MODELLING OF A BIOFLOCCULANT SUPPORTED DISSOLVED AIR FLOTATION SYSTEM FOR FATS OIL AND GREASE LADEN WASTEWATER PRETREATMENT

by

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DECLARATION

I, **Melody Ruvimbo Mukandi**, declare that the contents of this thesis represent my own unaided work, except where specifically acknowledged in the text, and that the thesis 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.

Signature ……………………… Date………………….. 19/12/2017

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ABSTRACT

In the recent past, the poultry industry in South Africa has grown due to an increased demand of poultry products as a result of population growth and improved living standards. Furthermore, this has led to poultry slaughterhouses generating high strength wastewater which is laden with a high concentration of organic and inorganic pollutants from the slaughtering process and sanitation of equipment and facilities. As a result, South Africa has promulgated restrictions and a set of quality standards for effluent discharged into the environment to minimize ecological degradation and human health impact. Hence, there is a need for improved Poultry Slaughterhouse Wastewater (PSW) pre-treatment prior to either discharge into municipal wastewater treatment plants (WWTP) or on-site secondary treatment processes such as anaerobic digesters. Additionally, amongst the pre-treatment methods for Fats, Oil and Grease (FOG) laden wastewater, flotation remains the most popular with Dissolved Air Flotation (DAF) system being the most applied. However, modelling and optimization of a biological DAF system has never been attempted before in particular for a bioflocculant supported DAF (BioDAF) for PSW pre-treatment. Process modelling and optimization involves process adjustment to optimize influential parameters. In this study, Response Surface Methodology (RSM) was used to develop an empirical model of a BioDAF for pre-treatment of PSW, for which a bioflocculant producer including production conditions, flocculant type and its floc formation mechanism, were identified.

Twenty-one (n = 21) microbial strains were isolated from the PSW and their flocculation activity using kaolin clay suspension (4g/L) was quantified, with a mutated *Escherichia coli* (*mE.coli*) [accession number LT906474.1], having the highest flocculation activity even in limited nutrient conditions; hence, it was used for further analysis in other experiments. Furthermore, the optimum conditions for bioflocculant production achieved using RSM were pH of 6.5 and 36℃, conditions which induced instantaneous bioflocculant production with the highest flocculation activity. The bioflocculant produced by the *mE.coli* showed the presence of carboxyl/amine, alkyne and hydroxyl functional groups, which was indicative that the bioflocculant contained both polysaccharides and some amino acids.

Subsequent to bioflocculant production studies, the mechanism for floc formation was assessed using RSM at pH 4 (min) and 9 (max) with a bioflocculant dosage between 1% (min) and 3% (max) v/v, which culminated in minimal zeta potential changes. However, results from electron microscopy analyses, indicated that at a pH 4 and bioflocculant dosage of 1% (v/v), floc

agglomeration was evident; hence, these conditions were used in the operation of a bioflocculant supported DAF system. As the charge neutralization mechanism was not the primary flocculating mechanisms as determined by zeta potential results, a floc bonding mechanism test using 10mM EDTA-2Na, 0.5M HCl and 5M urea was also conducted, elucidating bridging as the responsible mechanism for floc formation thus flocculation, i.e. for the bioflocculants produced by the *mE. coli*.

To evaluate the efficacy of the bioflocculants produced, i.e. for PSW pre-treatment, DAFs operated at a flow rate of 1mL/min with an HRT of 32hr were used, with only the pH being adjusted for bioflocculant supplemented DAFs (BioDAFs) while maintaining a 1% (v/v) bioflocculant dosage. The performance of the BioDAF was compared to conventional DAFs (ConDAFs). The ConDAF removed up to 45.43% FOG, 41.95% tCOD, 33.97% sCOD, 42.06% TSS, 28.1% tProtein, 6.11% sProtein, and 55.25% turbidity whereas the BioDAF removed up to 97.53% FOG, 65.85% tCOD, 26.56% sCOD, 83.1% TSS, 73.14% tProtein, 97.8% sProtein and 81.96% turbidity; thus demonstrating that the BioDAF was relatively efficient in pollutant removal as compared to a ConDAF. Additionally, a toxin test for the pre-treated wastewater was negative meaning, indicating minimal toxin production by the *mE. Coli* used.

Data generated from numerous analytical methods from the experimental trials was used in the generation of empirical models using RSM (Design-Expert Version 6.0.8) to mathematically describe the operation of bioreactor systems to produce the bioflocculant and in particular for the BioDAFs. To ascertain which parameter were influential in the BioDAFs operation, a standard deviation analysis for each parameter was assessed, which indicated that sCOD had the lowest standard deviation, thus was suitable to generate an empirical model for the BioDAFs. A linear model was derived and based on the Analysis of Variance (ANOVA), the model was deemed significant. Thus the primary objective of developing a mathematical model that describes the operation of a bioflocculant supported DAF system for the pre-treatment of PSW, was successful.

Keywords: Bioflocculant; Dissolved air flotation (DAF); Mathematical modelling; Poultry slaughterhouse wastewater; Response surface methodology

DEDICATION

To my mom TAMBUDZAI CONCILIA MUKANDI

You are my Hero!!!

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LAYOUT OF THESIS

The aim of this study was to model a bioflocculant supported Dissolved Air Flotation (BioDAF) system with improved pollutant removal efficiency for Fats, Oil and Grease (FOG) laden poultry slaughterhouse wastewater (PSW) pre-treatment using Response Surface Methodology (RSM) such that the treated wastewater will comply with legislated wastewater disposal standards. The thesis is divided into the following chapters:

Chapter 1: Introduction. The chapter provides a background on water shortages, generation of PSW and the need for a BioDAF system. Furthermore, it provides a hypothesis, the aims and objectives, including the significance and delineation of the study.

Chapter 2: This chapter discusses three primary areas that are; PSW, Dissolved Air Flotation (DAF) system and flocculation as a pre-treatment technique for pollutant separation in wastewater. Under the section focusing on PSW, the generation, composition and the regulatory requirements for PSW discharge are discussed. Furthermore, the DAF system as a pre-treatment technology is introduced focusing on its application, with the flocculation process being discussed as well as the use of chemical (synthetic) and natural flocculants, i.e, bioflocculants.

Chapter 3: This chapter is concerned with the theory behind mathematical modelling and factors that can affect a DAF systems' operation. Furthermore, RSM which is a modelling and optimization software that was used in this study, is discussed, focusing on the model that can be obtained from the software and their applicability in modelling bioprocess engineering systems.

Chapter 4: Materials and methods. The chapter provides the methods used in the isolation and identification of the microorganism obtained from the PSW for bioflocculant production with high flocculating activity. Furthermore, it lists all equipment and materials used in the setup for each phase of the research, BioDAF system design including operation and how RSM was used to generate the empirical models deemed suitable to predict system operation at each stage of the research. Additionally, methods herein defined as analytical techniques including parameters analyzed in order to assess pre-treated water quality, are also described.

Chapter 5: Results obtained from the experimental work carried out so as to achieve the aims and objectives of the research are given and discussed in this chapter.

Chapter 6: This chapter provides the overall conclusions and recommendations for future studies.

Chapter 7: This chapter lists references and the bibliography used to support the research undertaken.

Appendices: Lists auxiliary information which was deemed supplementary thus not needed for the body of the thesis.

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LIST OF SYMBOLS

Nomenclature

Symbol	Description	Units
A	Optical density of the control	nm
$\frac{A}{S}$	Air solid ratio	-
b	Model parameter (vector)	-
B	Optical density of the sample	nm
f	Pressurization system efficiency at pressure	0.8
k	Number of variables	-
m	Number of lines from the matrices	-
n	Number of columns from the matrices	-
P	Operating pressure	Kg/cm^2
R^2	Goodness of model fit	-
S_a	Air solubility	mL/L
$sCOD$	Soluble chemical oxygen demand	mg/L
$tCOD$	Total chemical oxygen demand	mg/L
x	Chosen design matrix	-
X	Influent solids concentration	mg/L
x_i	Variables	units not defined
X_i	Coded value	-
X_i	Coded independent variables	units not defined
x_{cv}	Centered point value	-
Y	Response variable	units not defined
	Greek letters	
ε	Residuals/errors	-
β_0	Constant	-
β_i	Linear coefficient	-
β_{ii}	Quadratic coefficient	-
β_{ij}	Interactive coefficient	-

GLOSSARY

Basic Terms and Concepts

Term	**Definition/Explanation**
Flocculants	:are chemicals that cause flocculation by aggregation of suspended particles and colloids, forming a floc (IUPAC, 1997),
Flotation	:is a dynamic process used in a wide variety of industries to reduce suspended solids, reduction of both turbidity and chemical oxygen demand of water in wastewater treatment, the recovery of minerals, amongst other activities (Chen *et al.,* 2000),
Bioflocculants	:are organic macromolecules produced by a wide variety of microorganisms (Manivasagan *et al.,* 2015),
Mathematical model	:is a representation in mathematical terms of the behaviour of real devices, systems and objects (Abramowitz & Stegun, 1968),
Removal efficiency	:is given by the formula, $R(\%) = [(In - Out)/In] * 100$ and can be calculated using monitoring parameters such as COD and FOG (de Nardi *et al.,* 2011),
Response Surface Methodology (RSM)	:is a set of statistical and mathematical techniques that are used for experimental design, modelling, evaluation of process variable effects and the determination of optimum condition for variables such as to predict the response (Montgomery, 2008).

Abbreviation	**Description**
BioDAF:	Bioflocculant supported Dissolved Air Flotation
BPM:	Bioflocculant Production Media
CCD:	Central composite design
CFD:	Computational Fluid Dynamics
ConDAF:	Conventional Dissolved Air Flotation
DAF:	Dissolved Air Flotation
FOG:	Fats, Oil and Grease
pH:	Potential of Hydrogen
PSW:	Poultry Slaughterhouse wastewater
rpm:	Revolutions per minute (rev/min)
RSM:	Response Surface Methodology
sCOD:	soluble Chemical Oxygen Demand

tCOD:	total Chemical Oxygen Demand
TDS:	Total Dissolved Solids
TSS:	Total Suspended Solids

CHAPTER 1

INTRODUCTION

CHAPTER 1

1. INTRODUCTION

1.1 General background

Globally, water scarcity has been associated with climate change, a growing global population and ineffective water management including industrialization, putting the availability of the natural resource under duress. Industrial activities, living standards, characteristics of wastewater and recalcitrant pollutants, have led many countries to adopt various techniques for wastewater treatment (Daigger, 2009). In the recent past, the poultry industry has also grown due to increased demand in poultry products, a major protein source in the human diet, thus leading to the generation of Poultry Slaughterhouse Wastewater (PSW) which contains a high quantity of suspended solids, nitrogenous compounds, fats, oil, grease (FOG) and detergents containing antimicrobial compounds, as a result of the slaughtering processes and sanitization of equipment including facilities (Amorim *et al.,* 2007). An increasing quantity of poultry slaughterhouse waste from production facilities has become one of the most critical environmental challenges due to potable water demand, thus the generation of wastewater containing pollutants that can affect human including environmental health because of pathogenic microorganisms in birds being slaughtered (Kalyuzhnyi *et al.,* 1998). Amongst treatment methods for oily wastewater containing FOG, flotation is considered the best option due to its operational ease, low cost, compact equipment with a minimized footprint and considerable efficiency; hence, the necessity to utilize such technology to ensure compliance with local wastewater regulations (standards) and environmental considerations (da Rocha e Silva *et al.,* 2015).

Flotation is a solids-water physical separation process used in a variety of industries to reduce suspended solids whilst contributing to the reduction of turbidity, chemical oxygen demand (COD), the recovery of minerals, amongst other activities (Chen *et al.,* 2000). In wastewater treatment, Dissolved Air Flotation (DAF) is applied for the removal of low density suspended solids. It is the most widely used flotation method for the pre-treatment of industrial wastewater (Shammas & Bennett, 2010), thus it can be used in PSW pre-treatment in conjunction with suitable flocculants.Most DAF systems use chemical flocculants. Recently Dlangamandla (2017) developed a bioflocculant supported DAF system classifying it as a BioDAF, with bioflocculants being used as primary agents of flocculation.

Generally, bioflocculants are environmentally benign as compared to some synthetic (chemical) flocculants which are considered to be harmful to humans and the environment. The use of a BioDAF system in the treatment of PSW will either have a higher or lower particle removal efficiency which in turn determines the quality of the final effluent; hence, in this study, models describing the BioDAF's performance thus efficiency, were developed using RSM, for the pre-treatment of PSW in order to predict the pollutant removal efficiency thus optimization of such an operation. The models developed resulted in the effective empirical description of a DAF process; hence, providing a basis for effective control of the BioDAF system for pilot plant studies.

1.2 Research problem

There is a continued decrease in the availability of freshwater which has made the objective in the wastewater treatment plant activities to change, from treatment for disposal, to treatment for recycling; thus, a high level of treatment efficiency is required. Furthermore, due to increased PSW production from slaughterhouses in South Africa and stricter treated wastewater disposal standards, there has been a lack of efficient environmentally benign pre-treatment processes for such wastewater; hence, there is a need for improvement and modelling of such technologies, as PSW is considered detrimental to the environment if disposed-off without treatment due to its complex composition due to constituents in the water such as FOG, proteins, blood, skin, feathers and carcass debris from the slaughtering process. Additionally, disinfectants and cleaning agents are also present in the PSW. To address; 1) the removal of such solids including absorbed chemical agents and 2) the need to use an environmentally benign system, a DAF system which is the most commonly used type of a pre-treatment system for the removal of low density suspended solids was proposed, i.e. using bioflocculants. Since such a DAF system used for PSW pre-treatment was never modelled and optimized, it was necessary to adequately describe the performance of the BioDAF system to effectively describe its efficiency for effective performance monitoring.

1.3 Hypothesis

H_0: The modelled bioflocculant supported DAF will not have improved particle removal efficiency when applied as a pre-treatment system for PSW.

H_1: The modelled bioflocculant supported DAF will have improved particle removal efficiency when applied as a pre-treatment system for PSW.

1.4 Research Questions

- Will the COD, TSS, FOG removal efficiency using bio-flocculant supported DAF be higher than when using a conventional system?
- Will the PSW be suitable for discharge, i.e. comply with the wastewater regulations after pre-treatment with the BioDAF system?
- Is the modelling of the BioDAF system feasible and an effective way to describe the pre-treatment efficiency of the system designed?
- Will such DAF pre-treated water, require further treatment using tertiary treatment systems to attain potable water quality standards?

1.5 Research Aims and Objectives

The research was divided into 3 Phases. Phase 1 (Aim 1): To isolate, from the PSW and identify a microorganism which produces bio-flocculants with high flocculating capabilities (flocculation activity); Phase 2 (Aim 2): To examine the effect of bio-flocculants on pollutant removal for a designed BioDAF system; and Phase 3 (Aim 3); To develop a mathematical equation/model for the BioDAF using environmental conditions as input parameters, i.e. focusing on the development of a model such that water quality parameters, i.e. COD, FOG, TSS and protein removal efficiency can be quantified as output parameters, subsequent to optimization of the BioDAF for the pre-treatment of PSW using RSM.

Phase 1: Aim 1: To isolate, from PSW and identify a microorganism which produces bio-flocculants with high flocculating capabilities (flocculation activity). To achieve this aim, this part of the study focused on the following objectives:

Objective 1: To isolate and identify a suitable microorganism using appropriate techniques to adequately produce a sufficient quantity of bio-flocculants with high flocculating capabilities for effective pollutant reduction from PSW.

Objective 2: To identify optimum environmental/production conditions for maximum bioflocculant production with a high flocculation activity.

Phase 2: Aim 2: To examine the effect of bio-flocculants on pollutant removal, for a designed BioDAF system. To achieve this aim, this part of the study focused on the following objectives:

Objective 1: To assess the effect of bio-flocculants produced by the isolate on pollutant removal from PSW.

Objective 2: To quantify zeta potential reduction (wastewater charge reduction), thus assessing bio-flocculants dosage and pH effect on pollutant removal, including mechanism of flocculation.

Objective 3: To identify optimum physico-chemical conditions (operational) for maximum flocculation activity using RSM for the BioDAF designed.

Phase 3: Aim 3: To develop a mathematical equation/model for the BioDAF using environmental conditions as input parameters, i.e. focusing on the development of a model such that water quality parameters, i.e. COD, FOG, TSS and protein removal efficiency can be quantified as output parameters, subsequent to optimization of the BioDAF for the pre-treatment of PSW. To achieve this aim, this part of the study focused on the following objectives:

Objective 1: To generate empirical (mathematical) models using RSM, which incorporates environmental factors such as pH and bio-flocculants dosage to simulate pollutant removal efficiencies focusing on COD, TSS, FOG and protein removal in a BioDAF System,

Objective 2: To assess the suitability of the models developed using statistical analysis (ANOVA), i.e. in order to determine the suitability of the model in describing the performance of the BioDAF,

Objective 3: To compare the models suitability to describe the performance of a conventional DAF (without bio-flocculants).

Objective 4: To determine whether the pre-treated PSW comply with industrial wastewater discharge limits as described by the City of Cape Town (South Africa) industrial discharge standards and to assess whether further treatment is required for the treated water to meet potable water standards.

1.6 Significance of the research

PSW is considered detrimental to fresh water sources if disposed-off untreated, due to its composition of FOG, proteins and other environmental pollutants. Thus, this has led to the adoption of a diverse quantity of techniques including DAF systems, used in the pre-treatment of such wastewater. However, the concept of a BioDAF elucidated herein, i.e. in this research, is fairly new, as such, a description of its proficiency for pollutant removal from PSW has never been conducted. Furthermore, system modelling, whether using empirical or theoretical models, has never been attempted for a bioflocculant supported DAF. This includes the performance and optimization of the BioDAF, taking into consideration, influential environmental parameters.

Therefore, in this study modelling and optimisation, so as to improve pollutant removal efficiency, was attempted, using bio-flocculants as a sole support mechanism for a DAF system, proposed to be used in the wastewater industry treating PSW in South Africa.

Overall, the use of such a DAF system on an industrial scale for PSW pre-treatment would culminate in a discharge that can comply with the appropriate regulations thus avoid fines and disposal charges for non-compliance, save water by promoting the reuse of the pre-treated PSW, while limiting the impact of the discharge on the environment, which will effectively reduce pollutants dispersion that contribute to ecological degradation.

1.7 Delineation of the research

- The scope of the research is solely focused on wastewater from one poultry slaughterhouse operating in Cape Town, Western Cape Province, South Africa.
- Data was gathered around what transpired prior and post PSW pre-treatment using a BioDAF system, with control studies being conducted using a conventional DAF system without flocculant supplementation.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2

2. LITERATURE REVIEW

2.1 Background: General water usage in relation to population growth

Sustainability of socio-economic development including the reduction of poverty is important and water plays a crucial role in such initiatives (South African Government, 2017). It is approximated that 75% of the earth's surface is covered by water, of which 97% of it is in oceans; thus, it is saline and unusable as it is, while the remaining 3% is freshwater. Of the 3% of freshwater, 30% is groundwater while only 0.3% is in rivers, reservoirs including lakes and it is the resource that is easily accessible to humans to meet their needs especially in developing countries. Overall, of all the earth's water, i.e. 99% is not readily available for use (Liu *et al.,* 2011).

Many activities such as industrial, recreational, agricultural and other anthropogenic activities are dependent on the availability of fresh water. As such, they also have an impact on the quality and quantity of wastewater generated (Duran-Encalada *et al.,* 2017). Much of the fresh water is used for commercial purposes, with two thirds of the global water supply being used for agro-industrial activities which in turn produces about 40% of the global food supply (Jagerskog *et al.,* 2016). According to UNDESA (2011), it is predicted that between 2011 and 2050, there will be a 33% growth in the world's population which will culminate in a 60% increase in global food demand. Generally, and due to this demand, there would be an increase in water consumption and living standards, which will further significantly, raise the water demand requirements (UNEP, 2011).

Water usages for domestic and industrial activities is expected to rise, mostly in countries that are experiencing rapid economic growth (WWAP, 2014) and as the demand of water is increasing, so is the quantity of wastewater being produced. It is estimated that, currently, two-thirds of the global population face water shortages for at least 30 days in a year in areas they live whereas half a billion people reside in places/localities where their water usage exceeds the total local potable water availability by a factor of two (WWAP, 2017). According to the WRG (2009), it is predicted that by 2030 there will be a 40% global water shortage based on the current portable water usage levels.

According to the UN (2015), the SDG goal 6.3 states that “By 2030, improved water quality must be achieved by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing treated water recycling and safe reuse globally” and also according to the Global Risks Report by the World Economic Forum (2016), water crisis has been listed as one of the major risks facing populations globally in the near future; hence, a global concern. Therefore, water management is important so as to maintain the limited resource, promote recycling and reuse, so as to adequately cope with water scarcity/shortages.

