of the tCOD, FOG, and TSS throughout the experiment. These are the raw values collected during the experiment, which may have erroneous in certain instances. Therefore, it was necessary to identify anomalies, i.e., identify and replace outliers, which might have had an influence on the distribution profile of the water quality parameters measured as this could affect the interpretation or the reproduction of an experiment. As previously alluded to, this outlier detection procedure could be achieved by boxplots, and was statistically interpretable using the inter-quartile range technique.

Previous studies on the EGSB reactor noted that it experiences clogging, sludge washout, and difficulties associated with the operation of the three-phase separator and the selection of the optimum up-flow velocity. These factors hinder the effectiveness of the EGSB, resulting in only a 65% treatment efficacy of the PSW [4]. In this study, the tCOD product concentrations from the EGSB fluctuated significantly in the early stages of PSW treatment, as expected. These results were consistent with the tCOD feed stream concentration, whereby during the initial stages of bioreactor operation, the feed contained higher concentrations of tCOD but gradually decreased towards the later stages of the treatment process. The highest percentage of tCOD removal was recorded at 76%, as shown in Figure 3.8. From these results it was noted that pretreating the PSW culminated in a significant increase in the treatment efficacy of the EGSB compared to the results from previous studies which only noted the upper limits of 65% tCOD removal without pretreatment.

Low concentrations of TSS were noted in the feed and product (Figure 3:H) and were consistently low throughout the experiment with spikes between day 20 and 40 which could be attributed to the fact that PSW samples were taken at a slightly different time to the previous batch. The percentage of TSS removal was also consistently above 50% and maintained above 90%, with the highest percentage removal recorded at 96%. The FOG feed concentrations were significantly lower due to the efficacy of the pretreatment tank, a clear indication of the impact of Ecoflush^{TM} in the hydrolysis of FOG. The FOG percentage removal fluctuated during the EGSB operation but was maintained above 50%. The average

FOG removal was 66% with a maximum recorded at 97%. The study by Cruz-Salomon reported that the performance of the EGSB bioreactor was improved by reducing the particle size in wastewater [27]. Other studies noted that at undiluted PSW significantly hindered the hydrolysis of FOGs and tCOD due to the accumulation of long-chain fatty acids in anaerobic digestors [25,28]. These results further emphasize the importance of a pre-treatment step prior to the anaerobic digestion as the EGSB did not experience any instances of sludge washout or clogging during the experiment.