2.2 Industrial portable water usage and wastewater

Globally, freshwater is used by four main sectors, with the agricultural sector being a leader followed by the industrial, energy and domestic sectors (Claudia, 2013). Water consumption within the manufacturing industry is increasing significantly especially in developing countries due to increasing manufacturing activities (OECD, 2017). Within the industrial sector, the food industry consumes a greater quantity of water for a ton of product produced. Water that is used in this industry is delivered by either a public supplier or by self. For industrial purposes, water is used for various activities that include sanitation of equipment and the production facility, cooling or heating, as a solvent, raw materials and final product washing and/or rinsing, incorporation into the product and also for transporting products in a production line. Industries that produce goods such as food, chemicals, paper and some metals, consume a significant quantity of potable water. The resultant wastewater that is generated is what is known as wastewater (USGS, 2017).

Water from these numerous industries, is discharged into rivers and sea, causing pollution and also into municipal/domestic wastewater treatment systems which in some instances culminates in their redundancy. These wastewater contains contaminants of concern such as heavy metals, nutrients, suspended solids, pathogenic microorganisms and other pollutants. Different industries produce wastewater with different compositions due to different types of contaminants based on the industry further requiring adaptability of the different types of treatment methods used (Metcalf, 2003). For instance, the poultry industry produces wastewater that has high suspended solids, fats, oil and grease (FOG) as contaminants from bird processing (Del Nery *et al.,* 2001). However, there is limited literature published about industrial water usage in the poultry industry in comparison to the agricultural and domestic sectors.

2.3 Portable water usage in poultry product processing

There is increased use of potable water in the poultry industry due to the high poultry product demand as a result of significant poultry product consumption. This is a direct result of population increases globally. It is projected that the global meat production will increase by 16% by 2025, compared to the previous decade which had an increase of 20%. Moreover, when compared to red meat, poultry meat is the major contributor to global meat production as it is cheaper (OECD/FAO, 2016). Fig. 2.1 and 2.2 illustrates global meat consumption in comparison to population increases in South Africa.

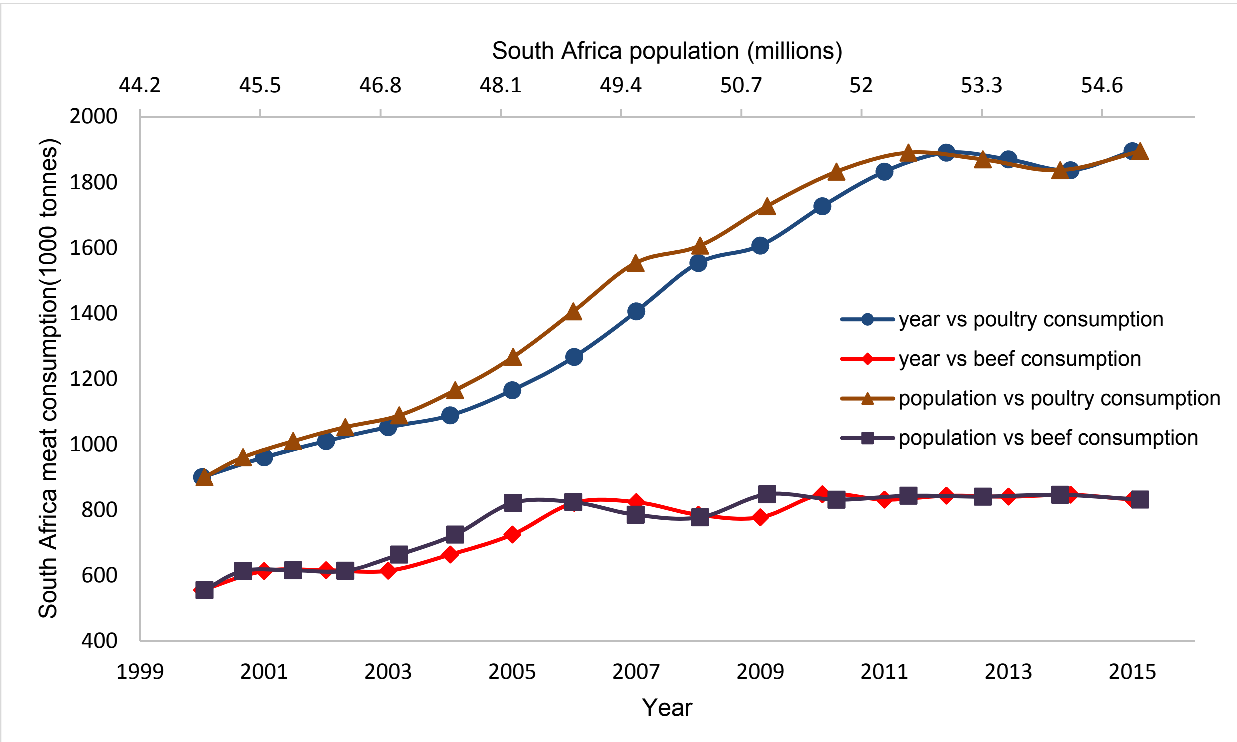

Figure 2.1: An illustration of poultry consumption in comparison to beef consumption and population increases in South Africa (OECD, 2017; The World Bank, 2017).

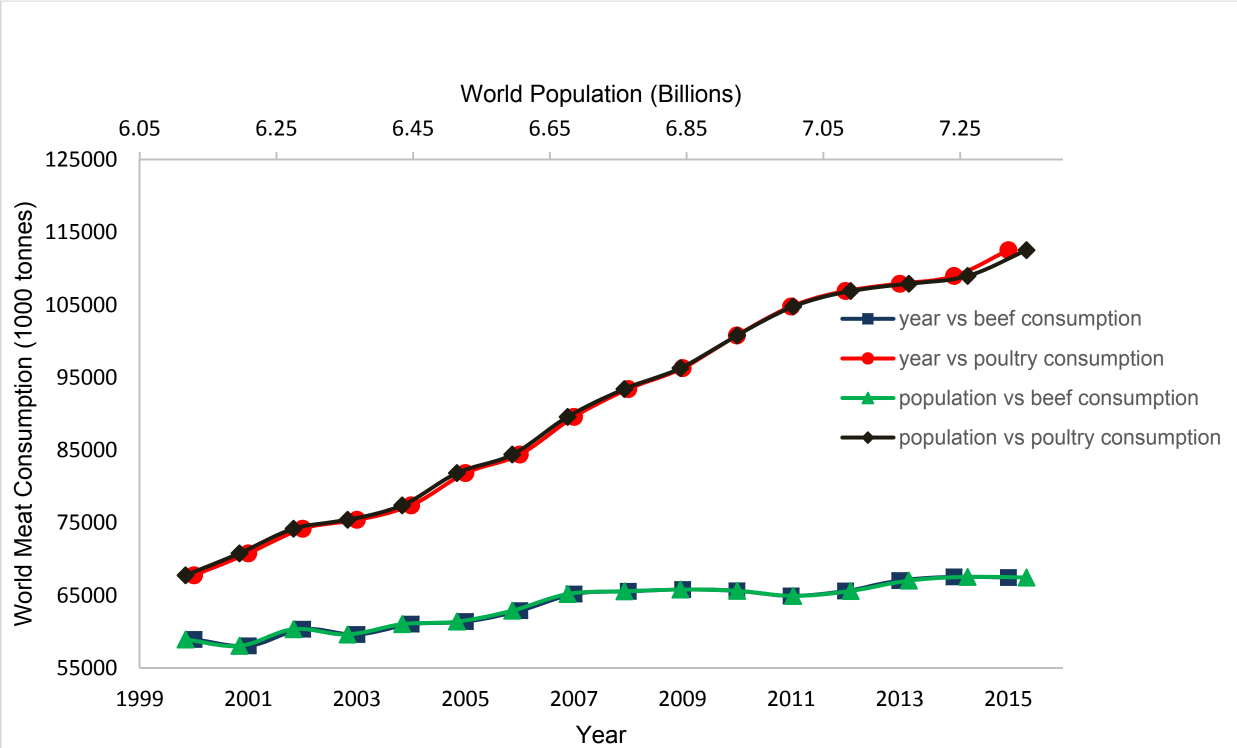

Figure 2.2: An illustration indicating global poultry consumption in comparison to beef consumption and global population increases (OECD, 2017; The World Bank, 2017).

Due to increases in local and global production including consumption of poultry products, a large volume of poultry slaughterhouse wastewater (PSW) is produced from the slaughtering processes, cleaning including sanitization of the facilities and processing equipment (Hrynets *et al.,* 2011). Northcutt and Jones (2004) reported that poultry processing plants use an average of 26.5L portable water per 2.3kg bird live weight (BLW) which cumulatively aggregates to a consumption of 18.9 to 37.8L potable water per bird slaughtered based on plant processes used during primary and secondary processing of live birds to meat products. Table 2.1 indicates the average portable water used at each processing step in the poultry industry. Due to requirements set-out in process validation processes such as Hazard Analysis and Critical Control Points (HACCP) and pathogen reduction requirements in poultry meat, poultry processing plants end-up using a large quantity of potable water which culminate in the production of an equivalent quantity of PSW (Kiepper, 2003; Northcutt & Jones, 2004). The aforementioned water produced, is laden with FOG, carbohydrates and proteins from skin, blood, meat debris and feathers which in-turn results in the wastewater having a higher tCOD and BOD concentration. Water used during the evisceration process also accumulates faecal matter and even pathogenic bacteria (Zhang *et al.,* 2007).

Table 2.1: Average potable water usage in a typical poultry processing plant (L/B: Litres per bird), (A) at individual stages and (B) as a cumulative sum of the total water usage (Avula *et al.,* 2009).

(A)

Primary Processes	**Water usage (L/B)**	**Secondary Processes**	**Water usage (L/B)**
Receiving	0.00	Chilling	2.12
Killing	0.19	Eviscerations	7.57
Bleeding	0.00	Whole bird wash	1.32
Scalding	0.95	Cut-up/De-bone	3.03
De-feathering	1.14	Pack-out	1.14
Final Bird wash	3.03		
Total water usage = 20.49			

L/B = Litres per Bird

(B)

Primary Processes	**Cumulative water usage (L/bird)**	**Secondary Processes**	**Cumulative water usage (L/bird)**
Receiving	0.00	Chilling	7.43
Killing	0.19	Eviscerations	15.0
Bleeding	0.19	Whole bird wash	16.32
Scalding	1.14	Cut-up/De-bone	19.35
De-feathering	2.28	Pack-out	20.49
Final Bird wash	5.31		
Total water usage = 20.49			

L/B = Litres per Bird

2.4 Characteristics of poultry slaughterhouse wastewater (PSW)

Poultry slaughterhouse wastewater (PSW) quality depends on a number of factors which include the size and structure of the processing facility used, the number of birds slaughtered per day, the efficiency of the facilities, blood capture procedures and also on how the facility manages water usage (De Nardi *et al.,* 2008). According to Kiepper (2003), PSW is characterized by uncollected blood, eviscerations, feathers and detergents used in the cleaning of the slaughtering area at the processing plant.

Therefore, the quality of the PSW can be characterized in terms of its biological, physical and chemical properties (Metcalf, 2003). Since PSW is laden with FOG, carbohydrates, proteinaceous matter, blood, bird skin debris and feathers (Fonkwe *et al.,* 2001a), these contaminants contribute to the high organic matter load and a notable quantity of suspended solids presence in the wastewater generated. The primary source of such matter in the PSW ranges from lipids released during scalding to faeces, skin and blood. The significant sources of nitrogen also present in PSW are urine, faeces and blood.

Additionally, blood, faeces, cleaning and sanitation products contribute to the phosphorus in this wastewater (Del Nery *et al.,* 2007). Furthermore, PSW is also contaminated with parasite eggs, pathogenic and non-pathogenic bacteria and viruses including a fair quantity of dirt and other inorganic matter (Franke-whittle & Insam, 2013). FOG in PSW makes-up greater than 67% of insoluble COD (Valladão *et al.,* 2011). The primary environmental problem associated with slaughterhouse wastewater is the large quantity of liquefied waste, suspended solids and also odour generating constituents (Mittal, 2006); hence, pre-treatment of PSW prior to discharge is essential to minimize environmental pollution and to reduce recurring fines from the relevant authority for exceeding prescribed wastewater discharge limits.

2.4.1 Fats, Oil and Grease (FOG) in wastewater

Effluent from food processing industries commonly contain wastewater which sometimes contains a stable oil emulsion containing suspended solids. FOG exists in five forms that are: chemically emulsified, physically emulsified, dissolved, free oil and oil wet solids (Bennett & Shammas, 2010). Fats oil and grease are problematic in downstream anaerobic process in wastewater treatment plants as they are difficult to digest and also usually cause formation of scum or crust (Cammarota & Freire, 2006). However, the removal of FOG from such process wastewater is known to be difficult, i.e. to bio-remediate. Although, the layer of FOG can be separated by gravity separators or using flotation processes, these processes are inefficient especially if the FOG is in the form of a fine particle dispersion or emulsion (Toyoda *et al.,* 1999). The separation of FOG using DAF systems requires suitable flocculants and a pressurized sparging system to form fine bubbles; hence, in the current research the removal of FOG from PSW using a bioflocculant supported dissolved air flotation (BioDAF) system (Dlangamandla, 2017) was analysed and modelled.

2.4.2 Suspended solids in wastewater

According to the APHA (1992), total solids (TS) in wastewater can be defined as residual material that remains in a container after evaporating and drying the sample at a specific temperature. These solids can be classified according to their particle size as either; 1) total dissolved solids (TDS), 2) total suspended solids (TSS), or by organic composition as 3) total fixed solids (TFS) and 4) total volatile solids (TVS), (CSUS, 1993).

If these solids are discharge into fresh water bodies, they cause turbidity increases which in turn reduce light penetration and dissolved oxygen transport. Their effect in fish is such that fish gills are clogged; hence, they are an environmental concern (Mittal, 2004). TSS can be made-up of colloidal, sedimentable or floatable matter. It is therefore important to characterize solids present in PSW (Metcalf, 2003). Amongst treatment methods for PSW, screens are the most commonly used, i.e. as preliminary physical treatment processes used in poultry plants to remove solid constituents in PSW. This type of treatment, usually removes solid particles greater than 500 μm (Kiepper, 2003) in order to avoid and reduce clogging including fouling of equipment. Merka (2004) reported that the mean particle size of particulate matter which makes up about 80% of in/organic material found in PSW is between 75 to100 μm which is classified as TSS above (CSUS, 1993); hence, the ideal pre-treatment system for such wastewater is a DAF system (de Nardi, *et al.,* 2008).

DAF systems have been previously applied in the removal of TSS and FOG in wastewater from the food industry (Manjunath *et al.,* 2000), with their removal efficiency being increased by the supplementation of chemical (de Nardi *et al.,* 2008) or bio flocculants (Dlangamandla, 2017) for flocculable matter reduction in PSW. Flocculants are added to PSW to promote coagulation, FOG flotation, including protein aggregation and precipitation (De Nardi *et al.,* 2011). According to Dlangamandla (2017), a bioflocculant supported DAF system (BioDAF) removed a higher percentage of TSS, proteins and lipids when compared to chemical DAF that was operated with 2% (v/v) alum while a conventional DAF was determined to be the least efficient when they were operated under similar environmental conditions such as HRT and sparging rate at ambient temperature including steady state conditions.

2.5 Dissolved air flotation (DAF) as a pre-treatment system

Pre-treatment process selection is dependent on the quality and type of the wastewater including the desired effluent requirements (Krofta, *et al.,* 1995).

Recently, the use of a DAF for the pre-treatment of different industrial wastewaters, has been advocated for due to the advances in the technology which have led to the expansion of its usage (Haarhoff & Edzwald, 2013); for instance, DAF uses different parameters such as pressure 400-500kpa for the pre-treatment of different types of wastewater particularly for the removal of organic matter (Ross, *et al.,* 2000). However, in this study, low pressure thus energy consumption was preferable.

A DAF system used for pre-treatment process usually consist of a flotation tank whereby the flocculation and separation of suspended matter takes place, with different inlet and discharge ports, with one port whereby the treated water is discharged and while the other acts as an inlet for the raw water to pass into the DAF tank. Although the DAFs' tank is the primary unit, there are several components that are important for the optimal operation of the DAF (Ross, *et al.,* 2000; Woo, 2016). Performance of DAF systems is normally affected by pre-treatment conditions such as the frequency and concentration of flocculants dosage, adjustments in pH, and the physical design of the system, to mention a few (Edzwald, 2010). Overall, industrial wastewater such as PSW, wastewater containing sulphur ions (Amaral Filho, *et al.,* 2016; De Nardi *et al.,* 2008), and many other pollutants, can be pre-treated using a DAF system prior to discharge, i.e. to remove contaminants that can results in deleterious impact on downstream wastewater treatment processes; hence, its use prior to secondary treatment processes, is recommended.

2.5.1 Background: Dissolved Air Flotation (DAF)

The underlying principle behind the DAF system is based partially on Henry's law which states that the solubility of air in water is directly proportional to the existing pressure in the system under evaluation (Schers & Van Dijk, 1992). The primary objective of a DAF system is to form positively buoyant air bubble-particle agglomerates by attaching particles onto the surface of bubbles. Thereafter, the agglomerates rise to the surface of the flotation cell whereby they accumulate and form a layer that can be subsequently skimmed-off using mechanical skimmers (Leppinen *et al.,* 2001). The air flotation system operational principle is based on micro-bubble formation in different forms, which are: dispersed air, dissolved air and electrolytic coagulation and floatation which can be supported using biological and chemical flocculants. To induce flotation, different micro bubbles, under different pressure, are required; hence, dissolved air flotation can be further divided into: dissolved pressurised air and vacuum air flotation (Zhu *et al.,* 2014).

Generally, flotation is mainly applied where the use of sedimentation is not attainable and it is dependent on the surface chemistry of the matter to be separated. This technique is used for the treatment of solid containing liquid effluents, especially those effluents in which the differences between the particle densities is minute (Couto *et al.,* 2004; Rodrigues & Rubio, 2007).

For such wastewater, DAF is a well-known pre-treatment separation process forming an aqueous pneumatic current saturated with air, at a pressure which is greater than atmospheric pressure, to form flocs which rise to the surface of the aqueous phase (Haarnoff & Edzwald, 2013). The total dissolved and suspended solids to be removed using a DAF system should be of minute sizes. The screening unit used to reduce large particles is usually preceded by a flocculation unit whereby dissolved and small suspended solids are flocculated into removable larger particles; hereafter, referred to as flocs (Edzwald, 2010). The dosing of an appropriate quantity of suitable flocculants culminates in particle surface chemistry changes; the particles become hydrophobic with the particle repulsion charge being reduced for ease of attachment, i.e. floc formation (Zhu & Zhou, 2014). However, there is minimal literature describing the flocculation mechanisms and/or bonding type, for bioflocculants used in BioDAFs.

2.5.2 Applications of the dissolved air flotation (DAF) systems

The primary application of DAF systems for the reduction of ion charge (zeta potential) and removal of fibres, solids and including other suspended materials from wastewater (Matis, 1995), can only be achieved if the materials have a greater tendency to float, i.e. that are easily suspended within the wastewater. Additionally, the application of DAF system culminates in the reduction of parameters such as tCOD, BOD, turbidity and others which are of primary concern in wastewater treatment plants (Al-Shamrani *et al.,* 2002). DAF system usage has been implemented for decades in different wastewater plants as an alternative to sedimentation. The primary advantage of flotation over sedimentation is that minute or particles with a lower density and with a propensity to slowly settle can be removed efficiently and rapidly (Casey & Naoum, 1986). As such, it is the most widely used flotation type method for the treatment of suspended solids laden wastewater, due to its pre-treatment efficiency, minimal cost including operators (personnel) technical know-how requirements.

Other advantages associated with the use of such a system include high air velocity, which permits for high suspension of solids independent of loading rates, a high floc formation rate including floc concentration attained (good thickening). This process can sustain the removal of low density particles which require long settling periods (Shammas & Bennett, 2010). Nowadays, DAF is applied in raw surface water and wastewater treatment for numerous industries including mineral processing, pulp and paper for plant fibre recovery, poultry industry for FOG and protein removal, de-inking of recycled paper and waste sludge thickening to name a few (Bahadori *et al.,* 2013). The use of bioflocculants has made the application of the DAF system favourable or feasible due to the environmental benignity of proposed bioflocculant usage (Tansel & Pascal, 2011).