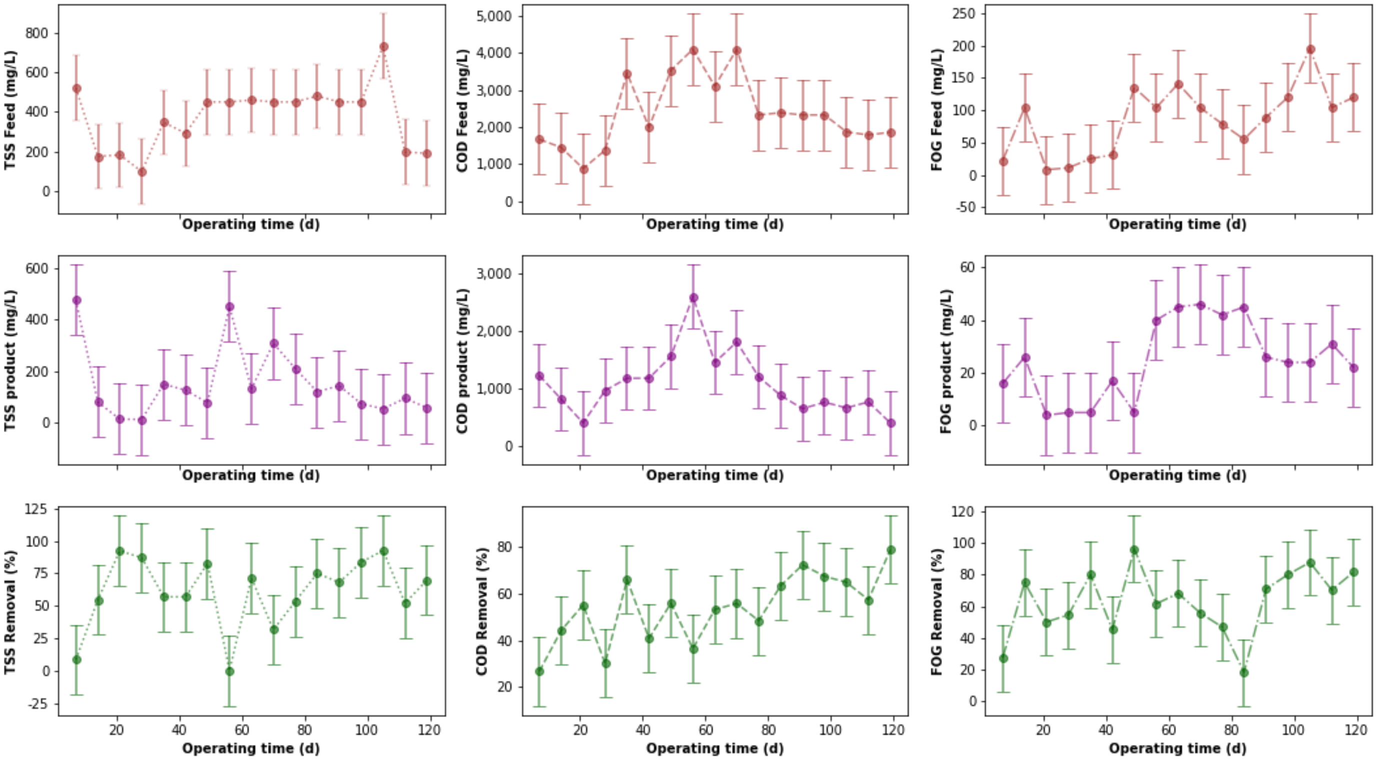
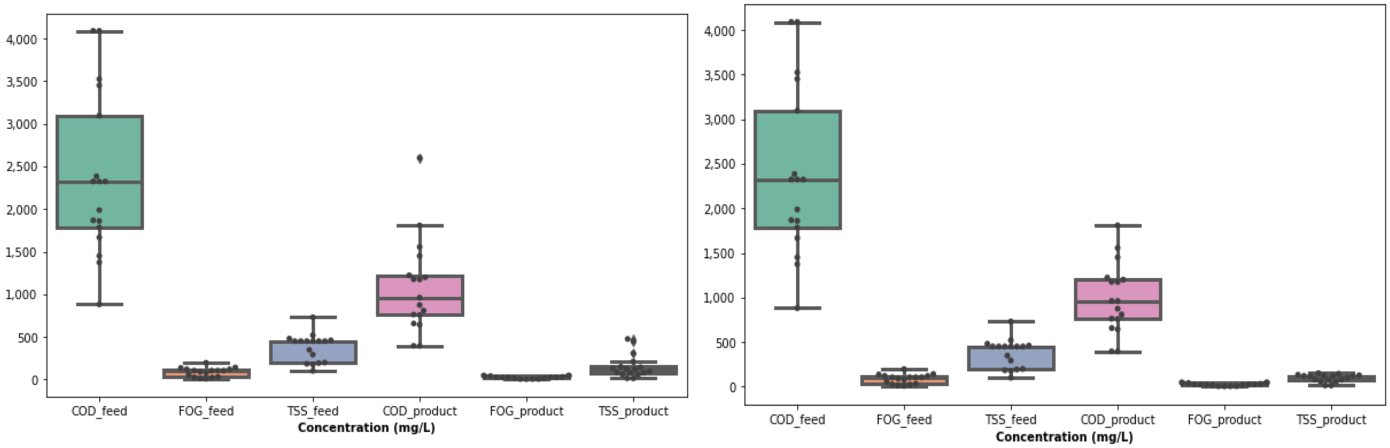


Figure 3:H EGSB Treatment performance before anomaly detection and correction

As depicted in Figure 3:I:a, outliers were identified in the tCOD and TSS outlet values. Their respective distributions were corrected by replacing outliers with median values of relevant distributions, which culminate in Figure 3:I.b, where no outliers appear.

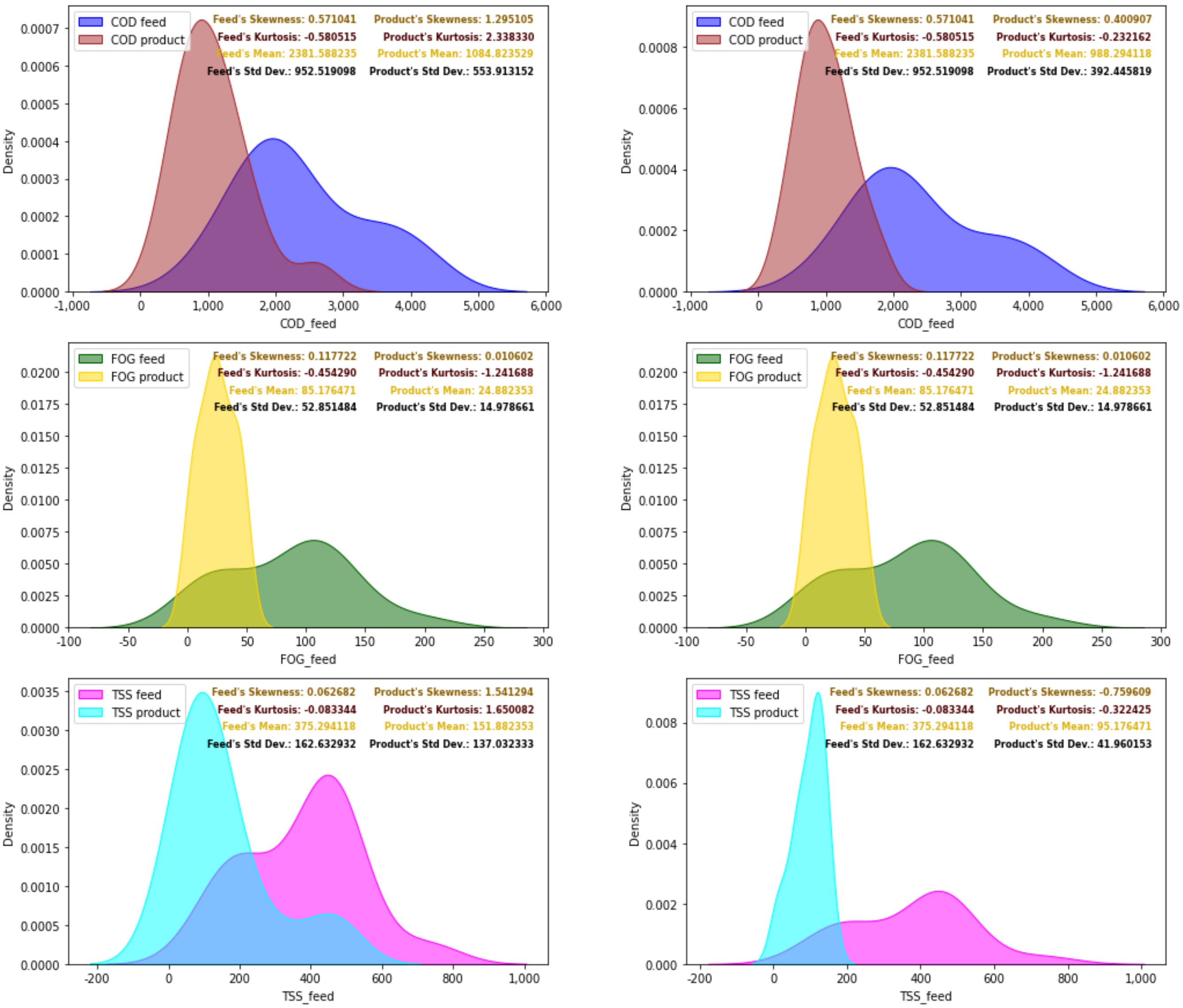


(a) (b)

Figure 3:I EGSB treatment parameters before and after anomaly detection. (a) Anomaly detection and correction. (b) After anomaly detection and correction

The effect of these outliers in each distribution before anomaly detection and correction is visualized by the comparison between their respective probability density functions, as depicted in Figure 3:J. The latter shows a clear reduction of the level of skewness of parameters corrected, attaining a distribution closer to normality. This change can also be noticed in the change in the values of the mean as well as standard deviation of the corrected distributions.

Ultimately, this correction led to a more conducive analysis of the performance of the EGSB based on the variation of the change in the concentration of contaminants at the inlet and outlet of the EGSB, as depicted in Figure 3:K. From the latter, the variation in the removal efficiencies with respect to the tCOD, FOG, and TSS can also be observed, and showcases a good performance of the EGSB, particularly for the removal of TSS where the EGSB removal efficiency was maintained above 60% despite various fluctuations of the organic loading rate (OLR).



(a) (b)

Figure 3:J Probability density functions of the EGSB before and after anomaly detection and correction. (a) Before anomaly detection and correction. (b) After anomaly detection and correction.