2.6 Flocculants

Flocculants are chemicals that facilitate flocculation by aggregation of suspended particles and colloids, forming flocs (IUPAC, 1997). They are used to destabilize and/or reduce particle charge for ease of attachment. Generally, destabilization is caused by an increase in the ionic strength which in turn reduces the zeta potential of the particle/ wastewater phase or by adsorbing counter ions on the suspended particles; thereby, neutralizing the particle charge (Crini, 2005). Flocculation is usually described by the following common mechanisms; sweeping flocculation, bridging, charge neutralization and electrostatic charge patching (Van Damme *et al.,* 2013), see Fig 2.3.

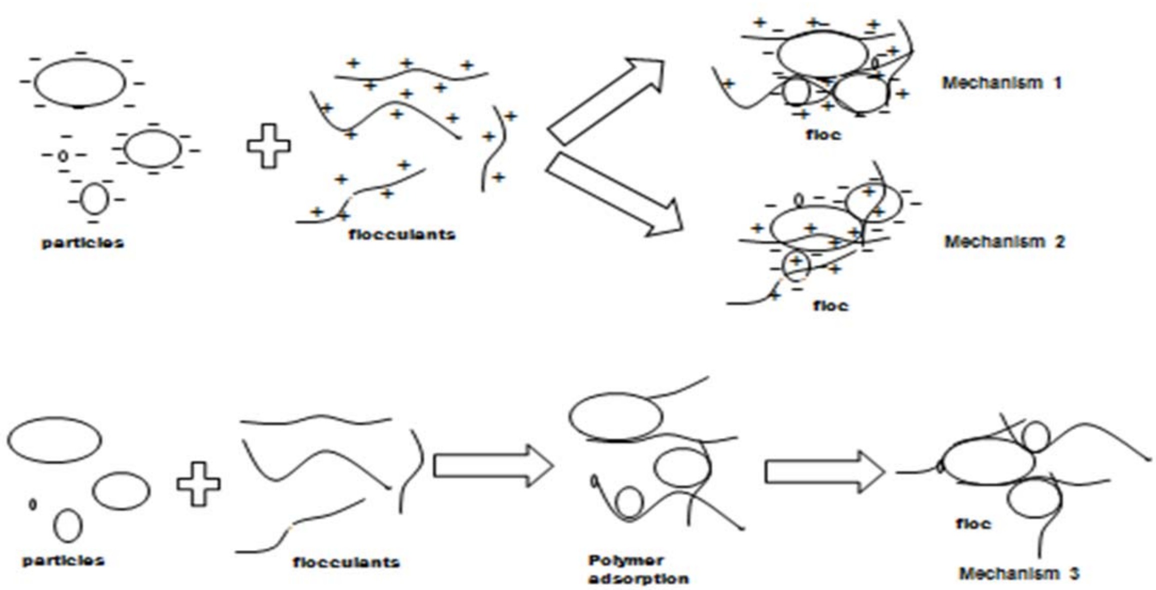

Figure 2.3: Representation of flocculation mechanism (1) charge neutralization, (2) electrostatic patch and (3) polymer adsorption and bridging (Dao *et al.,* 2016).

In large scale wastewater treatment plants, flocculants are used, so as to alter the physical properties of dissolved and suspended solids; hence, facilitating their removal (Mabinya *et al.,* 2011). Such flocculants can be of synthetic or natural origin (Hu *et al.,* 2006) with bioflocculants being preferred over synthetic flocculants, due to their low impact on the environment (Dlangamandla, 2017). The primary variables that are usually measured or assessed to quantify whether flocculants are efficient; include zeta potential reduction of the wastewater in comparison to pollutants removal percentage, reduction of turbidity, and others (Stechemesser & Dobias, 2005). Furthermore, flocculants can be categorized into three groups; organic synthetic, e.g. polyacrylamide derivatives; inorganic, e.g. alum, aluminium sulphate; and natural occurring flocculants, e.g. chitosan and protein based bioflocculants (Qin *et al.,* 2015; Roselet *et al.,* 2015).

2.6.1 Chemical flocculants

Chemical flocculants are predominantly inorganic and synthetic organic compounds. Their extensive usage has been restricted due to their perceived carcinogenicity and neurotoxicity (Dearfield *et al.,* 1988). Although inorganic flocculants are cost effective with ease of use and availability; they have their disadvantages. These include the production of a high quantity of metallic sludge that is not readily dehydrated, rapid increases in alkalinity in the wastewater, with floc formation reduction using flocculants such as alum in cold water, i.e. during winter. Furthermore, their functional properties are largely pH dependent and high suspended solid loading rates influences efficiency thus requiring a higher dosage. The use of alum and other aluminium salts for flocculation is now deemed controversial due to the association and/or probable negative clinical outcomes such as Alzheimer’s disease being associated with aluminium residue in treated water (Ali *et al.,* 2010).

Synthetic and/or flocculants of an organic polymeric origin have some advantages when compared to inorganic flocculants. These advantages include reduced dependence on pH, lower dosage requirements, in some cases, lesser residual sludge formation, increased and rapid separation rates due to larger agglomerate size formation and retainment of efficiency even at low temperatures. Other advantages include ease of tailorability as their molecular weight distribution; chemical structure including functional groups can be tailored for the treatment of a specific wastewater type.

Despite their advantages over inorganic polymers, they also have some disadvantages which include, polymer toxicity, high cost of tailoring and some are not non-biodegradable. When synthetic polymers are used in wastewater treatment, they can also produce residue and recalcitrant by-products which are classified as toxicants that would be as a result of unreacted chemicals from the production of the monomer units, e.g. dimethyl amine and formaldehyde and/or as residue of unreacted monomers, i.e. acrylamide and trimethylolmelamine including undesired end-products of the reaction during production (Bratby, 2006; Bolto & Gregory, 2007; Wu *et al.,* 2012). Some of the synthetic flocculants and their by- or undesired end-products have been suggested to cause carcinogenic effects, biomagnifying into the food chain (Ali *et al.,* 2010). For instance, acrylamide monomers are classified as being non-biodegradable carcinogenic and neurotoxic to humans (Ruden, 2004). In environmental engineering systems, synthetic chemical compound usage is undesirable (Tenney & Stumm, 1965); hence, the use of bioflocculants as an alternative to synthetic chemical flocculants is hereby proposed.

2.6.2 Bioflocculants

Microbial flocculants, termed bioflocculants are extracellular biopolymeric substances that are produced by fungi, bacteria, yeast and algae during cell growth and cell lysis (Salehizadeh & Shojaosadati, 2001; Manivasagan *et al.,* 2015). They are composed of macromolecular substances which interact with the contaminants in the wastewater being treated. Their constituents include a variety of functional groups such as proteins and polysaccharides (Zheng *et al.,* 2008; More *et al.,* 2014). Their composition and properties are dependent on the type and strain of the microbial producer, environmental conditions including the composition of the nutrient media used (Subramanian *et al.,* 2010).

Moreover, bioflocculant commercial application has been limited due to the high production cost, associated with substrates used in the nutrient media designed for their production, which is deemed as costly. Nevertheless, numerous ways of reducing the input/operational costs have been recently explored with promising results; such as the utilisation of various industrial wastewaters such as PSW, dairy and potato starch wastewater as a nutritional source for the production of bioflocculants as such wastewaters was determined to contain nitrogen and carbon sources that can replace conventional and/or refined substrates (Dlangamandla, 2017; Guo & Ma, 2015; Guo *et al.,* 2015; Wang *et al.,* 2007).

Agricultural waste that is rich in residual reducible sugars has been utilized as a cheap carbon source in the production of bioflocculants recently (Guo *et al.*, 2015a); an effective strategy, as such waste, usually results in pollution of the environment due to landfilling; hence, its beneficiation in the production of bioflocculants, is of economical and practical interest.

The primary mechanisms for floc formation using bioflocculants was determined to be achieved through charge neutralization and polymer bridging, i.e. mechanism 1 and 2, see Fig. 2.3. Polymer bridging suggests that bioflocculant chains and suspended solids primarily form flocs though ionic mediated bridging (Sobeck & Higgins, 2002). In charge neutralization, the charged bioflocculant, neutralize the charge of the suspended solids and colloids (Lian *et al.,* 2008); hence, such an electrostatic interaction would result in charge neutralization, leading to floc formation. There has been an increasing need of environmentally benign materials in surface water, wastewater treatment, including other environmental engineering applications and bioflocculants are a promising alternative to recalcitrant synthetic flocculants that are currently in use.

For the past decade, they have been more attention in downstream process efficacy in wastewater treatment plants with regard to the resultant effects of either chemical and/or bioflocculants (Cosa *et al.,* 2012; You *et al.,* 2008). Due to the green chemistry advocacy approach and implementation nowadays, bioflocculants have been studied for application in industries ranging from food production to biological waste reduction and/or treatment (Aljuboori *et al.,* 2014). For this research, bioflocculants will be used as an alternative of chemical flocculants in a DAF system for the pre-treatment of PSW such that the discharge will comply with wastewater discharge regulations.

2.7 Regulatory constraints associated with poultry slaughterhouse wastewater (PSW) disposal

PSW is considered detrimental to environmental health worldwide due to its composition as a result of the slaughtering process. Disinfectants and cleaning agents which contain antimicrobial agents are also present in such wastewater (Fonkwe *et al.,* 2001; Wu & Mittal, 2011; Bustillo-Lecompte *et al.,* 2014). Wastewater guidelines and regulations observance is important in mitigating the impact of PSW on the environment, particularly when being disposed-off into fresh water sources. Due to increased poultry slaughterhouse waste production and stringent environmental regulations, there has been a lack of efficient pre-treatment processes dedicated for PSW pre-treatment (Pierson & Pavlostathis, 2000). Amongst pre-treatment methods for oily water containing FOG, flotation is considered a suitable bioremediation pre-treatment method option due to its operational ease, low cost, compact equipment including high efficiency which facilitates and ensures compliance with wastewater discharge standards resulting in less environmental and ecological degradation, with probable treated wastewater recycling and reuse (da Rocha-Silva *et al.,* 2015). Such an initiative, i.e. such as the use of a modelled BioDAF system for the pre-treatment of FOG laden PSW, will not only promote environmental sustainability but ensure regulatory compliance.

2.7.1 South African wastewater legislation

Abattoir waste is managed by the National Environmental Management Act (NEMA) and the National Water Act (NWA) of 1998 (Act. 36 of 1998) (DWA, South Africa, 2009). According to the NWA act, as amended, which states "... water extracted for industrial purposes shall be returned to the source from which it was abstracted, in accordance with quality standards gazetted by the Minister from time to time", and "wastewater means water containing waste, or water that has been in contact with waste material.’’ The act requires that industries which produce wastewater keep the discharge under the regulatory limits with a pre-requisite registration for fresh water usage and wastewater disposal with the relevant department being essential. It also describes the management and quality requirements of discharging waste or water containing waste into a water resource [Sections 21(f) and (h)] (DWA, South Africa, 1998).

Industries in South Africa that are within demarcated municipalities discharge their wastewater directly into the municipality sewage system; hence, the municipality takes responsibility of monitoring the treatment and disposing-off of the wastewater generated (Hammer & Hammer, 2008). These municipalities can therefore penalize industries that have effluent which contain high levels of toxicants and/or pollutants. Strict effluent discharge standards have been set in an effort to preserve the environment and fresh water resources due to industrialization.

This has resulted in regulatory compliance monitoring being an important part of water conservation (Yetilmezsoy & Zengin, 2009); hence, in order to comply with these environmental regulations, most of the particulate organic and soluble matter in the PSW must be removed prior to discharge (Zhang *et al.,* 1997). In South Africa, the Department of Water Affairs regulates the industrial effluent discharge standards. Penalties have become common for industries which do not meet the minimum discharge limits; as such municipalities have by-laws to ensure that they recover material costs from individual polluters through the "polluter pays" initiative. According to the City of Cape Town, (Western Cape, South Africa) whereby this study was based, wastewater and industrial discharge by-law (2006), Schedule 1 (1) (2), discharge tariff (penalty) can be levied based on a formula as listed in Eq. 2.1.

$$Cost = Vw(SVC) + VieT(COD - 1000)/1500 + VieT(SF) \qquad 2.1$$

Where:

Vw = total volume (*kL*), of wastewater discharged from the premises during the period under assessment,

SVC = sewerage volumetric charge in terms of the sanitation tariff,

Vie = total volume (*kL*) of industrial effluent discharged from the premises during the period under assessment,

T = cost, as determined by the council, of treating 1kL of wastewater, and

COD = chemical oxygen demand (mg/L) of the effluent.

In the event of the COD being <1000 mg/L, the COD factor falls away, with a surcharge factor being another way to ensure compliance. A surcharge factor (*SF*) of the effluent can be calculated according to Eq. 2.2.

$$SF = (X - L)/L \qquad 2.2$$

Whereby:

X = concentration of one or more of the parameters listed in Schedule 2 (see Table 2.2), and

L = being the limit applicable to that particular parameter.

To monitor the effluent discharged into municipal wastewater systems, chemical parameters such as BOD, tCOD, pH, suspended solids, oxygen absorption, nitrogen and phosphorus are quantified and compared to the discharge standards as governed by the South African Water Act and SANAS (2014) standards (Metcalf, 2003). For the same by-law mentioned above, i.e. in Schedule 2, the parameters as indicated in Table 2.1, are prohibited from being exceeded when discharging wastewater into the sewer which further lists' averaged PSW quality parameters from a poultry slaughterhouse in Cape Town (Basitere *et al.,* 2016; City of Cape Town, 2016), i.e. for which the wastewater was obtained for this study.

Table 2.2: Prohibited discharge into sewers

Parameter	Not to exceed (mg/L)	PSW average values (mg/L)
COD	5000	2903
Settleable solids (60 min)	50	-
Suspended solids	1000	794
Total dissolved solids at 105˚C	4000	604
Total phosphates as P	25	17
FOG	400	406*

*Out of specification (Basitere *et al.,* 2016; City of Cape Town, 2016)

Generally, abattoirs usually have difficulties meeting the by-law wastewater quality standards for dissolved solids and FOG; hence, an on-site pre-treatment system is necessary so as to reduce the pollutant load from the PSW prior discharge, in order to comply with the relevant regulations.

2.8 Literature review: A summary

We are heading towards a water constrained era whereby the improper management of fresh water could easily culminate in water shortages. This is a result of improvement of living standards and population growth. Recently, the South African poultry industry has grown due to increased poultry products demand thus the generation of a large quantity of PSW which contains a high concentration of suspended solids, FOG, phosphorus, proteinaceous matter and detergents from slaughtering processes and sanitation of equipment, which are considered detrimental to humans and the environment. As a result this, regulatory compliance monitoring was promulgated due to a lack of efficient pre-treatment processes and minimal standards for effluent discharge, leading to the adoption of diverse techniques for wastewater pre-treatment. Wastewater guidelines and regulations observance is important in mitigating the impact of PSW on the environment, particularly when being disposed-off into fresh water sources thus pre-treatment of PSW prior to discharge is essential. Amongst treatment methods for oily wastewater, flotation is considered a suitable option due to considerable efficiency thus the proposal to utilize a Dissolved Air Flotation system (DAF) for PSW pre-treatment in conjunction with bioflocculants which when added to the PSW can promote coagulation, flotation, including protein aggregation.

Moreover, in environmental engineering systems, synthetic chemical compound usage is deemed undesirable; hence, the use of bioflocculants due to their environmental benignity as an alternative to synthetic chemical flocculants which are un-biodegradable, associated with carcinogenicity including neurotoxicity effects in humans. For the current research, a BioDAF system which was initially developed by Dlangamandla (2017) was modelled with minor adjustments, as there is minimal literature describing the modelling of a DAF system for PSW and in particular a BioDAF.

CHAPTER 3

MATHEMATICAL MODELING OF BIOLOGICAL PROCESSES AND OPTIMISATION

CHAPTER 3

3. MATHEMATICAL MODELLING OF BIOLOGICAL PROCESSES AND OPTIMISATION

3.1 Background

This chapter focuses on mathematical modelling of physical and biological processes. It defines what mathematical modelling is and gives advantages of process modelling. Additionally, it covers the applications of modelling, illustrates examples of models used for process engineering systems and lastly, it discusses software that can be used for modelling, specifically, Response Surface Methodology (RSM), which was used in this study.

3.2 Mathematical modelling

A mathematical model is a representation, in mathematical terms, of the behaviour of process units and whole systems (Abramowitz & Stegun 1968). Models represent real world problems in a mathematical form with some assumptions which aid in the understanding of process variables in a quantitative and fundamental manner (Das, 2014). Process components and variables are replaced with symbols when mathematical modelling is used. Mathematical models can be in the form of statistical models, dynamic system models and differential equations, among others. Various analytical and computational techniques are used for analyses and synthesis of possible outcomes once the mathematical model has been obtained. In the development of a model, assumptions including outcomes are made, culminating in the simplification of the models being used. Modelling assist in the identification of the underlying and influential process variables used to describe the functioning of the aforementioned processes. Formulation and the refinement of preconceived ideas are paramount for effective process representation in a model which can be used to assess effects of changes in a defined system (Dorf & Bishop, 2001; Ugwa & Agwu, 2012). Therefore, it makes it feasible to assess the interrelationship amongst process variables through manipulation of the model being used to describe such a system (Gershenfeld, 1999).

Furthermore, mathematical modelling can also assist in the development and testing of a theory, by taking advantage of the accuracy offered by mathematics. Models often integrate theory and practical outcomes from experimentation, with modelling being analogous to behaviours of a system that can be further analysed and optimised through comparison with the predicted behaviour of a process.

To qualify a model, the theoretical basis of the developed model must agree with the experimental results achieved. If such an agreement is not achieved, further refinement including validation and the development of an advanced theory describing the process is required. When a theory is being developed, the mathematical specifications might direct the theory into a new direction, making such theory evaluation impossible to attain. This can culminate in the use of appropriate software and the development of an empirical model from experimental data. Therefore, algorithms, mathematical expressions and other simulation procedures can be used to develop an empirical model (Bender, 2000; Cavagnaro *et al.,* 2013).

There are three types of models, namely: theoretical, empirical and semi-empirical models. In this study the development and application of an empirical mathematical model based on the experimental observations was pursued. The main advantage of using this approach is the development of a model and assessing its applicability for a new (novel) system over a wide range of operating conditions (Anon, 2013). Models are further classified into two classes that is white box, grey box or black box and dynamic or non-dynamic (Alqahtani *et al.,* 2016). The first are based on the availability of information needed to develop the model. For instance, the black box approach is used when there is minimal information available while the white box is when all the important or necessary information is available for model development and computation. Dynamic models are time frame prediction models. They are made up of numerous ordinary differential equations (ODE), which are based on known input and output variables within a defined system. The objective is to generate requisite information for either a steady and/or unsteady system (Lauwers *et al.,* 2013).

3.2.1 Benefits and application or uses of mathematical modelling

Models are useful in answering questions, predicting behaviour and solving industrial process engineering problems. Modelling assists in finding the most crucial characteristics of a system being studied, culminating in the abstraction of non-influential variables for a process unit or whole system. They give clear suggestions of the input and output variables. Model development and system organisation between variables, most often reduces unknown information about a system. It also assists in the formulation and testing of a hypothesis to get information about a system which is not readily available. Modelling reduces input and/or process development costs which are usually needed for studying a system directly (Novoseltsev & Novoseltseva, 2009), which is sometimes uneconomical if implementation of intervention measures is done without prior assessment or prediction of outcomes.

Modelling can further reduce changes, reworking of proposed solutions while minimizing errors to improve the standard or quality of a proposed solution to a problem. As the modelling of devices and natural phenomena is important to both science and engineering, thus a powerful tool used in research and development for scientific research, models obtained through pilot scale research studies can be used to control or predict the behaviour of a system in applied settings or at an industrial scale (Mazur, 2006). Nowadays, process engineers, physicists and economists, all use models to predict behaviour of defined phenomena (Dangelmayr, 2005), with some approaches using historical data. Such an approach is being used in water resources management, environmental studies focusing on pollutant dispersion, economics, population dynamics, drug design, climate change and many others (Das, 2014). For instance, in water resource management, modelling can be used to design DAF systems and optimise their operating conditions.

3.3 Models for DAF systems

The application of relatively simple and conceptually appropriate mathematical models is a substantial tool to identify, understand critical and influential parameters in a process. Fundamental principles and model development can improve our understanding of the design and operation of DAF systems which is largely dependent on generation of pilot plant data and experience (Edzwald, 2007). Various conceptual models of DAF systems have been developed and used so as to understand the complex variables that affect a DAF systems operation with a focus on suspended solids, and other wastewater quality parameters to be improved and air bubble generation including size (Haarhooff & Edzwald, 2001), to name a few.