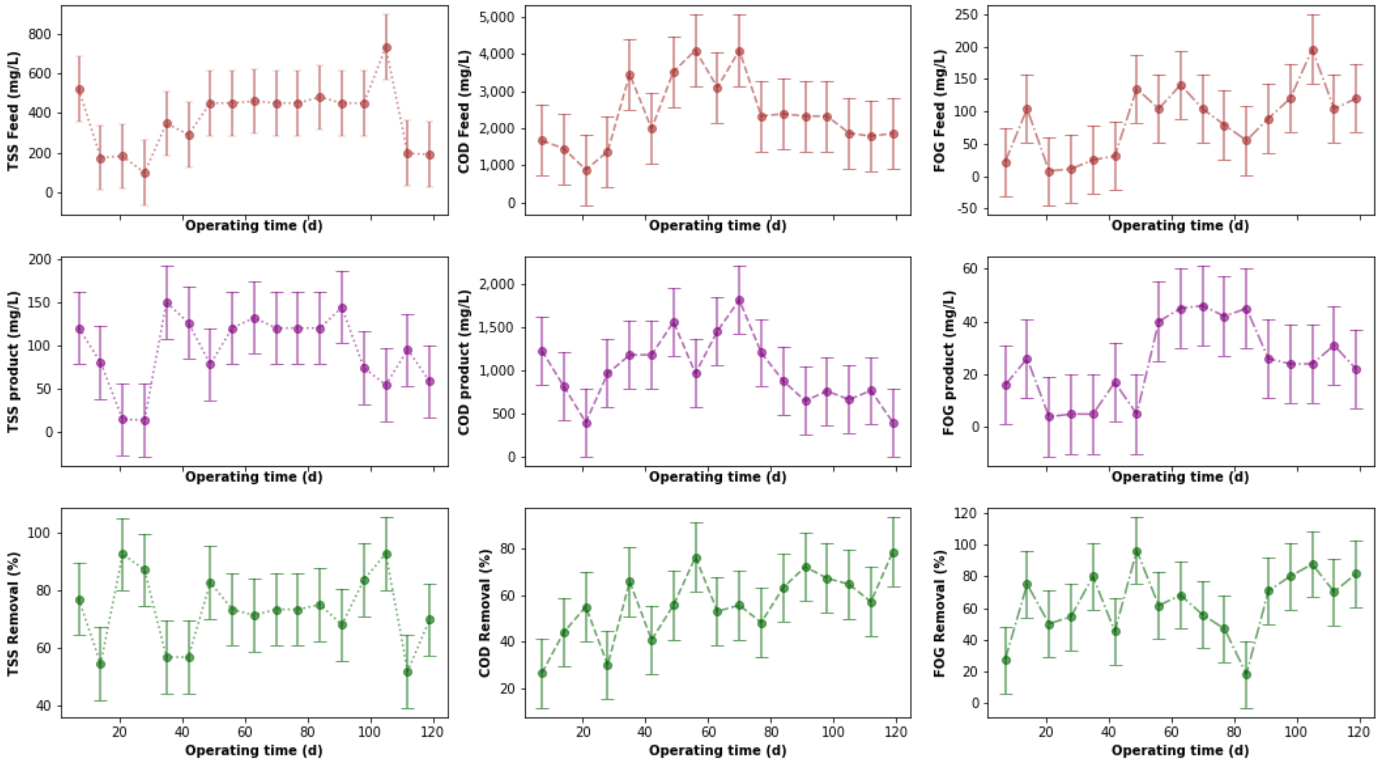


Figure 3:K EGSB treatment performance after anomaly detection and correction

A correlation matrix containing the Pearson correlation coefficient (*r*) and the p-value of each statistical analysis was used to correlate the performance of each of the evaluated removal efficiencies (see Figure 3:L). No correlation was found, with *r* values were relatively low when two removal efficiencies were compared, and p-values above 0.05 in each case.

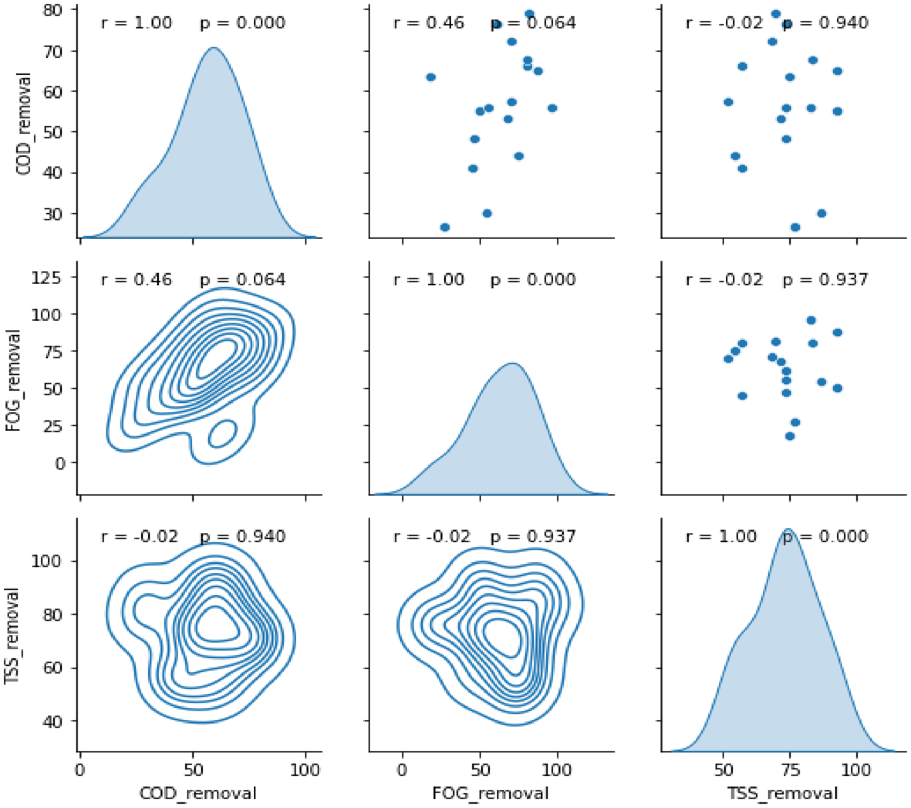


Figure 3:L Correlation matrix between the EGSB removal efficiencies

3.3.3 PSW pretreatment process coupled with an EGSB reactor

Figure 3:M provides the overall performance of the pre-treatment stage coupled with the EGSB for the distributions devoid of outliers. The analysis of the results focused on the impact of each individual treatment step, namely the pre-treatment step and the EGSB, as well as the combined uninterrupted treatment process characterized by comparing the feed before pre-treatment to the effluent collected at the end of the anaerobic treatment process. The combination used in the pre-treatment step achieved an average of 52% tCOD removal with the highest activity recorded at 76% in the pre-treatment tank alone. The percentage of tCOD removal in the EGSB alone was, on average, 53% with upper limits of 79%, as illustrated in Figure 3.8. This result was consistent with research by Basitere et al. [4], which noted an average of 57% tCOD removal for the EGSB reactor. These results showed that even though each stage achieved more than a 50% average removal efficiency individually for the measured parameters, running these steps individually might not yield the desired output in the remediation of PSW. This was further emphasized when assessing the combined treatment efficacy of the pre-treated PSW and the PSW effluent from the EGSB in Figure 3:M. The combination, as hypothesized, had a drastic improvement on the removal of tCOD, with an average percentage removal of 76% and upper limits of 91%. The study by

Williams et al. [29] recorded upper limits of 93% COD removal at an optimized organic loading rate (OLR) and hydraulic retention time (HRT) in an EGSB reactor. Mbulawa [30] also noted a 66% COD removal by crude lipases of the *Bacillus cereus* CC-1 strain and recommended the use of this strain for pretreating PSW prior to AD. These results further emphasized the importance of a pre-treatment step for tCOD-laden wastewater prior to anaerobic digestion and the use of combinational treatment processes to remediate PSW.