The flotation cells of a DAF system can be of any shape for instance El-Gohary *et al.* (2010) and de Nardi *et al.* (2008) used column cell whereas Behin and Bahrami (2012) used a rectangular cell in their different studies for pre-treatment of wastewater. However, the flotation process consist of two different influential parameters that have a direct impact of separation efficiency, i.e. at a microscopic level, whereby flocs and bubbles interact including floc-bubble agglomerate formation and also at a macroscopic level whereby general flow pattern and the tank geometry are influential (Crossley & Valade, 2006). DAF which is a common type of flotation process is mainly comprised of two zones (see Figure 3.1) that are; 1) the reaction or contact and 2) the separation, zones. The contact zone is whereby the air bubbles come into contact with suspended solids subsequent to the adherence of the particles to form flocs which results in the formation of stable buoyant particles. The separation zone provides conditions for particle-bubble agglomerates to rise to the surface of the wastewater whereby they aggregate and are subsequently skimmed-off (Moruzzi & Reali, 2010).

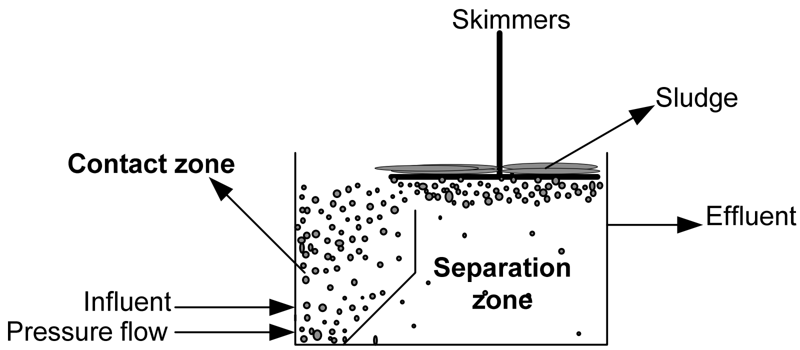

Figure 3.1: Schematic illustration of the contact and separation zones of a dissolved air flotation system (Behin & Bahram, 2012; Edzwald 2010)

In the separation zone, most of the models are based on the rising rate of particle-bubble aggregates, i.e. buoyance of the flocs, for example Lakghomi *et al.* (2012) modelled the separation zone by analysing bubble aggregation and the importance of a stratified flow using computational fluid dynamics (CFD) software. They reported that by increasing the quantity of sparging, culminated into positive results, as it improved bubble movement and also created a favourable horizontal stratified flow pattern.

Similarly, efficiency models can then be used to describe the efficiency of a DAF process based on the interaction between suspended particles input and improvement of the treated water in terms of quality characteristics (pollutant removal efficiency). Furthermore, they are contact zone models which usually focus on the attachment of a floc to a bubble and also the formation of the floc-bubble aggregates (Edzwald, 2010). Bondelind *et al.* (2013) demonstrated that the modelling of the contact zone by presenting a model that described aggregates formation and to estimate their sizes can be achieved. Their model constituted of five forces which are buoyancy, electric, van der Waals, hydrophobic and hydrodynamic repulsion. Some of the assumptions they made are that the bubbles rise as rigid spheres and in the contact zone they have a constant size and they also assumed pneumatic bubble movement as a mixing mechanism. Their model indicated that the aggregate sizes calculated were dependent on the shape and size of interacting flocs while air bubble characteristics including their surface potential had a direct influence on the density of the suspended solids which had an effect on the flocculation outcomes observed.

3.3.1 Factors affecting DAF system operations

There are a number of factors that are considered when designing a DAF system, i.e. the type and quantity of wastewater being treated, the nature of contaminants in the wastewater, the level of treatment to be achieved and also the subsequent downstream secondary treatment process to be used (Telang, 1996). Furthermore, such factors are indicative of the operation parameters (input) to be used during modelling, i.e. they are input process variables. These parameters can include air flow rate, solids retention time, flocculants concentration thus dosage and air dissolution pressure, amongst others. These factors affect flocculation, attachment of bubbles to suspended particles, buoyancy rate of aggregates, as they directly have an influential role in the quality characteristics or outcomes of the wastewater being treated (Han *et al.,* 2001). The overall removal efficiency of the pollutants is affected by a number of parameter such as wastewater flow rate (organic loading rates), tank geometry, surface properties (charge) of the materials, i.e. suspended solids as FOG, and bubble geometry (Bondelind *et al.,* 2013).

Furthermore, an important parameter which affects the overall performance of the DAF system is the air-solid ratio as it has an effect on particle-bubble collision frequency, buoyancy (eddy) velocity and pollutant removal rates. A mass balance for air-solid ratio can be represented by Eq. 3.1 (El-Gohary et al., 2009).

$$\frac{A}{S} = \frac{1.3S_a(fP-1)}{X} \qquad 3.1$$

Where:

$\frac{A}{S}$ = air-solid ratio (kg air/kg solids),

S_a = air solubility (mL/L),

P = operating pressure (kg/ cm^2 or Pa)

f = pressurisation system efficiency at pressure 0.8, and

X = influent solids concentration (mg/L).

This research focused on the following input parameters; pH, and flocculants concentration thus dosage all of which affect TDS and FOG removal including other pre-treated wastewater characteristics such as tCOD and protein removal. Therefore, the modelling of the BioDAF for PSW pre-treatment would be valuable in trying to achieve high particle removal efficiency by identifying key and influential parameters involved in the process, since, the modelling and optimization of a BioDAF system has never been attempted before. The models generated for the BioDAF can thus be further used to develop theories for such a process.

3.3.1.1 Bubble formation for DAF systems

There are three common classes/ways of bubble generation and the most commonly used is the one whereby compressed air is dissolved in the wastewater. An alternative is through power generation using ultrasound to instigate cavitation reduction of wastewaters' density forming ultrasonic waves; hence, bubble formation. The latter delivers air under low pressures and the bubble formation is aided by additional features such as pneumatic wastewater oscillations or mechanical vibrations. One of the benefits of micro bubble formation is solid-micro bubble interaction which facilitates flotation (Zimmerman *et al.,* 2008). DAF system utilizes air bubbles which are basically supplied through three different flow sheets i.e. i) partial pressurization, ii) total pressurization of the influent and iii) recycle pressurization in which the clarified effluent is pressurized and then mixed with influent again (Zouboulis and Avranas, 2000). The latter is the most widely used form but for the present research full air will be supplied directly into the tank through air diffusers which will cause the formation of air bubbles.

Most sparging systems used in DAF units are operated at pressures between 400-600kPa. In a DAF, bubbles are formed from cavitation when the pressure drops upon introduction into the system. The sudden reduction of pressure causes air to be released into the wastewater as micro-bubbles with a size of 10 to 100μm (Edzwald, 2010). There are two steps for bubble formation, i.e. nucleation and growth. Nucleation occurs prior to pressure reduction at the nozzle and then the secondary step involves bubble growth that starts after the excess air in the saturated liquid is conveyed from the dissolved to gaseous phase (De Rijk & den Blanken, 1994). Minute bubbles of <100μm usually rise as rigid spheres; hence, they are applied in DAF system operations and modelling. Bubble size affects particle to bubble attachment performance and also the bubble rise velocity. In a DAF system, bubble size distribution is affected by a number of factors such as the design of the diffusers used, sparging rate and bubble growth. Air bubbles in flocculant free wastewater have a negative charge, thus a negative zeta potential. In DAF applications, the surface charge of bubbles can be altered through the addition of flocculants (Edzwald, 2010) with a BioDAF being supplemented with bioflocculants. Additionally; computer software has been used to study bubble properties such as size as an influential (input) parameter.

3.4 Software in process modelling

Over the past four decades, the reliability of models has improved due to increased computational power that has been provided for by modern computing. Computer software are currently being used to do the numerical computations (Cavagnaro *et al.,* 2013), while a decade ago as indicated by Krofta *et al.* (1995), an attempt was made to model a DAF system using Partial Least Square Regression, reporting that the mathematical model obtained although predicted the results with a reasonable accuracy, computing methods such as CFD could have improved the accuracy of the models including the determination of flow patterns in the DAF studied. Behin and Bahrami, (2012) used CFD to model an industrial dissolved air flotation tank through the use of residence time distribution curve to model the flow rate, in which they used a coloured tracer injection method to obtain mathematical equations. They found out that an increase in inlet flow will result in an increase in mixing thus decreasing the volume of the dead zone. The data obtained agreed to the empirical models developed to a reasonable extent. Also, Bondelind *et al.* (2010) used a CFD model to predict DAF operation focusing on turbulence, bubble size and the DAF geometry with a 2D model indicating that there was a need for adjustments in the geometry and parameters regulating the flow, with a 3D model accurately improving the modelling outcomes. The study also reported that bubble size had an effect in the separation zone than in the contact zone. Similarly, RSM has been used in chemical and biochemical process optimisation and evaluations (Shahrezaei *et al.,* 2012). Montgomery defined RSM as a set of statistical and mathematical techniques that are used for experimental design, modelling, evaluation of process variable effects and the determination of optimum conditions for variables such as to predict a response provided there are changes in input environmental factors (Montgomery, 2008).

One of the advantages of using RSM is that it can be applied to a set of or a response of interest that is influential instead of changing one parameter at a time when other parameters are constant which simply means more experimentation is required when one factor at a time is utilized, culminating in more time being used in experiments (Bezerra *et al.,* 2008). RSM can define the independent variables effect as individuals or in a combination and also generates an empirical mathematical model which can be utilized to describe the process being modelled (Anjum *et al.,* 1997). Adlan *et al.* (2011) used RSM for the optimization of a DAF system for the treatment of semi-aerobic landfill leachate and reported that the experimental results obtained were consistent with the ones from the predicted model. For the current study, RSM was used to generate a model for the BioDAF for the pre-treatment of PSW so as to achieve high pollutant removal efficiency. This study is the first to make such an attempt. The model development steps that were followed are highlighted in Fig. 3.1.

STEP 1: Selection of independent variables

↓

STEP 2: Experimental design (conduct experiments as per experimental matrix)

↓

STEP 3: Fit the polynomial equations to the experimental data

↓

STEP 4: Evaluate models fitness

↓

STEP 5: Verify the need/not for performing a displacement towards the optimal region

↓

STEP 6: Obtain optimum values of the variables used

Figure 3.2: Steps involved in mathematical modelling using RSM.

3.4.1 Developing models using RSM

The first step involves input (influential parameter) variable selection, i.e. variables with major effects on the process being modelled must be selected. The range of the independent variables is usually determined based on the literature reviewed and preliminary studies, i.e. empirical observations. In the second step, which is the experimental design, the best model that will suit the research undertaken for this study was selected to evaluate process outcomes, which can be TSS, FOG and protein removal efficiency for a BioDAF system in order to determine critical and/or the influence of process/environmental conditions on the outcomes, most which can be determined using wastewater quality analytical analyses. The chosen function is such as that illustrated in the Eq. 3.2 (Bas & Boyaci, 2007).

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \varepsilon \qquad 3.2$$

Where:

k = number of variables, which are pH, flocculants dosage

β_0 = constant term

β_i = coefficients of linear parameters

x_i = is the variables

ε = residuals associated to experiments

Furthermore, coding is important as it allows the selection of the independent variables' range which affect the DAFs' output variables with 1 (high), 0 (average) and -1 (low) values being known, which can culminate in Eq. 3.3 being used (Bezzera *et al.,* 2008), i.e. to determine the coded value. From the literature reviewed, Table 3.1 lists coded parameters that have previously been determined to be influential on DAF systems.

$$X_i = \frac{x_i - x_{cp}}{\Delta x_i} \qquad 3.3$$

Where:

X_i = coded value,

x_i = real value,

Δx_i = value of variable change and

x_{cv} = real value of centered point.

Table 3.1: Coded selected parameters/independent variables using RSM design

Selected parameter/ independent variable	**Coded levels**			**References**
	1	**0**	**-1**	
pH	4	6.5	9	Dlangamandla, 2017
Microbial bioflocculants dosage	n/d	n/d	n/d	n/a

n/d = not previously determined, n/a – not applicable

In the third step, the data obtained from the experiments is then computed into the mathematical model that describes the behaviour of the output variables for the DAF system, with a model-see Eq. 3.4 (Bas & Boyaci, 2007).

$$y_m x_m = X_m x_n b_n x_1 + \varepsilon_x x_1 \tag{3.4}$$

Where:

y = output variable (vector), which can be wastewater quality characteristics such as FOG, TSS, COD, BOD and protein,

m = number of lines from the matrices,

n = number of columns from the matrices,

b = parameter of the model (vector),

x = matrix of the chosen design and

ε = the residual

Thereafter, a method of least squares can be used to solve Eq. 3.3 to attain the lowest residual possible. Additionally, to evaluate the model suitability, an analysis of variance (ANOVA) can also be used. After this, optimal conditions can be determined, to generate a descriptive empirical model such as the one shown in Eq. 3.5 with its differential format being that shown in Eq. 3.6.

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 \tag{3.5}$$

$$\frac{dy}{dx} = b_1 + b_2 + b_{12}(x_1 + x_2) \tag{3.6}$$

The model can then be solved to get the values of the independent parameters which give the highest and the lowest response (Tir & Moula-Mostefa, 2008), with further comparative analyses to assess model suitability being determined by comparing the modelled and experimental values achieved using other statistical correlation, such as a correlation coefficient as shown in Fig. 3.2, to determine the suitability of the model.

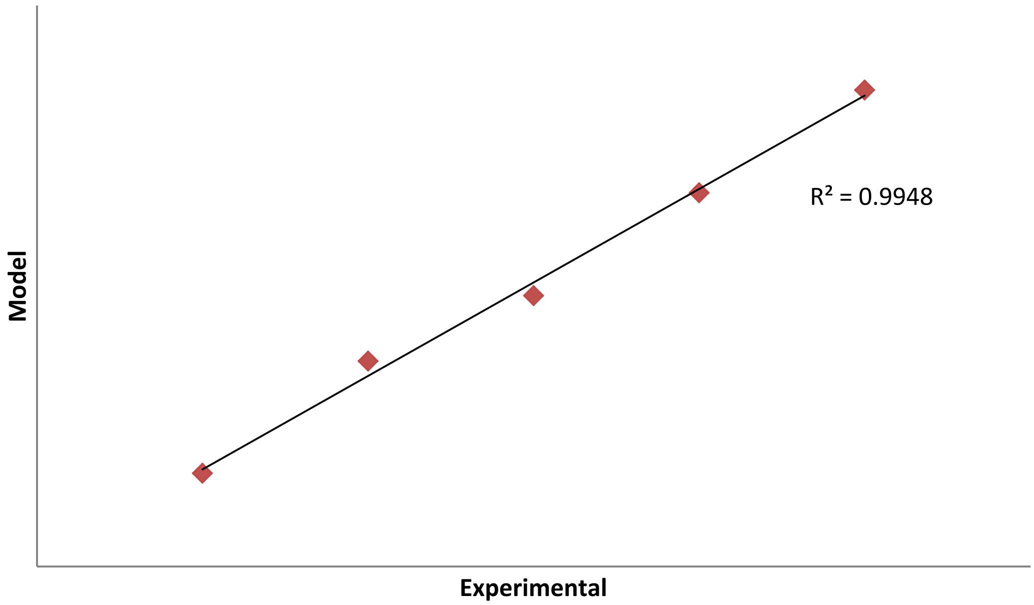

Figure 3.3: An example of the assessment of the mathematical model describing the correlation between the model and actual experimental values (Ghafari *et al.,* 2009).

3.5 Mathematical modelling: A summary

During the industrial revolution, real devices and systems were improved based on plant data and personnel experiences; however, fundamental principles and model development has improved our understanding of process systems without the construction of miniaturised systems (pilot scale). The behaviour of real devices and systems can now be presented in mathematical terms, i.e. mathematical modelling, with various analytical and computational techniques being used for analysis and synthesis until a suitable design is obtained.

For the current research, RSM which is a set of statistical and mathematical techniques that are used for experimental design, modelling, and optimization can be used to model a BioDAF for PSW pre-treatment for high pollutant removal efficiency. Thereafter, a predictive empirical model, i.e. which empirically predicts the BioDAF's performance, can be obtained or can be used to identify the determination of critical points, in particular, from responses generated by influential environmental parameters. Since modelling and optimization of a BioDAF for PSW pre-treatment has never been attempted prior to this research, a methodological attempt has to be made, in order to advocate for a green chemistry approach for the operation of DAFs in large scale systems.

3.5.1 Limitations of current research studies include (but are not limited to):

Minimal and/or limited research on:

- Modelling of Dissolved Air Flotation systems:
  - for use in poultry slaughterhouse wastewater pre-treatment, and
  - In particular, a BioDAF, which uses biological flocculants for pre-treatment of PSW.

Furthermore, previous studies have also indicated the need to:

- Perform bioflocculant kinetics, identify flocculation mechanisms and to generate suitable models for DAF systems so as to improve operational efficiency of such systems.

CHAPTER 4

MATERIALS AND METHODS

CHAPTER 4

4. MATERIALS AND METHODS

4.1 Phase 1 Experiments

4.1.1 Microbial isolation and identification

Numerous microorganisms were isolated from the poultry slaughterhouse wastewater (PSW) which was collected in 20L sterile polypropylene containers every fortnight from a poultry slaughterhouse in Cape Town, Western Cape, South Africa, subsequent to storage at 4℃ prior to use. A volume (1mL) of the PSW was serially diluted (10^{-3}) using sterile distilled water (sdH_2O) with 0.1mL (100µL) of the diluted PSW being used to culture numerous isolates on nutrient agar (31g/L). The petri dishes were incubated at 30℃ for 24hr, with single colonies being sub-cultured on fresh agar until pure cultures were obtained. The pure isolates were individually assessed for flocculation activity prior to the identification of an organism which produces bioflocculants with a high flocculation activity, analysed using a standardized medium including method as reported by Zhang *et al.* (2007). Primarily, and to ascertain the suitability of the organism selected, sustained bioflocculant production was periodically assessed daily, i.e. 24hr intervals, for a production period of 72hr. Furthermore, both rapid production of bioflocculants to reduce the fermentation cycle and high flocculation activity at low dosage, were also considered to be of utmost importance. This strategy, i.e. to isolate a suitable organism from the PSW itself, was implemented to minimize gene flow, thus limit the transfer of modified genes into the local environment.

The isolate which consistently produced bioflocculants with the highest flocculation activity was initially characterized using morphological attributes, i.e. structure; colour, gram staining, under a microscope (Olympus CX21FS1 microscope, Olympus Corporation, Tokyo, Japan) at 100X magnification. Furthermore, 16s rDNA sequence analysis was conducted by an external SANAS accredited laboratory (Inqaba Biotech™) for identification. This procedure was duplicated to ascertain the identity of organism of interest, as mutations can occur culminating in misidentification. For DNA sequencing, universal primers 5’ AGAGTTTGATCMTGGCTCAG 3’ and 5’ CGGTTACCTTGTTACGACTT 3’ for forward and reverse reactions, respectively, were used which culminated in sequence analysis using a CLC Main Workbench v.7. The sequence results obtained were compared to other available sequences in the GenBank from the National Centre for Biotechnology Information (NCBI) database (htt://www.ncbi.nlm.nih.gov).

Further biochemical and confirmatory analysis was performed using a VITEK 2 system v07.01 (BioMérieux Inc., France) designed for automated phenotyping using colorimetric reagent cards (Pincus, 2006), following a procedure described by the manufacturer for fermenting and non-fermenting Gram-negative bacilli cultures.

4.1.2 Media and Inoculum preparation: bioflocculant production

Bioflocculants were produced using a bioflocculant production media (BPM) formulated by Zhang *et al.* (2007) with minor modifications. A loopful of the isolate was inoculated into 250mL Erlenmeyer conical flasks with 50mL BPM which consisted of 0.1g yeast extract, 0.5g peptone, 0.1g glucose, 0.2g $(NH_4)_2SO_4$, 0.02g $MgSO_4.7H_2O$, 0.1g K_2HPO_4, 0.7g $CaCl_2$ and 0.01g NaCl in 100mL sdH_2O. The flasks were incubated (33℃) in a rotary (121rpm) shaker (Labwit ZWYR-240 shaking incubator, Labwit Scientific, Australia) for 24hr, with the overnight cultures (5mL) being used as an inoculum to inoculate 45mL of BPM for the experiments. Experimental trials were analogous to the inoculum preparation procedure, albeit periodic sampling (24hr) was instituted for a bioflocculant production period of 72hr for which collected samples (4mL) were analysed for microbial growth rate and flocculation activity as an indication of bioflocculant production. An adequate volume (stock solution) of the BPM was prepared to minimize variations and to ascertain reproducibility of the results, with inoculum preparation constituted by pooled aliquots from a set (n = 3) of flasks.

4.1.3 Partial determination of bioflocculant activity

Flocculation activity was performed according to a method developed by Kurane *et al.* (1994), with minor adjustments. A volume (50mL) of 4g/L kaolin clay suspension to which $CaCl_2$ (1.5mL, 1% w/v) was supplemented, was aliquoted to a 250mL flasks with a volume (1 mL) of the crude bioflocculant sample being added to the suspension. The mixture was swirled subsequent to aliquoting the mixture into glass measuring cylinders (50mL) followed by a resting period (5 min). The supernatant (top layer) was recovered for absorbance, i.e. optical density (OD) reading (OD_{550nm}), using a spectrophotometer (Jenway 7305 Spectrophotometer, Bibby Scientific Ltd, United Kingdom). A similar procedure was followed for reference (control) experiments in which a bioflocculant free BPM was used. Overall, the quantification of flocculation activity was reported as an average from duplicates and flocculation activity was calculated using Eq. 4.1.

$$\%\ Flocculation\ Activity = \frac{A-B}{A} * 100 \qquad 4.1$$

Where:
A= absorbance of the control, and
B= Absorbance of the sample.

4.1.4 Response surface methodology for optimum bioflocculant production conditions

Design Expert software (Design-Expert Version 6.0.8) was used to generate an experimental design which was followed by the analyses of data obtained. Furthermore, for optimization of bioflocculant production conditions, a Central Composite Design (CCD) was selected. To generate conditions for bioflocculant production, two predetermined parameters which were deemed influential as observed in a previous study (Dlangamandla, 2017) and preliminary experiments, i.e. temperature and pH, were assessed as input parameters (interdependent) with flocculation activity being the output parameter (outcome). A pH and temperature of 4 (min) to 9 (max), and 33 (min) to 39℃ (max) respectively, were selected for the CCD, generating thirteen (n = 13) experimental conditions which were assessed in a rotary (121rpm) shaker (Labwit ZWYR-240 shaking incubator, Labwit® Scientific, Australia) with periodic sampling (4mL) at 2hr interval for the first 10hr, with the last sample being withdrawn after 27hr, for a production period not exceeding 30hr, reduced from the initial 72hr (see section 4.1.1). The samples were analysed for microbial growth rate (OD_{660nm}) and flocculation activity (OD_{550nm}) using a spectrophotometer (Jenway 7305 Spectrophotometer, Bibby Scientific Ltd, United Kingdom). Flocculation activity (Y) as an output parameter was described by second order model, with a minimum residual achievable determined using a least square method, with model suitability analysis being conducted using ANOVA. To ascertain reproducibility of the experimental outcomes by the model, i.e. flocculation activity, a coefficient of correlation was determined.

4.1.5 Optimum conditions: Bioflocculant production, extraction, purification and characterization

Bioflocculants were produced (see section 4.1.2), using 36℃ as the optimum temperature, which was determined from the optimization of biofloculant production using RSM. After incubation for 24hr, the recovered fermentation broth was centrifuged (4000rpm) for 30min to remove biomass. The recovered supernatant was mixed with cold ethanol (4℃) using a 1:2 ratio subsequent to swirling and further centrifugation (4000rpm) for 30min. The precipitate was rinsed and dialyzed using sdH_2O overnight subsequent to vacuum drying in a desiccator (5.8L Duran desiccator DN12491, Duran® group, Germany).

Fourier-transform infrared spectroscopy (Spectrum Two FT-IR™ spectrometer, PerkinElmer Inc., USA) was used to identify functional groups, among which organic, polymeric, inorganic constituents can be identified, in the purified bioflocculant, in a spectral range of 4000-500 cm^{-1}.

4.2 Phase 2 Experiments

4.2.1 Response surface methodology for optimum physicochemical conditions for maximum flocculation activity

As in section 4.1.4, for maximum flocculation activity, two parameters, i.e. pH and bioflocculant dosage, were assessed with zeta potential (mV) being the output parameter (*Y*). For effective floc formation, a degree of electrostatic repulsion between flocs, must be quantified, with an ultimate objective to ascertain whether the electrostatic repulsion force is effectively reduced. A pH of 4 (min) and 9 (max), including a bioflocculant dosage of 1% (v/v, min) and 3% (v/v, max) were selected for the CCD, generating experimental trials (n = 13) analogous to those observed in section 4.1.4, using a second order model to predict experimental outcomes (*Y*) with a similarity index in the form of a correlation coefficient being a suitable statistical adequacy determinant.

4.2.2 Zeta potential measurements

A kaolin suspensions (4g/L) were added to 250mL Erlenmeyer conical flasks in 50mL aliquots whereby the pH of each solution was adjusted using 1M of either NaOH or HCl, depending on the required pH as determined by the CCD. Prior to pH adjustments, a volume (1mL) of a 1% (w/v) $CaCl_2$ was also added to both bioflocculant free and bioflocculant containing suspensions. Subsequent to the addition of all required constituents in an individual mixture, thorough swirling was instituted with a resting period of 8 min after which the top layer of the supernatant was withdrawn for analytical measurements.

A Zetasizer (Zetasizer Nano Z.S, Malvern Instruments Ltd, United Kingdom) was used for zeta potential measurements, for the following suspensions; 1) kaolin suspension, 2) kaolin/$CaCl_2$ suspension and 3) kaolin/$CaCl_2$/bioflocculant suspension; with the bioflocculant supplemented suspensions being at a predetermined concentration and pH. The standard operating procedure had water as the dispersant, kaolin clay as the material and DTS1060 cell was used for the measurements.

Furthermore, a drop (100 µL) of each suspension was rapidly recovered immediately after the addition of components and swirling to fix it onto slides, for visual microscopic observations using an electron microscope (Olympus CX21FS1 microscope, Olympus Corporation, Tokyo, Japan).

4.2.3 Flocculation mechanism (Bonding type) determination

Samples for the determination of bonding mechanism, i.e. flocculation mechanisms, were prepared in a similar manner to that used for flocculation activity as described in section 4.1.3, whereby suspensions containing kaolin clay/$CaCl_2$/bioflocculants were allowed to sediment. The supernatant was removed from the measuring cylinders- with the exception of the reference experiment (control), such that some sedimented flocs, i.e. residue, at the bottom of each measuring cylinder are dried at ambient temperature, with further treatment by the addition of (45mL), of 10mM EDTA-2Na, 0.5M HCL and 5M urea, to each measuring cylinder, with qualitative observations being made (He *et al.,* 2009).

4.3 Phase 3 Experiments

4.3.1 Experimental design: Dissolved air flotation setup

A continuous system was used whereby the PSW was continuously fed into the DAF system with the pre-treated wastewater being continuously recovered. The DAF system was similar to that designed by Dlangamandla (2017). The experimental set-up (see Fig. 4.1 and 4.2) consisted of a tank in which floc formation ensued, a collection tank beneath the primary tank in which the skimmed flocs, i.e. sludge/solid residues overflow, were collected, a storage feed tank from which the supplied raw PSW was pumped into the DAF tank using a Gilson peristaltic pump and a pre-treated wastewater storage tank. The Gilson peristaltic pump was used to maintain steady state conditions, with the in- and out-flow rate being pumped at similar rates. Also an air pump (Resun air pump, AC-9906, Resun®, China) that supplies 16000Pa was used to supply air to the specifically designed air diffusers (see Fig. 4.2) that further ensured pneumatic mixing including sufficient air distribution while generating micro-bubbles. Two out of six ports were used and at the lowest pressure supply so as to maintain low pressure thus low energy consumption. All system components were connected using silicone tubing, with polypropylene being used for storage tanks; while the DAF tank constructed using polyvinyl chloride (PVC) had a diameter of 16cm and a length of 29.5cm.

Figure 4.1: Photographic illustration of the DAF bench scale set up.

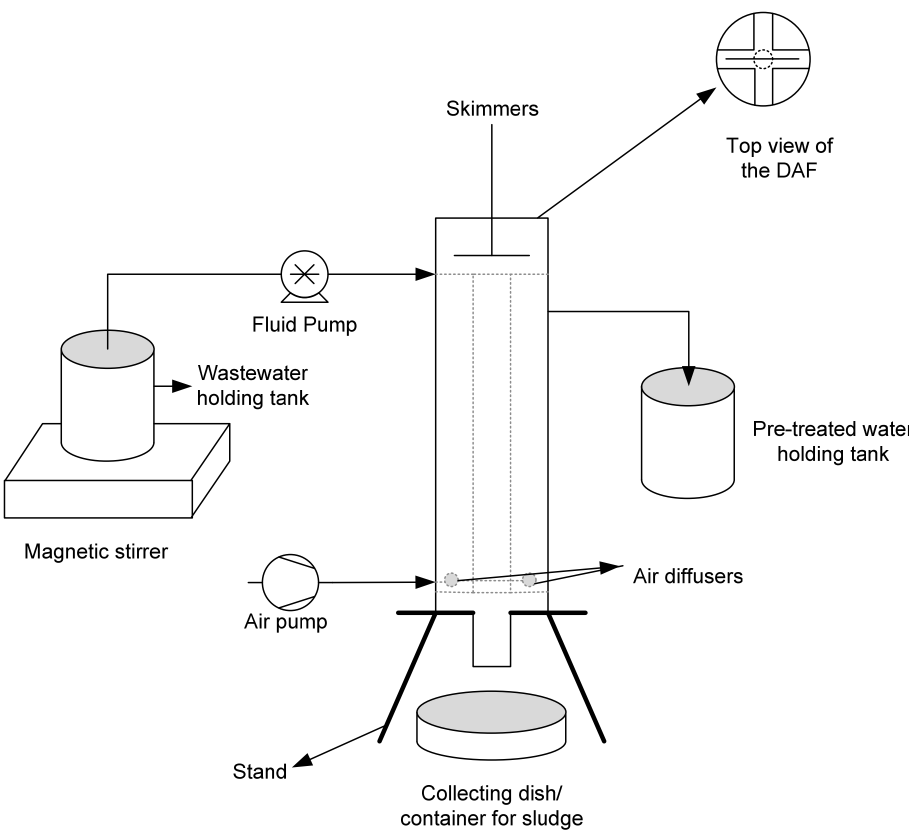

Figure 4.2: Schematic illustration of the DAF bench scale set up.

The air diffusers (Mott element 6500, Mott Corporation, United States of America) with a porous diameter of 1.27, a porous length of 2.32cm and a length of 2.54cm were made of stainless steel which has advantages of being resistant to corrosion, heat and chemical damage; hence, enabling constant air flow, smooth surface which prevent fouling and have added weight thus they do not float but can easily submerge into the solution such that bubble are supplied from the bottom of the tank ensuring sufficient distribution of air bubbles. Notably they were made of Porous Material 316LSS, Media Grade = 40 which is favourable for fine micro bubble generation.

Figure 4.3: Specifically designed air diffusers used in the DAF system.

4.3.2 Sample collection and analysis

Poultry slaughterhouse wastewater (PSW) was collected from a poultry slaughterhouse in Cape Town, Western Cape, South Africa in 20L polypropylene containers and was stored at 4℃ prior to use. The DAF system was operated at a high throughput rate with a HRT of 33hr at an influent and effluent of 1mL/min. The conventional DAF was operated neither with pH adjustment nor bioflocculant supplementation whereas for the BioDAF, the pH was adjusted to 4 and bioflocculants dosage was at 1% (v/v) as determined in phase 2 experiments (section 4.2). Bioflocculants used in the BioDAF were produced as outlined in phase 1 experiments (section 4.1). Furthermore, a toxicity test was conducted using a Microcystins test strip kit for finished drinking water since the bioflocculants used in the BioDAF were produced by an isolate deemed to have been mutated, as such, biotoxin production had to be determined.

During sample collection, aseptic methods were employed at all times to minimize cross contamination which can influence the results, with analysis being conducted rapidly thereafter to also reduce changes, which can occur during sample storage.

All samples were analysed in duplicates as to attain a representative averaged value for each parameter assessed. For the DAF system, sampling was done at initiation of the experiment (t = 0hr) and at 8hr intervals, thereafter. The wastewater was analysed for common water quality parameters such as sCOD, tCOD, TSS, TDS, total/soluble protein, FOG concentrations including turbidity and pH (APHA, 2005).

4.3.3 Analytical methods

PSW samples were withdrawn prior and post pre-treatment with the DAF system with both tCOD and sCOD being analysed using Merck solutions: A (1.14679.0495) and B (1.14680.0495) and also a Merck low range test kit (1.14541.0001), with readings being done on a Merck spectroquant® NOVA 60. Turbidity was quantified using the turbidimeter TN-100 (Wirsam Scientific & Precision Equipment (Pty) Ltd, South Africa) with pH and TDS being analysed using a PCSTester 35 multi parameter instrument (Wirsam Scientific & Precision Equipment (Pty) Ltd, South Africa). TSS was quantified using EPA Method 160.2 (see Appendix C4) with total and soluble protein concentrations being quantified using the BIO-RAD Quick Start™ Bradford protein assay kit (Bio-Rad Laboratories Inc., USA - see Appendix C5). FOG analysis was conducted at an external laboratory in accordance with EPA (2005) standards (City of Cape Town, Scientific Services, and South Africa).

4.3.4 Response surface methodology for removal efficiency quantitation model development

BioDAF system was operated using the physico-chemical conditions determined in phase 2 of this research study. All data generated from numerous analytical methods were used in model development. This was done initially to ascertaining the standard deviation for all parameters evaluated with the lowest standard deviation being observed for sCOD, which was used for model development using Design Expert software (Design-Expert Version 6.0.8). ANOVA was then used to evaluate the model suitability and validity.

CHAPTER 5

RESULTS AND DISCUSSION

CHAPTER 5

5. RESULTS AND DISCUSSION

This chapter is divided into three phases

- **Phase 1 (Aim 1):** To isolate and identify a suitable microorganism from the PSW which rapidly produces bio-flocculants with high flocculation capabilities (flocculation activity), and to identify suitable optimum conditions to produce the bioflocculants;
- **Phase 2 (Aim 2):** To examine the effect of bio-flocculants on pollutant removal from the BioDAF system, focusing on the identification of environmental conditions in which the bioflocculants will function optimally; and
- **Phase 3 (Aim 3):** To develop an empirical mathematical equation/model which describes the BioDAF operation under defined environmental conditions which are used as input parameters in the CCD such that pollutant removal efficiency can be quantified as an output parameter, for the pre-treatment of the PSW.

5.1 Phase 1: Microbial isolation and identification of bioflocculant producing isolate

5.1.1 Introduction

Bioflocculants are extracellular polymeric substances that are produced by different microbial species in different environmental matrices such as water and soil (Xia *et al.,* 2008; Zhang *et al.,* 1999). Recently, bioflocculants have been determined to have advantageous attributes due to their environmental benignity, as compared to chemical flocculants which are non-biodegradable, having been determined to be harmful to both the environment and humans (Liu *et al.,* 2015). Bioflocculants have been applied in numerous industrial applications which include wastewater treatment operations. Generally, wastewater is known to be a depository of numerous pollutants, including organic compounds, with some pathogenic microorganisms proliferating in such wastewater, due to the availability of rapidly metabolisable nutrients (Gupta & Thakur, 2015). Although, bioflocculants produced by microorganisms isolated from PSW including their application in PSW pre-treatment, has rarely been reported. Hence, in this part of the study, a bioflocculant producing microorganism (E1) was isolated from the PSW, with the purpose of assessing its capabilities to rapidly produce bioflocculants with a higher flocculation activity for PSW pre-treatment.

5.1.2 Aims and objectives

The aim of this part (phase 1) of the study was to isolate and identify a microorganism which produces bio-flocculants with high flocculating capabilities (flocculation activity) from the PSW. The objectives were to:

- Isolate and identify a suitable microorganism using appropriate techniques to adequately produce bio-flocculants with high flocculating capabilities for effective pollutant reduction from the PSW,
- Identify optimum environmental, i.e. production, conditions for rapid and maximised bioflocculant production with a high flocculation activity.

5.1.3 Microbial Isolation

Numerous microbial species (n =21) were isolated from the PSW and their flocculation activity using a kaolin clay suspension was assessed. However, a few isolates (n = 3) were deemed to rapidly produce bioflocculants with high flocculating activity, with isolate E1, showing the highest flocculation activity even in limited nutrient conditions, satisfying the selection criteria as elucidated in section 4.1.1; hence, it was solely selected and used for further analyses and experiments.

5.1.4 Microbial characterization and identification

Isolate E1 was identified to be gram-negative, cocci shaped with an appearance of scattered single cells with mucoid cream-white colonies when grown on nutrient agar. Furthermore, the molecular analysis based on the 16S rDNA sequencing, confirmed the isolate to be a mutated *Escherichia coli* (*mE. coli*) assigned accession number LT906474.1. Generally, *E. coli*, which is associated with its proliferation in the gut of warm blooded animals, such as *Gallus gallus domesticus* (domesticated chicken), morphological attributes are distinct, i.e. albeit gram-negative (confirmed in this study), the bacterium is rod-shaped, with the isolate E1 being observed to be a coccoid bacterial species – see Fig. 5.1.

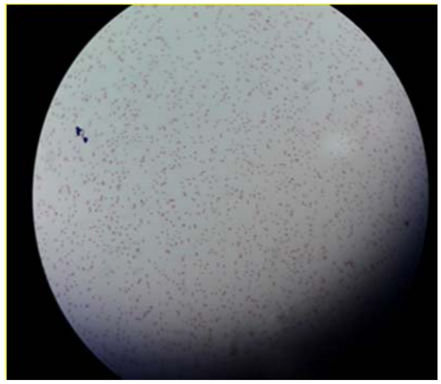

Figure 5.1: Microscopic image showing the characteristics of E1 from a gram stain

Due to the morphological irregularity of the isolate selected, as compared to the generic and thus common morphology of *E. coli*, further confirmatory analysis was required, which resulted in the reanalysis of the 16S rDNA, which confirmed that the isolate was *mE. coli* (accession number CP024862.1). A secondary assessment/analysis, using the VITEK 2 system v07.01 (BioMérieux Inc., France), was also conducted, with the results confirming a 92% probability that the selected organism (E1) was *mE. coli* (see Appendix A2 for biochemical test results). These results suggested that the isolate E1 was a mutant; with mutation having occurred. Mutations are known to be the origins of variations in heritable traits of evolution for organisms, with environmental conditions having a direct influence on the inherited traits of mutated species. As such, the characteristic changes, i.e. environmentally facilitated directed evolution, can lead to changes in physiological responses and the genetic stability of some species (Kram & Finkel, 2014), a primary reason for the observance of the cocci shaped *E. coli*. Some researchers have indicated that *E. coli* mutants can attain a temperature dependent round cell shape (Iwaya *et al.,* 1978), with cell division patterns being parallel rather than perpendicular (Cooper, 1997), an attribute dependent on cellular membrane crystallinity which generally underlies most microbial cellular divisions.

Furthermore, Ferrer-Miralles *et al.* (2009) reviewed how *E. coli* including its mutants has been used as microbial cellular factories, which reflects the acceptability of using *E. coli* in for the production of numerous bio-products, including bioflocculant production for this study. *E. coli* is known to be a facultative anaerobe which is partly due its habitat as it forms part of the natural intestinal microbiota of warm blooded animals including humans. Previously, it has been determined to be essential in the maintenance of the physiology of the environment it proliferates in, i.e. such as that of PSW; hence, it was cautiously used in this study.

Generally, most *E. coli* strains are regarded as harmless although they can be opportunistic pathogens (Conway, 1995); hence, the need for toxicity tests. It was hypothesised that the *mE. coli* was from the intestinal biota of slaughtered birds, culminating in the strain forming a part of the bacterial community in the PSW. In the literature reviewed, there is minimal information on the *E. coli* facilitated bioflocculant production, with most studies reporting on their production from organisms such as *Bacillus* spp., *Klebsiella* sp. (ISO4), *Staphylococcus* sp., *Pseudomonas* sp., and *Salmonella* spp., isolated form wastewater samples (Mathias *et al.,* 2017). In this study, the *mE. coli* (E1) was utilised for the purpose of producing bioflocculants for use in a BioDAF system to aid with floc formation as part of the pre-treatment process for PSW.

5.1.5 The interaction between culture conditions and bioflocculation production

5.1.5.1 Optimisation of bioflocculant production conditions

Extracellular products produced during cell growth can be expressed as bioflocculants. These bioflocculants are capable of influencing solid particles interactions in a wastewater to form flocs (Kasan *et al.,* 2016). To investigate optimum conditions for bioflocculant production with a high flocculation activity, the effect of temperature and pH was analysed by assessing the flocculation activity. A temperature of 33℃ (min) to 39℃ (max) in conjunction with a pH range of 4 (min) to 9 (max) were evaluated. The results (Table 5.1) depicted that an optimum pH of 6.5 and a temperature of 36℃ were favourable for the production of bioflocculants that had a high flocculation activity instantaneously produced during incubation. At acidic pH, the flocculation activity was minimal as compared to alkaline pH. Furthermore, there was an increase in flocculation activity with an increase in incubation period, indicating an increase in bioflocculant production with culture age. This concurred with observations made by Deng *et al.* (2005) who stated that, cumulative polymeric flocculants production increases with culture age.