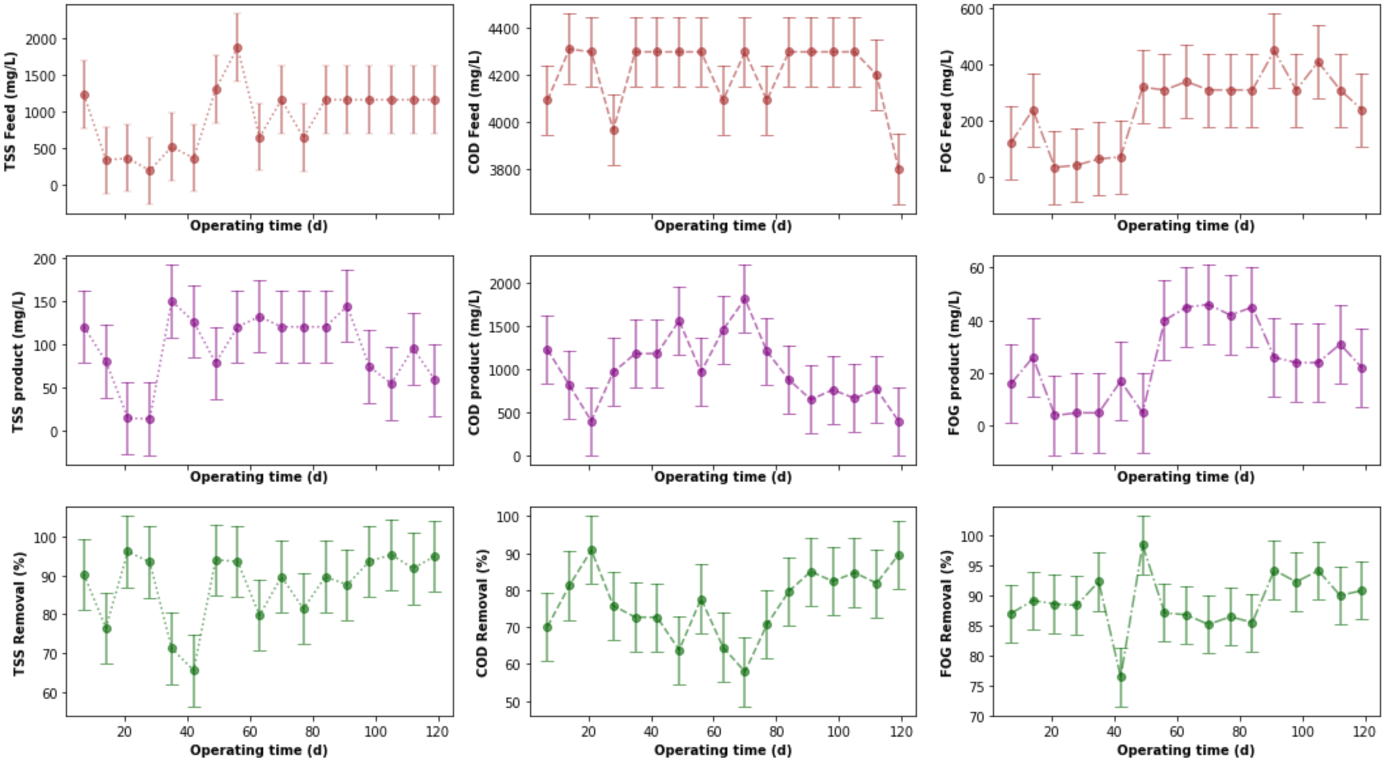


Figure 3:M Performance of the pre-treatment stage coupled with the EGSB

Initially, low-percentage reductions were noted for TSS in the pre-treatment tank at an average of 48% which was not a significant reduction; however, it could be noted that there was no previous data on the activity of Ecoflush™ on TSS removal in a pre-treatment tank for PSW remediation. Moreover, the percentage of TSS removal could, therefore, mostly be attributed to the flocculation activity and filtration which reduced the concentration of the suspended solids in the pre-treated PSW before feeding them into the EGSB. The EGSB had a much better impact in the remediation of TSS in the system. The average removal achieved was 68%, reaching a high of 93%, as seen in Figure 3:K. The high concentrations of TSS in the effluent were noted to cause a decrease in the working volume of the bioreactor. Such solids could also decrease the useful characteristics of the anaerobic granular sludge and this in turn reduced the bioreactor's performance [25]. The combined remedial action of the pre-treatment step and the EGSB averaged 87% TSS removal with peaks of 96% TSS removal, showing an effective, combined, remedial performance as illustrated in Figure 3:N. This result further demonstrated that the combined treatment process enhanced the efficacy of the PSW treatment system.

Similarly, FOG removal levels in the pre-treatment tank for PSW averaged approximately 66% with an upper limit of 96% FOG removal, while the average for EGSB had an upper removal limit of 97%. The combined FOG treatment efficacy consisted of a mean of 88% with an upper limit of 98%, as depicted in Figure 3:N. From these results, it could be noted that the EGSB, coupled with a pre-treatment step, proved effective for reducing the FOG levels in PSW. This reduction was a result of bio-flocculation-coagulation caused by the Ecoflush™. These results are in line with the manufacturer's observation that Ecoflush™ actively reduces the COD levels and remediates FOGs in organic waste [21]. Valladão et al. [31] also noted no clear reduction in the treatment efficiency of an unpretreated effluent containing FOG, whereas a pre-treated FOG effluent showed effective AD bioreactor performance results, emphasizing the need for pre-treatment to maintain the process efficiency. Commercially available FOG hydrolyzing agents with a flocculation-coagulation activity can be used in pretreatment systems for abattoir wastewater with a high FOG content to aid AD systems used as primary organic matter digesters. Such a strategy, as reported herein, resulted in the treatment efficiencies of 90% with upper limits of 98%.

The probability density function of the removal efficiencies and the water quality assessment parameters, both at the inlet and the outlet of the combined system are depicted in Figure 3:N, showing the mean and standard deviation of each distribution.

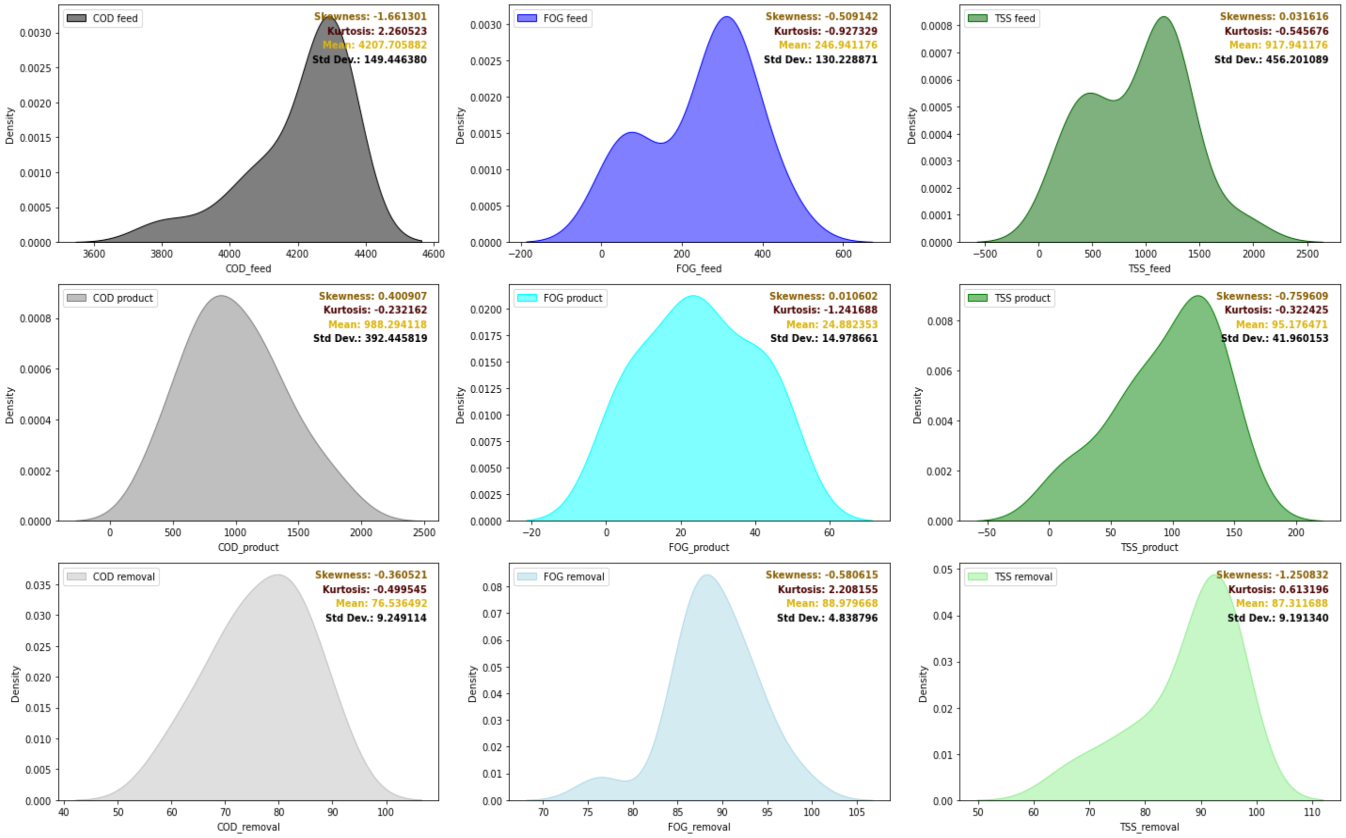


Figure 3:N Probability density function of the inlet and outlet of the overall system, including their removal efficiencies