Table 5.1: Central Composite Design with 13 experimental runs for bioflocculant production and flocculation activity

Run	Factor 1 A:pH	Factor 2 B:Temperature (℃)	Response 1 Y: Flocculation Activity (%)
1	6.5	36	100
2	4	39	61.4
3	6.5	40.2426	100
4	9	33	99.32
5	9	39	76.67
6	6.5	31.7574	100
7	2.96447	36	0
8	6.5	36	100
9	4	33	17.11
10	6.5	36	100
11	6.5	36	100
12	6.5	36	100
13	10.0355	36	73.51

5.1.5.2 Effect of pH and temperature on bioflocculant production

Since environmental and/or bioreactor conditions are known to affect the growth rate of microorganisms, it was prudent to also assess the effect of pH and temperature on bioflocculant production, since, pH occasionally affect bio-product activity including nutrient utilisation (Xia *et al.,* 2008). According to Aljuboori *et al.* (2014) bio-product production by most microorganisms can either increase in-between minimum to optimum pH, then decrease in between optimum to maximum pH. However the bioflocculants produced by the *mE. coli* (E1) showed that the bioflocculants produced had a higher activity from optimum to maximum pH rather than minimum to optimum pH. This can be a reflection of ionic changes which influences nutrient uptake and metabolic reactions, which supports the notion that optimal pH differentiation can occur within a single microbial specie depending on the physiological conditioning traits of the residual bio– and by-products in a culture broth (Luo *et al.,* 2016). Li *et al.* (2009) reported that optimal pH for bioflocculant production by *B. linchenformis* was 6.5 to 9.0 with the highest production being at pH 7 whereas Zheng *et al.* (2008) reported the highest bioflocculant production by *B. megaterium* being at pH 9.

For the *mE. coli* (E1), the highest production was observed at pH 6.5, indicative of the influence of pH on bioflocculant production, which largely depends on a number of environmental (external) factors such as bioreactor operational conditions, i.e. pH including temperature, among others. Temperature affects microbial activity and metabolic processes of microorganisms. Most bioflocculant producing microorganisms have an optimum temperature of 25 to 37℃ (Wu & Ye, 2007). Additionally, optimum pH is critical to support the production of bioflocculants as well as maintain suitable microbial growth rates for bioflocculant production, there have been reports that sub-optimal temperature favours higher production of bioflocculants (Moreira *et al.,* 2000). This concurred with results presented in this study, as the pH was near neutral while the temperature was slightly sub-optimal for *E. coli* growth.

5.1.5.3 Process optimisation by RSM

RSM was used for optimisation of bioflocculant production for high flocculation activity. Table 5.2 enlist the ANOVA of the quadratic model obtained.

Table 5.2: Analysis of Variance (ANOVA) for Response Surface Quadratic model parameters used to estimate the optimum conditions for maximum bioflocculant production with a high flocculation activity

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	13807.58	5	2761.52	177.43	< 0.0001	Significant
A-pH	*5072.12*	*1*	*5072.12*	*325.89*	*< 0.0001*	
B-Temperature	*58.54*	*1*	*58.54*	*3.76*	*0.0936*	
AB	*1120.24*	*1*	*1120.24*	*71.98*	*< 0.0001*	
A^2	*7488.95*	*1*	*7488.95*	*481.17*	*< 0.0001*	
B^2	*9.82*	*1*	*9.82*	*0.63*	*0.4531*	
Residual	108.95	7	15.56	-	-	
Lack of Fit	*108.95*	*3*	*36.32*	-	-	
Pure Error	*0.000*	*4*	*0.000*	-	-	
Cor Total	13916.53	12				

R^2 = 0.9922   Adjusted R^2 = 0.9866   Predicted R^2 = 0.9443   C.V. % = 4.99

Adequacy of the model describing bioflocculant production was determined using ANOVA. ANOVA showed that a 2nd order model described the results better than those of other orders.

Since a coefficient of correlation (R^2) is indicative of variations in the response as predicted by the model (Gupta & Thakur 2016), confirmatory analysis between the model and experimental data is required. The correlation coefficient (R^2 = 0.9922) revealed that only minute, i.e. 0.0088%, variations cannot be explained by the model (Ahamad *et al.,* 2005). The predicted R^2 of 0.9443 was in agreement with the adjusted R^2 of 0.9866, with differentiation being < 0.2; implied that the comparison between the empirical model and the actual data culminated in a suitable fit (Elkisibi *et al.,* 2014). Moreover, the model was significant as some of the F> prob values were > 0.05, while a coefficient of variance (CV), which is the ratio between standard error estimate and response mean value, was used to determine the reproducibility of the model (Gupta & Thakur 2016), with the CV (4.99%) being <10; hence, indicating that the model can be reproduced.

An empirical correlation between flocculation activity and other factors (pH and temperature) was obtained as given in Eq. 5.1.

$$Y = -912.94759 + 158.64645A + 24.97016B - 2.23133AB - 5.24974A^2 - 0.13201B^2 \qquad 5.1$$

When factor coefficients are in a coded equation/model they reveal the effect of individual factors and their interaction on the response (independent factor). Furthermore, a negative coefficient value indicates that the individual or interaction factor affects the response in that test range negatively while the opposite is also true (Gupta & Thakur 2016).

The three dimensional surface plot (Fig. 5.2) is an illustration of the interactive effect of temperature and pH on the production of the desired bioflocculants as determined by flocculation activity as a response. This illustration shows a deep forward skewness for the response, depicting the suitability of near neutral pH values for a better response (flocculation activity). It also shows that the optimum conditions of bioflocculant production was at pH 6.5 and temperature 36℃, thus these conditions were used in further experiments.

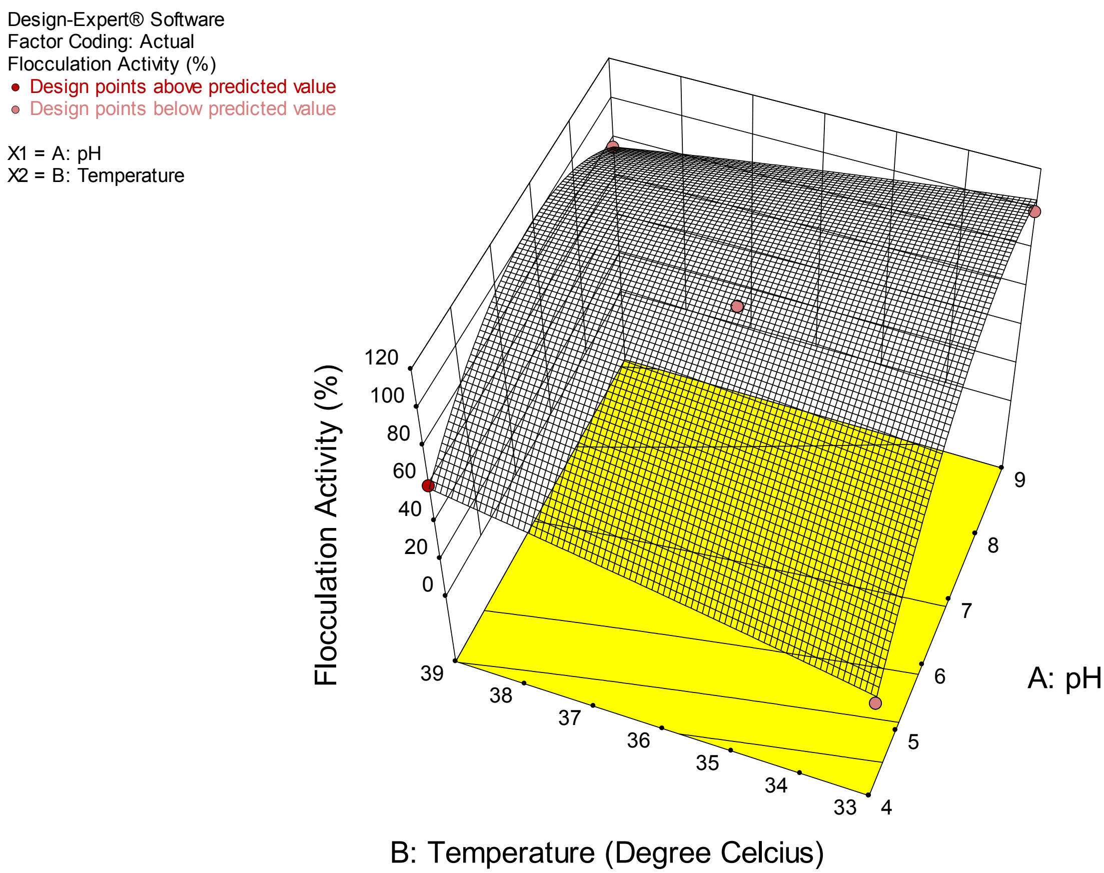

Figure 5.2: 3-D surface plot showing the interaction of temperature and pH on flocculation activity

5.1.6 Characterisation of bioflocculant produced by *mE. coli* (E1)

FTIR was used to determine the functional groups prevalent in the bioflocculant produced by the *mE. coli* (E1) used in this study. The spectrum (Fig. 5.3) displayed a peak at 3309.15 cm^{-1}, which is an indication of hydroxyl groups that results from the vibration of O-H and N-H bonds present in carbohydrate rings of polysaccharides. A weak bend depicting presence of alkynes was also observed at 2132.51 cm^{-1}. Another spectral peak was present at 1636 cm^{-1} which indicated the presence of alkenes and/or amines. The peak observed at 1174cm^{-1} is indicative of the presence of amines/carboxylic acids. All these results suggest that the bioflocculant contains both polysaccharides and some short chained proteins (Yin *et al.,* 2014). The functional groups present in this bioflocculant are known to be preferred for flocculation purposes due to their hydrophilicity which aid in the extension of polymer chain and also for floc formation of suspended particles (Wang *et al.,* 2011; Tang *et al.,* 2014).

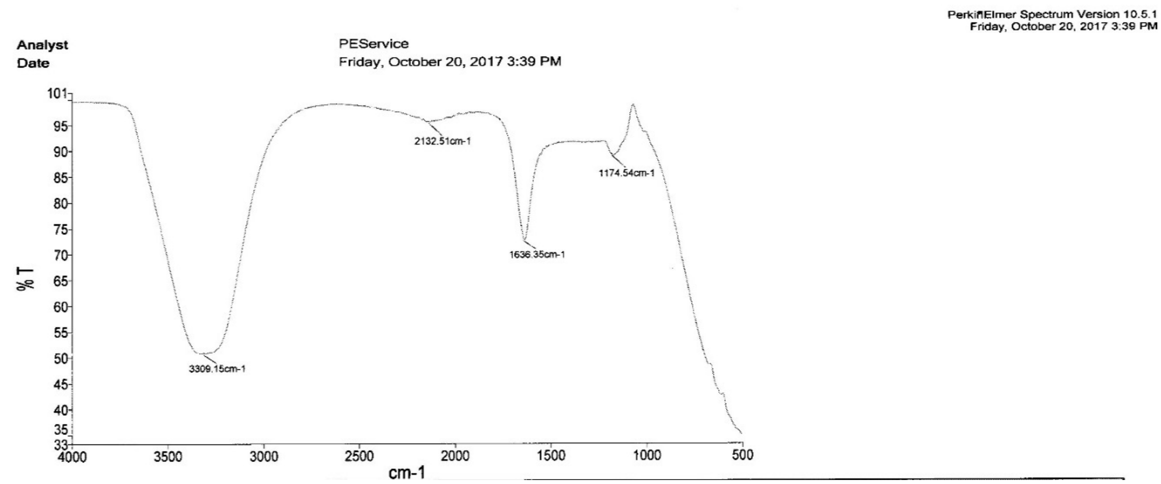

Figure 5.3: IR spectrum of bioflocculant produced by the *mE. coli* (E1) in this study

5.1.7 Summary

In this part of the study, bioflocculant producing microorganisms were isolated from PSW and the isolate *mE. coli* (E1) producing bioflocculants with the highest flocculation activity was used for further studies. Microbial identification using 16s rDNA and VITEK 2 system v07.01 revealed that the microorganism was mutated *E. coli* strain. RSM was then used to determine the optimal operating conditions for the production of bioflocculants, indicating that the optimum pH of 6.5 and a temperature of 36℃ were favourable conditions for the instantaneous production of bioflocculants with highest flocculation activity.

One of the requirements for any bioprocess design and development is to assess the functionality and mechanisms of the bio-products, i.e. the bioflocculants produced; on pollutant removal was deemed necessary, and thus the next phase of the research studies.

5.2 Phase 2: Bioflocculant effects on pollutant removal

5.2.1 Introduction

The primary flocculating mechanism of bioflocculants has been proposed to be bridging and/or charge neutralization (Li *et al.,* 2009). However, it is believed that most bioflocculants are negatively charged thus charge neutralization rarely occurs; hence, floc formation mechanism attributed to microbial flocculants is less understood and needs to be investigated unlike that imparted by synthetic chemical flocculants for which flocculation mechanisms are well understood (He *et al.,* 2009).

Currently, a low flocculation capability of bioflocculants has been one of the hindrances in the practical application of bioflocculant including redundancies in suitable bioprocess design development as a way of overcoming these challenges. Previously, although bioflocculant producing organisms have been isolated from various environments with some studies reporting on flocculation mechanism observed (He *et al.,* 2009); this has not been reported for mutated *E. coli* strains such as the *mE. coli* (E1). Hence, in this part of the study, bioflocculant effects from *mE. coli* (E1) on pollutant removal including floc formation mechanism were studied so as to further use usability in a BioDAF system to pre-treat the PSW.

5.2.2 Aims and Objectives

The aim of this part of the study was to examine the effect of bio-flocculants on pollutant removal, from a BioDAF system. The objectives were to:

- Assess the effect of bio-flocculants produced by the *mE. coli* (E1) on TSS removal from the PSW using a DAF system,
- Quantify the zeta potential reduction (wastewater charge reduction), thus assessing bio-flocculants dosage and pH effects on floc formation for TSS removal, and
- Identify optimum physicochemical conditions for maximum flocculation using RSM

5.2.3 Effect of bioflocculants on Total Suspended Solids (floc) removal

Bioflocculant producing organisms are capable of producing bioflocculants that induce flocculation of solid particles in wastewater. Bioflocculants do not only aid aggregation of particles but they also influence other physicochemical properties of the wastewater; hence, promoting contaminants/particle removal (Liu *et al.,* 2004). In the current study, the effect of bioflocculants on TSS, i.e. floc formation, was evaluated by analysing the zeta potential imparted by the bioflocculants, thus determining a flocculation mechanism- at different pH and bioflocculant dosage using a kaolin suspension as indicated by using the CCD in RSM to generate experimental conditions. Furthermore, flocs were fixed onto slides and viewed under an electron microscope, to confirm floc aggregation. This further confirmed the physicochemical conditions identified for maximum flocculation activity.

5.2.3.1 Zeta potential analysis

In order to determine if charge neutralisation was the primary flocculation mechanism responsible for floc formation by bioflocculants produced by *mE. coli* (E1), zeta potential measurements at different pH and bioflocculant dosage were determined. The results (see

Appendix B1) showed that at pH 2.96 the kaolin suspension had a zeta potential of -36 mV whereas at pH 9 it had a zeta potential of -50.1 mV. This depicted that the zeta potential of the kaolin suspension had increased with an increase in pH; hence, some researchers have indicated that whenever an alkali is supplemented to a suspension, mobilized particles acquire a higher negative charge (Li *et al.,* 2009); however, a well-defined environment as defined by the CCD is required to effectively ascertain charge density changes, i.e. the wastewaters' pH, ionic strength, dosage of the bioflocculants must be known, as reported herein. The observed negative charge can results in the formation of an electrical double layer that causes the particles to remain suspended in the solution, with repulsion being sustained (He *et al.,* 2009). Subsequent to the addition of $CaCl_2$, the zeta potential was reduced to -11.3 mV at pH 2.94 and -16.3 mV at pH 9. The supplementation of bioflocculants using varying dosages while maintaining a known concentration of the kaolin in suspension, albeit at different pH, resulted in minimal changes in the zeta potential observed, which remained negative. For example, at pH 2.96 after the addition of bioflocculants, the zeta potential slightly increased to -17.3 mV, which suggested that the bioflocculant might be having a negative zeta potential.

By increasing the bioflocculant dosage within the same pH range resulted in slight increases of zeta potential. This might be due to the increase of static repulsive forces between the kaolin particles. Liu *et al.* (2015) investigated the flocculation mechanism of cation independent bioflocculants in a study whereby a charge neutralization assay was utilised; reporting that the zeta potential of the kaolin suspension decreased slightly subsequent to bioflocculant supplementation, indicative of charge non-neutralization. In this study, it was evident that charge neutralization was not the primary flocculation mechanism for floc formation. This required that a bonding type test be conducted. Furthermore, the conditions for maximum flocculation activity could not be clearly optimized using RSM as the ANOVA indicated model unsuitability.

Table 5.3: Analysis of Variance (ANOVA) for Response Surface Quadratic model parameters used to estimate the optimum conditions for maximum flocculation activity

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	20.54	5	4.11	10.87	0.0034	significant
A-pH	*5.08*	*1*	*5.08*	*13.43*	*0.0080*	
B-Flocculant Concentration	*1.33*	*1*	*1.33*	*3.53*	*0.1024*	
AB	*2.500E-003*	*1*	*2.500E-003*	*6.615E-003*	*0.9375*	
A^2	*10.65*	*1*	*10.65*	*28.19*	*0.0011*	
B^2	*5.17*	*1*	*5.17*	*13.69*	*0.0076*	
Residual	2.65	7	0.38			
Lack of Fit	*2.65*	*3*	*0.88*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	23.19	12				

R^2 = 0.8859 Adj R^2= 0.8044 Pred R^2 = 0.1888

The ANOVA (refer to Table 5.3) for the response surface quadratic model showed that the predicted coefficient of correlation (R^2) was 0.1888, which was minuscule, thus depicting that the model was inadequate with the difference between the predicted R^2 and adjusted R^2 being >0.2, which further meant that the empirical model and the actual data were not a fit. However, the Eq. 5.2 was obtained, for which the response (*Y*) was the zeta potential.

$$Y = -5.56692 - 2.91264 * A - 3.92321 * B + 0.010000 * AB + 0.19800 * A^2 + 0.86250 * B^2 \quad 5.2$$

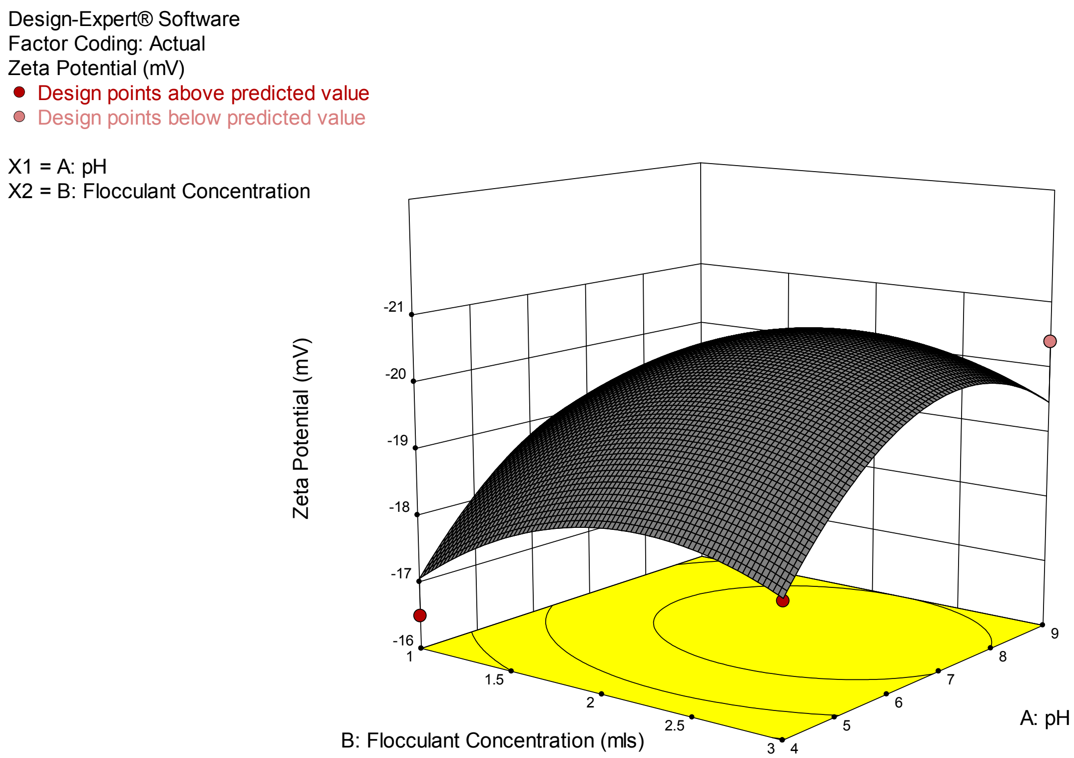

Figure 5.4: 3-D surface plot showing the interaction pH and bioflocculant dosage on zeta potential

The interactive effect of the independent variables (pH and bioflocculant dosage) on flocculation activity was analysed using a 3-D illustration (Fig. 5.4) using the obtained model. The plot showed a rudimentary interrelationship between pH and bioflocculant dosage, though slightly reflecting the importance of a lower pH and a lower bioflocculant dosage, so as to obtain lower zeta potential values which are favourable for charge neutralisation.

Moreover, as zeta potential results were inconclusive in terms of highlighting suitable conditions for maximum flocculation activity, microscopic imaging (see Fig. 5.5) was considered. It was clear that a pH of 4 with a bioflocculation dosage of 1mL (1% v/v) was suitable for floc aggregation when compared to other conditions; hence, these conditions were selected as suitable conditions for maximum flocculation activity which can be used in phase 3 experiments for this research. This concurred with results reported by Yim *et al.* (2007) who indicated that a bioflocculant named as p-KG03, with maximum flocculation activity at pH 4; indicating that bioflocculant concentration influences flocculation performance; hence, it is an important factor when elucidating flocculation activity for novel bioflocculants (Zheng *et al.*, 2008).

At high dosage concentration, floc formation was hypothesized to have been affected because of a blockage of binding sites on the kaolin particles constituting the clay thus depriving stronger bridging mechanisms between diverse particles and the bioflocculant supplemented to the suspension (He *et al.,* 2009). From the images, floc formation under different conditions at the same pH and different bioflocculant dosage indicated that it was advisable to dose at a low concentration as a comparison was made between bioflocculants free and supplemented suspensions. This supported the notion that bioflocculants produced by the *mE. coli*, would have a positive effect on floc aggregation thus pollutants removal potential, albeit at low pH.

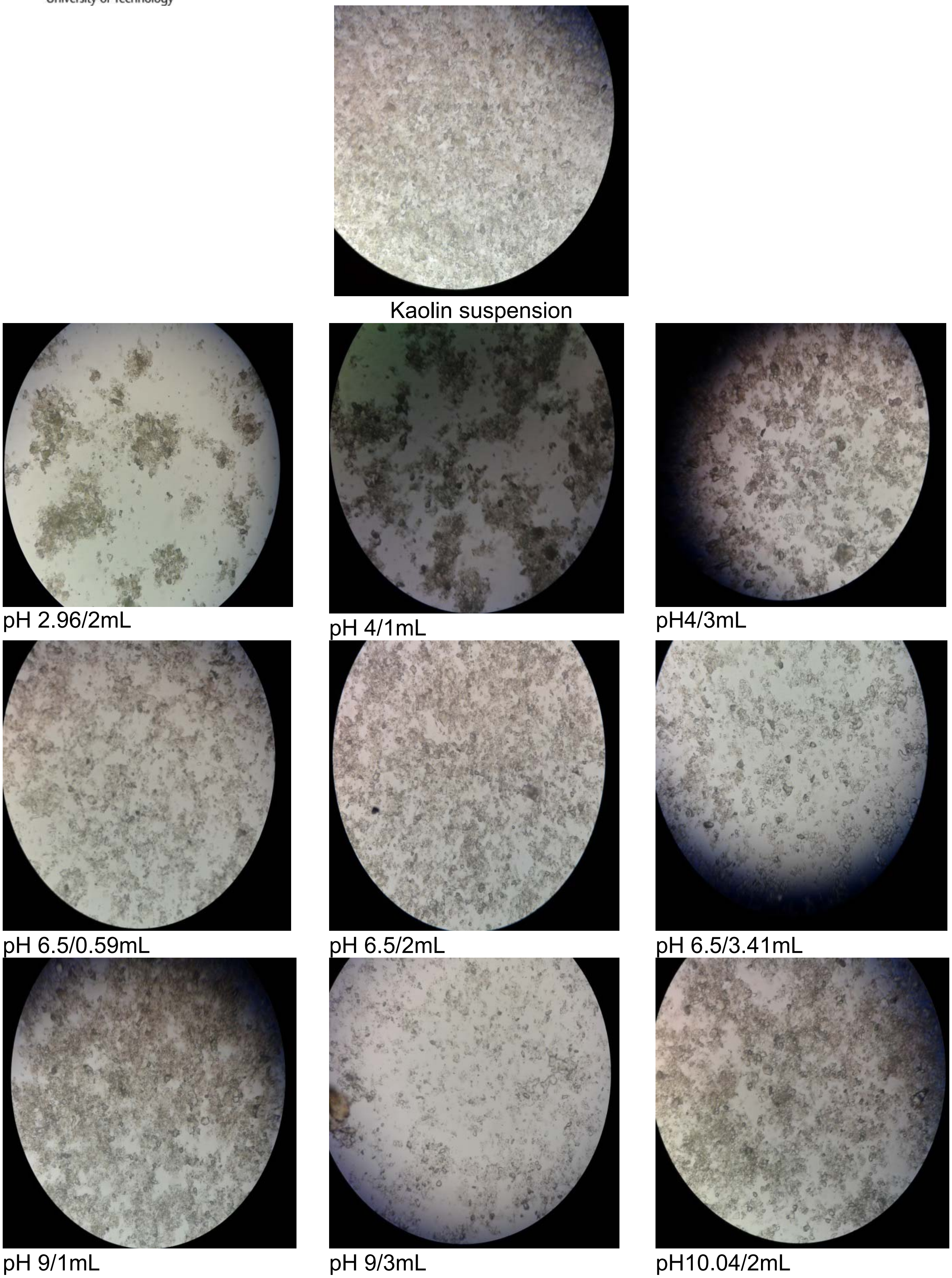

Figure 5.5: Photographic illustration of the kaolin suspension, illustrating floc formation at different pH and bioflocculant dosage.

5.2.3.2 Bonding type assay

In a bonding type assay was conducted using three (n =3) different solutions which constituted individualised chemical treatments, using urea, HCl and EDTA-2Na. The solutions of HCl and EDTA-2Na are known to disrupt ionic bonds whereas urea abolishes hydrogen bonds (He *et al.,* 2010; Hu *et al.,* 2009). After the addition of EDTA-2Na into the flocs, the formation was disintegrated thus suggesting that the EDTA-2Na solution might have interacted with Ca^{+} ions or functional groups within the bioflocculants whereas flocs that were treated with a solution of HCl were not disrupted, thus agreeing with the fact that the bonding type was not completely ionic in nature. Additionally, for urea treated flocs, the solution became cloudy thus suggesting that there were hydrogen bonds which have been disrupted. These results suggested that the responsible mechanism for flocculation for the bioflocculant produced by *mE. coli* was bridging. However, for a bridging mechanism, dependency on functional groups and chemical constituents in the flocculants, is influential (Tang *et al.,* 2014). Therefore, the hydrogen bonds might have been between the bioflocculants and kaolin particles which were destroyed by the urea culminating in the formation of new bonds which might have formed between the carbonyl group in the urea and clay particles; thereby, causing the kaolin suspension to become cloudy (Guo *et al.,* 2014). This was also supported by the IR spectrum (refer to phase 1 results) which shown the presence of hydroxyl groups within the bioflocculant structure which in turn favours the possibility of hydrogen bonding.

It is known that for flocculation activity to take place, the electrostatic repulsion forces must be minimal when compared to attractive forces between particles; hence; the use of Ca^{+} which acts as a neutralizer. The functional groups such as OH^{-} and $COOH^{-}$ in the bioflocculant and the combination between H^{+} and OH^{-} on kaolin particles will then form hydrogen bonds. Hence, a bridging mechanism takes place (Gao *et al.,* 2006) when these particles adsorb onto the bioflocculant functional groups. Therefore, these results ultimately brought about the conclusion that the mechanism imparted by the bioflocculants produced by the *mE. coli* is initially by charge neutralization, although observed to have minimal influence, in which Ca^{+} ions neutralize the electrical charge of kaolin clay particles, which was then followed by bridging, in which the functional groups that make up the bioflocculant (absorbance or adherence) aid in hydrogen bonding of bioflocculants to kaolin particles thus resulting in floc aggregation or flocculation.

5.2.4 Summary

This study focused on determining the effect of bioflocculants on pollutant removal by using RSM to determine optimal physico-chemical conditions (pH and bioflocculant dosage) for maximum flocculation activity through zeta potential analysis as an output variable, thus to determine the flocculation mechanism. Zeta potential results depicted that the addition of bioflocculants at different dosages to kaolin suspensions with different pH resulted in minimal changes; hence, proving that charge neutralization was not the primary mechanism thus flocculation mechanism was determined using chemical treatment of formed flocs. The results suggested that the responsible mechanism of flocculation was bridging. Therefore, the mechanism used by the bioflocculants produced by *mE.coli* was first by minimal charge neutralization followed by bridging, in which the functional groups that makes up the bioflocculant (adsorb or adhere) aid hydrogen bonding of bioflocculants and kaolin clay particles thus resulting in floc growth or flocculation.

From microscopic (qualitative) analysis of the flocs at the different pH and bioflocculant dosage, indicated that a pH 4 at a bioflocculant dosage of 1% v/v promulgated the formation of bigger, dense flocs than the rest of the experiments; hence, these conditions were selected as the conditions for maximum flocculation activity and these conditions were used in phase 3 of this research. Overall this supported the fact that bioflocculants produced can culminate in pollutant removal from the PSW.

5.3 Phase 3: Development of a mathematical equation/model for the BioDAF using environmental conditions as input parameters and removal efficiency as output parameter, subsequent to optimizing the BioDAF for the pre-treatment of PSW using RSM

5.3.1 Introduction

Poultry slaughterhouses generate high strength wastewater which is laden with organic and inorganic pollutants from the slaughtering process and cleaning of equipment including production facilities. In order to reduce the effect of pollutants present in this wastewater on the environment and humans, legislative restrictions on effluent discharge have been imposed; hence, the need for PSW pre-treatment prior to discharge into fresh water sources (Del Nery *et al.,* 2007). Additionally, amongst the treatment methods currently in use, flotation remains the most popular method, with DAF systems being the most applied. However, the modelling and optimisation of a biological DAF system has never been attempted before, in particular for PSW pre-treatment under optimum conditions. Process optimisation involves process adjustment so as to optimize influential parameters. Response optimisation which is normally affected by inputted independent variables can be achieved through proper experimental design (Aslan & Cebeci, 2007). For this to be achieved, RSM has been widely applied in the optimisation of flocculant production, with numerous studies such as that of Sun *et al.* (2015) reporting on the use of RSM to optimise influential flocculant parameters, with kaolin clay suspensions being used as a representative for TSS contamination, while simultaneously reducing/removing toxins such as microcystis from microbial contaminants. In this part of the study, CCD in RSM was used to develop experimental runs and an empirical model which describes a BioDAF operation for process control purposes.

5.3.2 Aims and Objectives

The aim of this part of the study was to develop a mathematical equation/model for the BioDAF using environmental conditions as input parameters for pollutant (tCOD, FOG, TSS and proteins) removal efficiency, which can be quantified as output parameters, subsequent to optimising the BioDAF for the pre-treatment of PSW using RSM. The objectives were to:

- Generate empirical (mathematical) models using RSM, which incorporates environmental factors such as pH and bio-flocculants concentration to simulate pollutant removal efficiency focusing on tCOD, TSS, FOG and proteins in a BioDAF system,
- Assess the suitability of the model developed using statistical analysis (ANOVA), i.e. in order to determine the suitability of the model in describing the performance of the BioDAF,
- Compare the models generated and asses as to whether they are suitable to describe the performance of a conventional DAF (without bio-flocculants), and
- Determine whether the pre-treated PSW comply with industrial wastewater discharge limits as described by the City of Cape Town industrial discharge standards.

5.3.3 DAF system operation

The PSW used in this study was first filtered using a metallic sieve 9.51 mm aperture size so as to rid the wastewater of all feathers and course solids as required in a large scale system. One of the requirements was such that the DAF systems were operated at a high throughput rate with an HRT of 33hr being used. The conventional DAF was operated without flocculants and the pH was also not adjusted, i.e. at a pH of 7.48, which was the pH of the feed whereas for the BioDAF, bioflocculants were added with the pH being adjusted to 4. These were the conditions determined by RSM optimization in phase 2 of the experiments. It was noted that the significant changes occurred after 8hr of BioDAF operation, which henceforth was the focus of the study.

5.3.3.1 Pollutant removal

The removal of pollutants was evaluated by quantifying wastewater parameter such as TSS and COD using analytical methods.

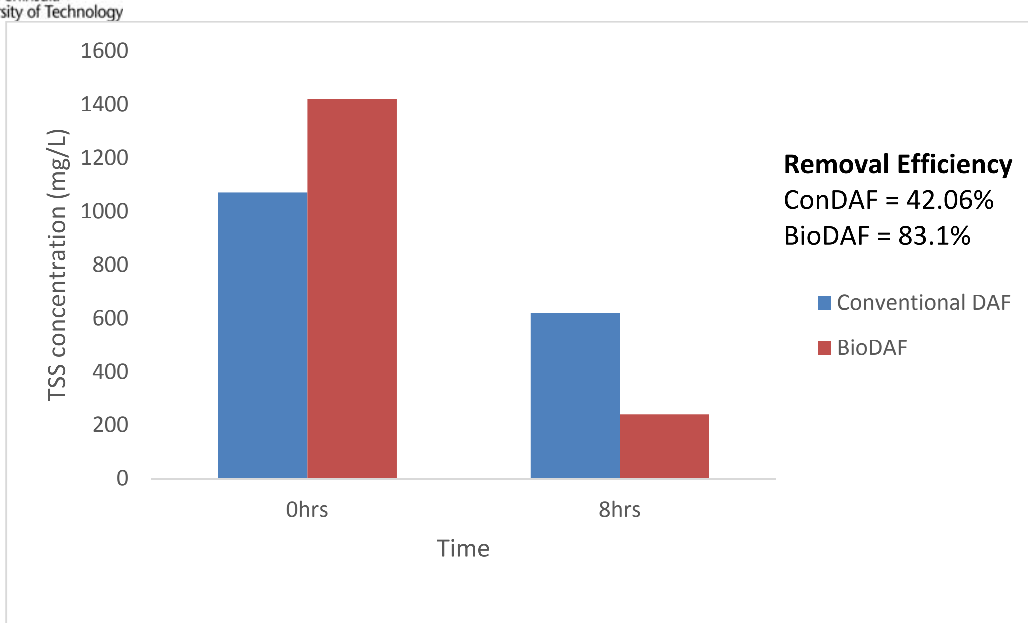

Figure 5.6: Graphical representation of TSS concentration reduction (ConDAF vs BioDAF)

Preliminary screening removed large solids with a 1 to 3cm diameter (Mittal 2006). Fig. 5.6 shows the initial concentration of TSS 8hr for both the ConDAF and BioDAF. The initial TSS for the conventional DAF was 1070mg/L and after 8hr the effluent had TSS of 620mg/L, a removal efficiency of 42.06% whereas the BioDAF had an initial TSS concentration of 1420mg/L which was reduced to 240mg/L culminated in 83.1% removal efficiency after 8hr, a ~100% increase when compared to the ConDAF. Considering that the PSW was from the same source minute differences in the initial concentration would have been expected albeit a difference of 350mg/L, constituted a significant difference. Such a difference was attributed to the addition of bioflocculants and a low pH used to acidify the feed of the BioDAF hypothesized to have influenced colloid aggregation thus an increase in the size of the aggregated particles which in turn increased the concentration of suspended solids in the supernatant sampled.

Furthermore, the higher TSS removal efficiency observed for the BioDAF was deemed to be as a result of bioflocculants supplementation which led to the aggregation of colloids and enlargement of flocs thereby promoting attachment to bubbles which were subsequently removed by scrapping. The use of flocculants has been reported to aid in the removal of suspended solids (de Nard et al., 2008). De Nardi *et al.* (2008), demonstrated that, after enhancing the functionality of DAF system with the aid of 24mg/L PAC, i.e. a chemical flocculant, removal efficiency of 74% for TSS was achieved which was lower than the 83% obtained in this current study whereby bioflocculants were applied.

This indicated that the use of bioflocculants has a potential as they performed better than some of the currently used synthetic chemicals with an added advantage of being environmental benign.

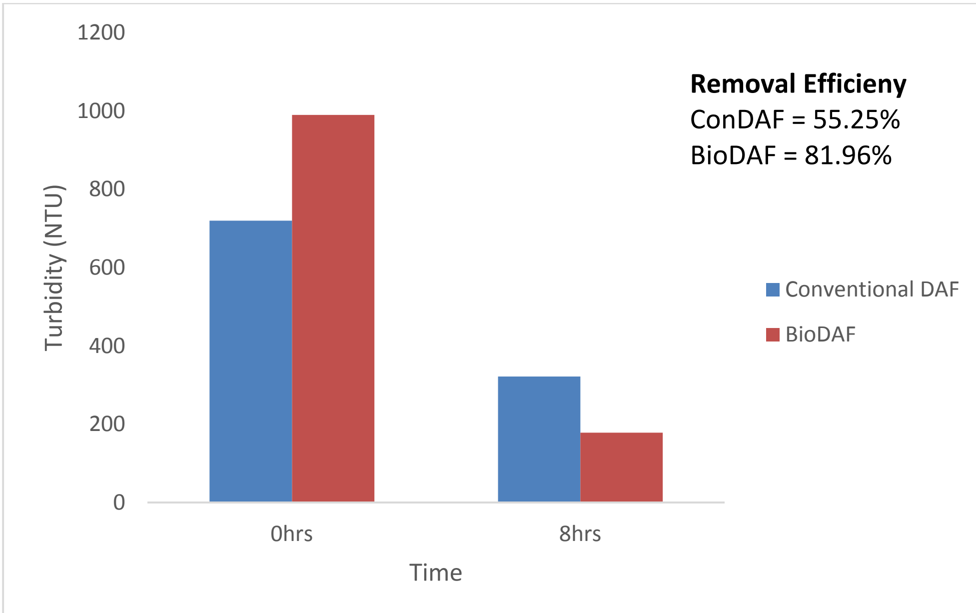

Figure 5.7: Graphical illustration of turbidity reduction (ConDAF vs BioDAF)

Fig. 5.7 illustrates the initial turbidity of 719.5 and 989.5 NTU which was reduced to 322 and 178.5 NTU for a ConDAF and BioDAF, respectively. Similar to TSS results, the difference in the initial turbidity was attributed to bioflocculant addition into the BioDAF which resulted in the precipitation of proteins, oil emulsification and aggregation of particulate matter thus slightly turbid wastewater. The turbidity reduction of the pre-treated wastewater for the BioDAF was higher than that of a ConDAF. This was a result of higher removal of suspended solids, and semi-emulsified oils in a BioDAF culminating in a clarified effluent as compared to the ConDAF.