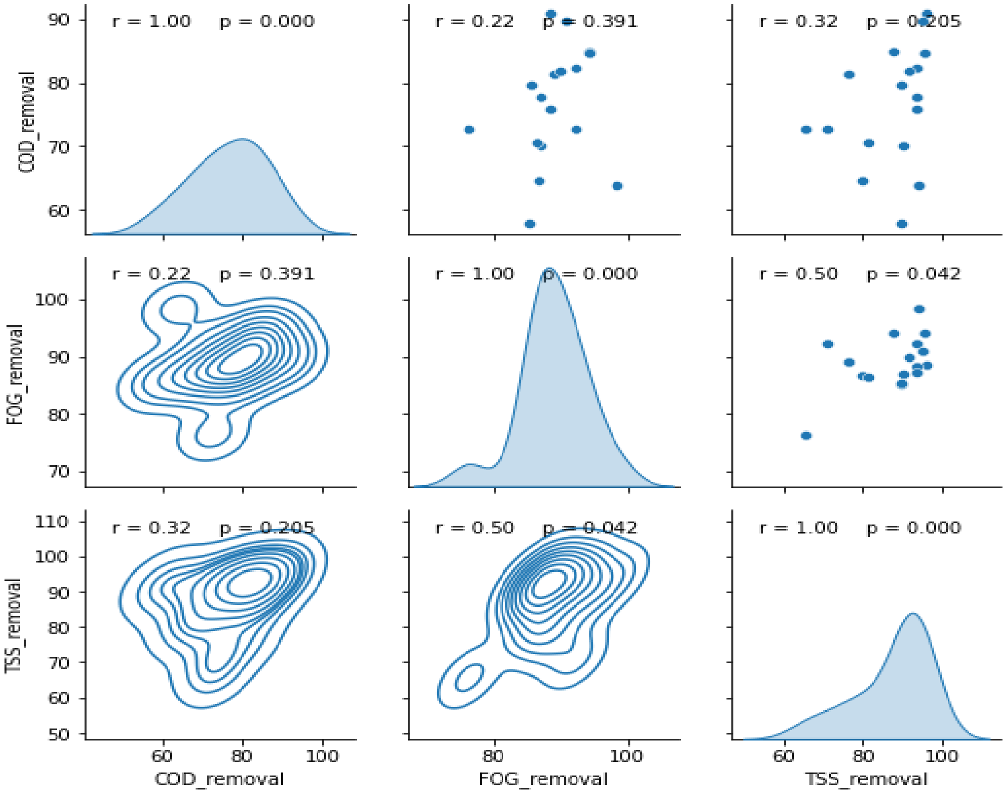


Figure 3:O Correlation matrix between the removal efficiencies of the pre-treatment stage coupled with the EGSB

Lastly, the correlation matrix in Figure 3:O, between the COD, FOG, and TSS removal efficiencies showed no strong correlation between these parameters for the combined system. Therefore, rejecting the hypothesis of possible similarity in the removal pattern of the respective water quality assessment parameters.

3.4 Conclusions

It was observed that the overall performance of a combined pre-treatment tank dosed with an eco-friendly, FOG-hydrolysing agent, in combination with an EGSB, performed satisfactorily for COD, TSS and particularly FOG removal. The removal efficiencies were consistently above 60%, with maximum values above 90% in certain cases for the individual parameters measured for the PSW. This performance highlights the importance of a pre-treatment stage prior to anaerobic digestion, as recommended by Bustillo-Lecompte and Mehrvar [23] and Njoya et al. [24], since this stage contributed to the precondition of FOG-laden PSW. However, further research should focus on (1) identifying the optimum conditions for FOG hydrolysis and agent-facilitated, pre-treatment tanks and (2) the optimum conditions for other commercially available flocculation-coagulation products, such as Eco-Flush™, that can be used by micro, small and medium poultry slaughterhouses in combination with an EGSB.

3.5 Summary

The treatment of poultry slaughterhouse wastewater (PSW) with an Expanded Granular Sludge-Bed Bioreactor (EGSB) is hindered by the accumulation and washout of sludge, and difficulties associated with the operation of the three-phase separator and the determination of the optimum up-flow velocity for sludge-bed fluidization. This results in a poor reactor functionality, and thus a poor performance due to fats, oil and grease (FOG) in the PSW being treated. Hydrolyzing the FOG content with a bio-delipidation, enzyme-based agent in a pre-treatment unit would significantly improve the effectiveness of the EGSB. In this study, PSW was pre-treated for 48 h with a biological mixture containing bioflocculants and bio-delipidation constituents. The pre-treated PSW was further treated in an EGSB. The PSW FOG, total chemical oxygen demand (tCOD) and total suspended solids (TSS) content were determined to assess the effectiveness of the pre-treatment process as well as to observe the remedial action of the combined pre-treatment-EGSB system. An increased treatment efficacy was noted for the combined PSW treatment system, whereby the COD, FOG and

TSS removal averaged 76%, 88% and 87%, respectively. The process developed is intended for micro, small and medium poultry slaughterhouses.

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CHAPTER FOUR

CONCLUSION AND RECOMMENDATIONS

4 CHAPTER FOUR: CONCLUSIONS AND RECOMMENDATIONS

The accumulation of organic matter within the EGSB reactor has been noted to result in clogging and sludge washout due to organic matter accumulation within the bioreactors. This study therefore sought to identify a pretreatment process that would help reduce the organic load before remediation in an anaerobic digestion process to effectively treat PSW. This study evaluated each treatment process in the remediation of PSW as well as the effectiveness of bio-physico-pretreatment coupled with EGSB reactor in treating PSW.

The average pretreatment percentages for COD, FOG and TSS were 43%, 66% and 59% removal, respectively. The percentage removal for the EGSB recorded upper limits of 76% COD removal, upper limits of 96% were recorded for TSS and FOGs removal peaked at 97% with an average of 66%. An increased treatment efficacy was noted for the combined PSW treatment system, whereby the COD, FOG and TSS removal averaged 76%, 88% and 87%, respectively. The process developed is intended for micro, small and medium poultry slaughterhouses.

Future research should focus on the following aspects:

- Optimization of Ecoflush^{TM} to identify the most effective concentrations for optimum treatment percentages
- More parameters such as BOD, alkalinity and biogas production should also be investigated to better understand the hydrolysis process employed by Ecoflush^{TM}.
- Optimizing the HRT and OLR proved to be somewhat of a challenge therefore identifying suitable pumps should be prioritized for future studies.

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APPENDICES

A. APPENDICES

APPENDIX A: Pretreatment performance

Days	COD_feed	FOG_feed	TSS_feed	COD_effluent	FOG_effluent	TSS_effluent	%COD_removal	%FOG_removal	%TSS_removal
7	4092	123	1230	1667	22	520	59	82	58
14	4310	240	340	1450	104	176	66	57	48
21	3210	35	365	881	8	184	73	77	50
28	3965	43	198	1374	11	100	65	74	49
35	5900	65	520	3452	25	348	41	62	33
42	4298	72	365	1988	31	292	54	57	20
49	4650	320	1302	3525	135	940	24	58	28
56	5120	730	1870	4092	370	1173	20	49	37
63	4092	340	650	3096	141	460	24	59	29
70	13250	2870	3100	4092	1478	2650	69	49	15
77	4092	310	645	2324	79	450	43	75	30
84	9850	1478	2650	2385	55	480	76	96	82
91	6700	450	2650	2324	89	450	65	80	83
98	4750	640	1160	2324	120	450	51	81	61
105	3484	410	1160	1868	196	730	46	52	37
112	4200	310	1160	1785	104	198	58	66	83
119	3800	240	1160	1860	120	192	51	50	83

APPENDIX B : EGSB performance

Days	COD_feed	FOG_feed	TSS_feed	COD_effluent	FOG_effluent	TSS_effluent	%COD_removal	%FOG_removal	%TSS_removal
7	1667	22	520	1223	16	476	27	27	8
14	1450	104	176	809	26	80	44	75	55
21	881	8	184	395	4	14	55	50	92
28	1374	11	100	961	5	13	30	55	87
35	3452	25	348	1174	5	150	66	80	57
42	1988	31	292	1176	17	126	41	45	57
49	3525	135	940	1556	5	78	56	96	92
56	4092	370	1173	2602	40	450	36	89	62
63	3096	141	460	1450	45	132	53	68	71
70	4092	1478	2650	1809	46	308	56	97	88
77	2324	79	450	1200	42	210	48	47	53
84	2385	55	480	874	45	120	63	18	75
91	2324	89	450	643	26	144	72	71	68
98	2324	120	450	757	24	74,0	67	80	84
105	1868	196	730	657	24	54	65	88	93
112	1785	104	198	762	31	95	57	70	52
119	1860	120	192	394	22	58	79	82	70

APPENDIX C : Overall performance

Days	COD_feed	FOG_feed	TSS_feed	COD_product	FOG_product	TSS_product	COD_removal	FOG_removal	TSS_removal
7	4092	123	1230	1223	16	476	70	87	61
14	4310	240	340	809	26	80	81	89	76
21	3210	35	365	395	4	14	87	88	96
28	3965	43	198	961	5	13	75	88	93
35	5900	65	520	1174	5	150	80	92	71
42	4298	72	365	1176	17	126	58	76	65
49	4650	320	1302	1556	5	78	66	98	94
56	5120	730	1870	2602	40	450	49	94	76
63	4092	340	650	1450	45	132	64	86	79
70	13250	2870	3100	1809	46	308	86	98	90
77	4092	310	645	1200	42	210	70	86	67
84	9850	1478	2650	874	45	120	91	97	95
91	6700	450	2650	643	26	144	90	94	94
98	4750	640	1160	757	24	74,0	84	96	93
105	3484	410	1160	657	24	54	81	94	95
112	4200	310	1160	762	31	95	82	90	91
119	3800	240	1160	394	22	58	90	90	95