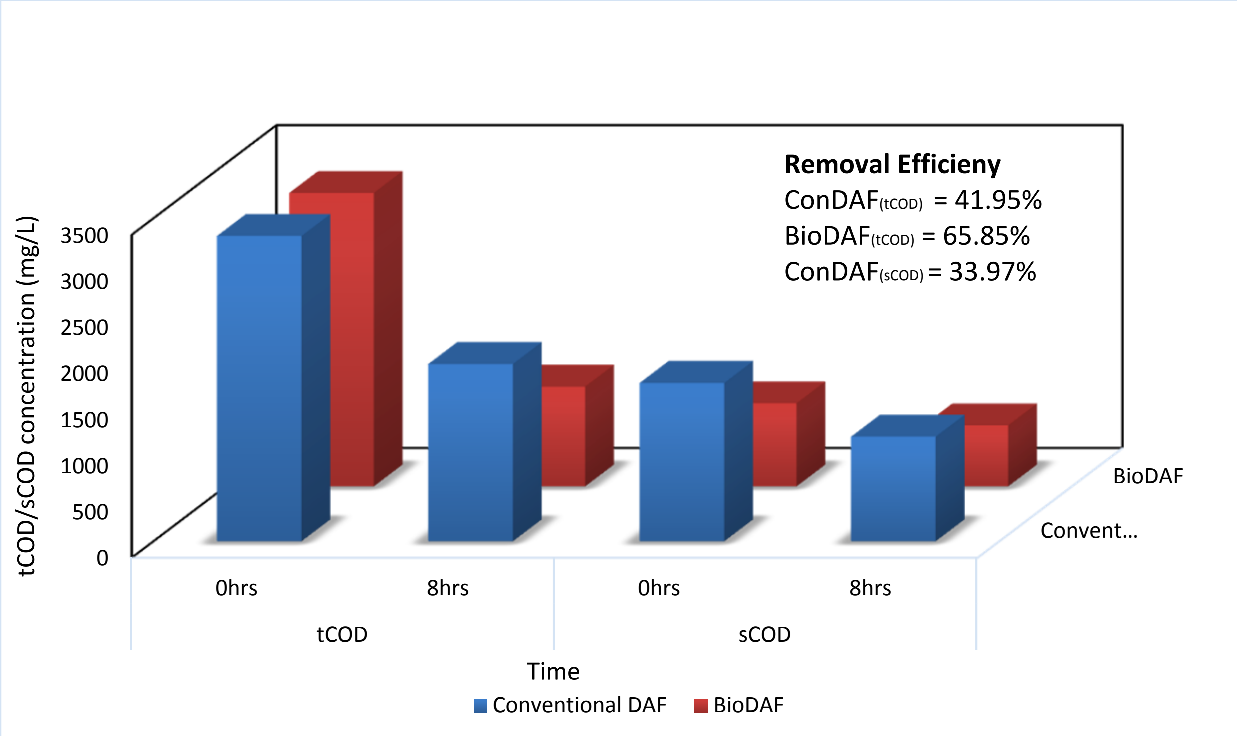

Figure 5.8: Graphical profile of tCOD and sCOD reduction (ConDAF vs BioDAF))

Similarly to the turbidity results, Fig. 5.8 illustrates both initial tCOD and sCOD reduction for the ConDAF (3307.5mg/L) and BioDAF (3180mg/L), which were reduced to 1920 and 1081 mg/L, respectively, which was indicative of the instantaneous and efficacy of the bioflocculants produced. Similarly, for sCOD which was initially at 1715 and 900mg/L, was reduced to 1132.5 and 661 mg/L for the ConDAF and BioDAF, respectively. The lower initial sCOD in a BioDAF was attributed to the flocculation of some of soluble substances, including soluble solids which were indicative of the bioflocculants adsorbance to the solids on the solid-liquid interfaces, but also the reduction in the net charge on liquid-liquid interfaces (Zouboulis & Avranas, 2000).

FOG, represented by the analysis of Fats, Oil and Grease, including total protein which was either quantified as soluble or total protein, can contribute to high tCOD concentrations; hence, as the protein and FOG concentration decreased so the tCOD concentration. The BioDAF had a lowly protein removal efficiency which was unexpected when compared to other removal efficiencies for TSS and FOG. This might have been because of the low pH (4). At lowly pH, hydronium (H^+) increase thereby sharing/adhering to some functional groups apportioned by the bioflocculants such as the carbonyl and carboxylic functional groups thus resulting in the decline of the tCOD removal rate (Guo *et al.,* 2013).

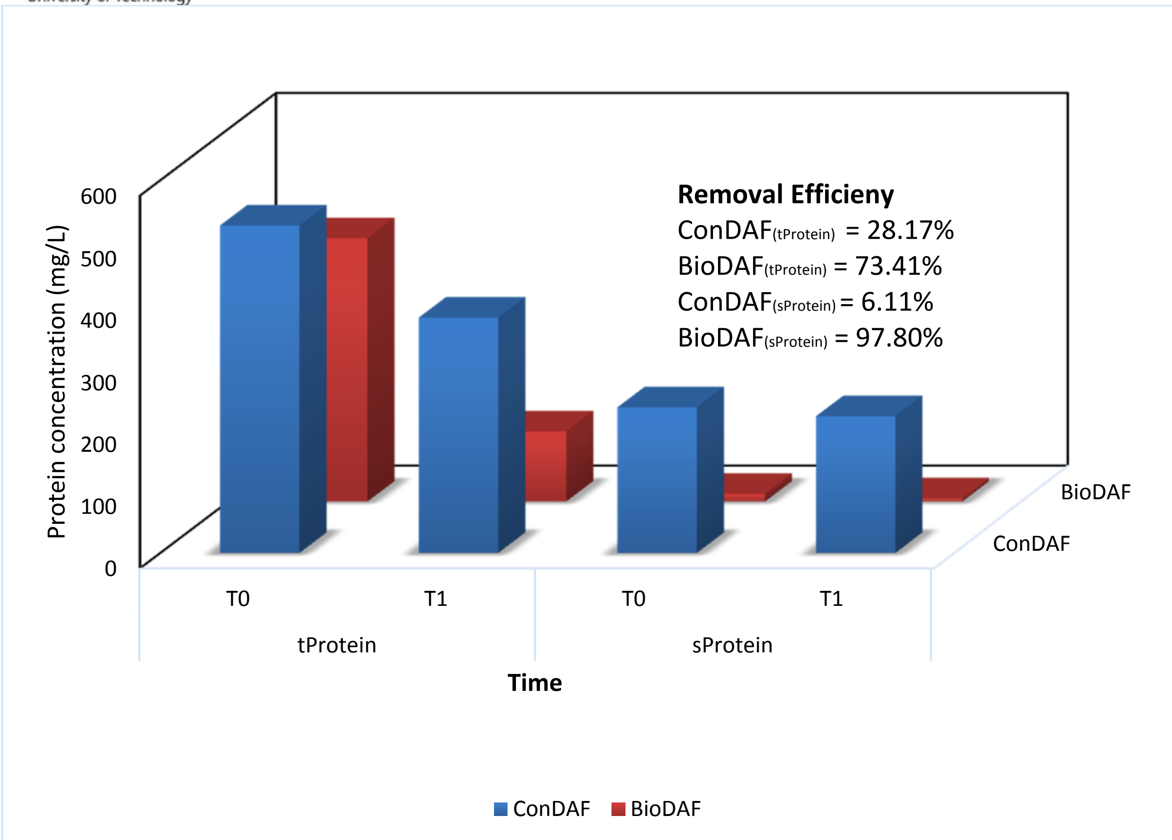

Figure 5.9: A representation of protein concentration reduction (ConDAF vs BioDAF)

Fig. 5.9 showed that changes in proteinaceous matter during the evaluation period, with total protein concentration 527.13 and 423.82 mg/L being reduced to 378.66 and 112.7 mg/L, respectively for ConDAF and BioDAF, after 8hrs of DAF operation. The initial soluble protein in the BioDAF system was 12.45mg/L, which was indicative soluble protein precipitation by the bioflocculants used; hence, the low concentration observed.

5.3.3.2 Fats, Oil and Grease (FOG) reduction

Since the PSW was laden with FOG (Table 5.4), it was impractical to apply other treatment methods such as sedimentation as fat globules do not possess settling properties as their density is similar to that of the wastewater; hence, the application of a DAF system advocated for in this study, which is effective in removing FOG. This separation method is also aided by the attachment of macro-air-bubbles to floatable matter, which effectively improves the buoyance of agglomerated particles or fats and grease in FOG. It has been hypothesised that FOG removal efficiency was increased by agglomerated globules while minimizing emulsification and maintaining macro-bubble sparging in the BioDAF, attributes hypothesised to be imparted by the bioflocculants used.

Table 5.4: FOG results for BioDAF and ConDAF

	FOG (mg/L) 0hr	FOG (mg/L) 8hr	Total FOG (mg/L) removed	Removal efficiency
ConDAF	427	233	194	45.43%
BioDAF	647	16	631	97.53%

Since FOG is associated with numerous challenges in biological treatment processes, particularly secondary (anaerobic) treatment systems, dissolved gas transfer rates must adequate, to reduce bulking and the proliferation of undesired microorganisms. Overall, Table 5.4 shows FOG removal efficiency using a ConDAF (45.43%) and BioDAF (97.53%) thus indicating that the BioDAF had a higher FOG removal efficiency. Pre-treatment of PSW with the BioDAF system was deemed to confer positive attributes which can be beneficial to downstream treatment processes, perhaps improving the biological degradation of residual oils in the FOG laden wastewaters, which can culminate in downstream process efficiency improvements and process control (Cammarota & Freire, 2006). By pre-treating the PSW with bioflocculants resulted in the flotation of some Fats and proteins, which would reduce the overall organic load rates for secondary treatment process. As the feed contained some emulsified constituents, their destabilisation, led to the breakage of the FOG emulsion, followed by partial re-coagulation of the buoyant FOG which attached to the macro bubbles, floating to the top where they were subsequently removed (Willey, 2001).

5.3.4 Conventional DAF (ConDAF) vs bioflocculant supported DAF (BioDAF)

The evaluation of the experimental results indicated that the BioDAF has better particle removal efficiency as compared to the conventional DAF. When Del Nery *et al.* (2007) utilised a conventional DAF system in the pre-treatment of PSW, they achieved a lowly 38, 51 and 37% removal efficiency for tCOD, FOG and TSS, respectively, which were similar to the results obtained using the conventional DAF system used in the current study which yielded 41.95, 45.43 and 42.06% for tCOD, FOG and TSS removal. However, when the BioDAF was employed, an improved performance resulted in 65.85, 97.53 and 83.1% removal efficiency for tCOD, FOG and TSS, was observed respectively. The improvement was attributed to bioflocculant supplementation which promoted the aggregation of particles matter through the formation of bridges amongst dispersed particles, resulting in the formation of particles with a size sufficiently big to be attached to the macro-bubble surface generated by sparging. Such an improvement in the DAF system effluent quality characteristics can be maintained through managing of process operating parameters (de Nardi *et al.,* 2008). Previously, the application of DAFs has proved to be having challenges, requiring long HRTs and thus large reactors to compensate for low throughput (treatment) rates (Asselin *et al.,* 2008), while the BioDAF with bioflocculants from the *mE. coli* can be operated using a shortened HRT.

5.3.5 Response Surface Methodology

The results obtained from the experimental trials were used to generate an empirical model using RSM. To ascertain which parameter to utilise due to the variation in wastewater quality of the samples obtained from the slaughterhouse, the standard deviation for each parameter was assessed and compared with the parameter with the lowest standard deviation being used to model the BioDAF. The experimental data obtained, correlated to a first order model. Using the Fisher's distribution test, the proposed model was verified, and was determined to be significant. From Table 5.4 it was observed that the bioflocculant dosage is insignificant, particularly for the sCOD which was selected to be the parameter to be modelled, as TSS including tCOD showed a higher variability thus a higher standard deviation, which would result in model redundancy. The determination coefficient was used to determine the fit quality proposed, i.e. between the predicted and actual sCOD concentrations. An correlation coefficient (R^2) of ~1, as with most linear trends, demonstrated that the linear model obtained was satisfactory to represent the sCOD reduction by the BioDAF system. The low %CV of 1.65 indicated reliability of the model.

Table 5.5: Analysis of variance (ANOVA) for the linear model

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	1.385E+005	2	69272.92	4.802E+010	< 0.0001	significant
A-pH	*1.385E+005*	*1*	*1.385E+005*	*9.604E+010*	*< 0.0001*	
B-Biodosage	*0.000*	*1*	*0.000*	*0.000*	*1.0000*	
Residual	1.443E-005	10	1.443E-006			
Lack of Fit	*1.443E-005*	*6*	*2.404E-006*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	1.385E+005	12				

R^2 = 1 %CV = 1.65

The mathematical model that described the reduction of sCOD as a function of pH and bioflocculant dosage was described by the regression quadratic equation which yielded an empirical model with minimal residual as shown in Eq. 5.3, with a representative contour plot being illustrated in Fig. 5.10 while a graphical illustration of modelled sCOD in comparison to actual sCOD removal efficiency being highlighted in Fig 5.11.

$$Y\ (sCOD) = +4065.83410 - 797.56814 * A + 5.88984E - 012 * B \qquad 5.3$$

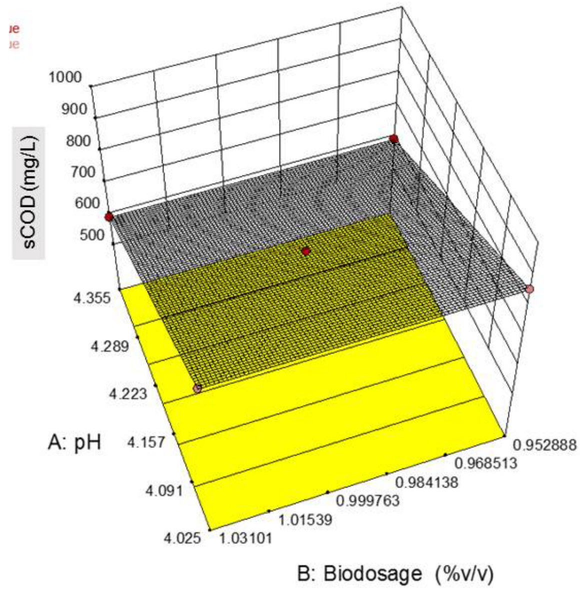

Figure 5.10: 3-D surface plot showing the interaction of bioflocculant dosage and pH on sCOD removal.

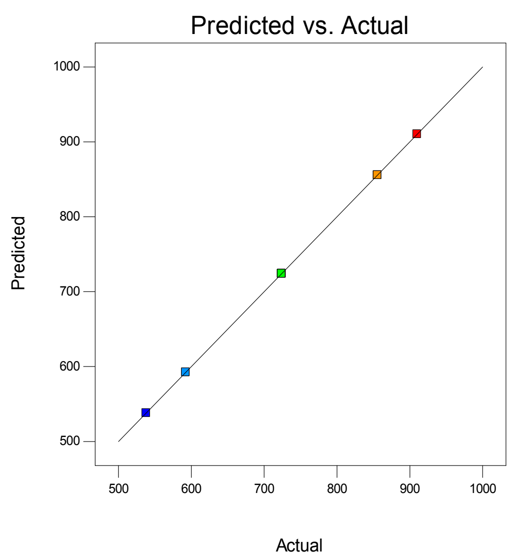

Figure 5.11: Graphical representation of predicted vs actual sCOD removal efficiency

To further determine if the experimental data is described by the model, residuals are examined so as to determine the adequacy of the model. This is done by plotting the actual versus the predicted response and if the plot forms a straight line then the model is adequate (Noordin *et al.,* 2004). Fig 5.11 above shows that a straight line was obtained thus indicating that there were no significant or major deviations, hence proving the model to be adequate.

Focusing on the RSM results the development of a model describing the operation of a DAF based on pollutant removal was deemed feasible as the derived model was significant.

5.3.6 Wastewater quality improvements

Regulatory compliance and observance is important when dealing with wastewater such as PSW (del Nery *et al.,* 2007). The PSW used in this study had average initial values of 537 mg/L FOG, 3244mg/L tCOD, 1715mg/L sCOD, 1245mg/L TSS, 475mg/L tProtein, 234mg/L sProtein, 855 NTU turbidity and a pH of 7. These values clearly indicates that TSS and FOG were out of specification as they exceeded the maximum permitted discharge limits of 1000mg/L and 400mg/L respectively (refer to literature review section 2.7.1 and (see appendix C2) according to the City of Cape Town, (Western Cape, South Africa) whereby this study was based, including those listed by the wastewater and industrial discharge by-law (2006), Schedule 2.

Furthermore, the etermination of whether the microorganisms produces toxins was assessed using test strips (Microcystines test strip kit for finished drinking water, Abraxis, United States of America) using milliQ water as a control, focusing on raw PSW and bioflocculant treated water (see appendix C2). The test was based on the competition for antibody binding site between the toxins in the mobilised agents on the test strips. Toxin presence would be indicated by colour change on the positive test line. The test strip has a control band which is used to validate the test and to confer a comparison of the intensity of the test line (Humpage *et al.,* 2012). For the present research, the test for toxicity of bioflocculant treated wastewater was negative as the intensity of the test line was similar to that of the control line thus suggesting that there were minimal toxins in the pre-treated wastewater.

Overall, after pre-treatment the PSW has most parameters below the permitted disposal limits with DAF having minimal concentration depicting that most of the pollutants have been removed. However, the effluent from BioDAF system cannot be disposed directly into the municipal sewer system as the pH-4.2 was still below the permitted limit of 5.5 to 12; hence, pH adjustment using alkali dosing or a secondary anaerobic pre-treatment system, is essential.

5.3.7 Summary

PSW was pre-treated using a ConDAF whereby there was no adjustment of pH or supplementation with any flocculants while the BioDAF system in which the pH was adjusted to 4 with bioflocculants dosage of 1% (v/v). The ConDAF system had low pollutant removal efficiency as compared to the BioDAF system, sole attributed to the efficacy of the bioflocculants used. Moreover the pre-treated water from a BioDAF showed the absence of toxins when tested for toxicity.

RSM was used to simulate an empirical model that describes the BioDAF process using a single parameter (sCOD), with the lowest deviation, used as a reliable parameter to simulate.. The results depicted that the empirical model developed was significant and can reproduced sCOD results for the BioDAF achieving a coefficient of correlation (R^2) of ~1 and the 1.65 %C.V which was indicative of the model reliability.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

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6.1 Conclusions

Isolate *mE. coli* (E1) was isolated from PSW for bioflocculant production for use in a bioflocculant supported dissolved air floatation system (BioDAFs) that was modelled for the pre-treatment of PSW. The isolates identification was confirmed by 16S rDNA sequencing, and verified using a VITEK 2 system v07.01. Response Surface Methodology (RSM) was used to determine the optimal bioflocculant production conditions, i.e. pH of 6.5 and a temperature of 36℃, conditions which facilitated rapid bioflocculant production.

Furthermore, the effect of the bioflocculant produced on pollutant removal was similarly analysed using RSM, to determine optimal operational physico-chemical conditions (pH and bioflocculant dosage) in which maximum flocculation activity can be achieved during dissolved air flotation, with zeta potential analysis being used as a variable to ascertain the flocculation mechanism. The zeta potential results depicted that there was an insignificant change in the charge density of the suspensions (kaolin clay, 4 g/L) when bioflocculants were dosed at different concentration, an effect observed at different pH which suggested that charge neutralization was not the primary mechanism for floc formation. From microscopic observations, the desired pH and bioflocculant dosage was observed to be at pH 4 and bioflocculant dosage of 1% (v/v), conditions which formed denser agglomerates, i.e. flocs, which was indicative of suitable conditions for maximized floc formation when operating a DAF. As such, these conditions were adopted for utilization in the BioDAF designed. Moreover, under the identified conditions, the flocculation mechanism as quantified using a bonding type test, confirmation a bridging mechanism.

The BioDAF designed was operated at a flow rate of 1mL/min with an HRT of 32hr; with a ConDAF being used for comparative analysis to assess the efficacy of bioflocculant supplementation. The ConDAF was operated with neither pH adjustment nor flocculant supplementation whereas the BioDAF was operated at a pH 4 and a bioflocculant dosage of 1% (v/v), with the BioDAFs performance being satisfactory with better pollutant removal, i.e. 97.53% FOG, 65.85% tCOD, 26.56% sCOD, 83.1% TSS, 73.14% tProtein, 97.8% sProtein, reducing the turbidity of the wastewater by 81.96%, than the ConDAF.

For process control purposes and to predict performance, an empirical model describing the operation of the BioDAF was developed using RSM (Design-Expert Version 6.0.8) from the data generated from numerous analytical methods. Since, sCOD had the lowest standard deviation due to the high variability of other quality characteristics of the wastewater generated from the slaughterhouse, i.e. as a parameter to be used to predict the performance of the BioDAFs, it was used in model development.

6.2 Recommendations for future research

Future studies on the BioDAF should include the evaluation of other parameters such as diffuser design and variation in sparging rate, influence of higher suspended solids loading, performance efficacy at even reduced HRTs, in order to ascertain that the proposed design can be scaled-up to a pilot plant size. Furthermore, since the microorganism used in this study i.e. *mE. coli* (E1) is a mutant, virulence studies need to be done on it to ascertain its usability.

CHAPTER 7

REFERENCES

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7. REFERENCES

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APPENDICES

Appendix A: Microbial Isolation and Identification

Appendix A1: Gram staining procedure

- A loop of E1- 24 hour old culture was transferred onto a clean slide and mixed or smeared together with water,
- It was then heat fixed onto the slide by passing it through the flame 3 times,
- Crystal violet was added and the slide was allowed to stand for 60 seconds,
- It was rinsed with running tap water and iodine was then added and the slide was allowed to stand for 60 seconds,
- The slide was then rinsed again with running tap water and a few drops of acetone were added and rinsed immediately,
- Safranin was then added onto the slide and allowed to stand for 60 seconds and was rinsed with running tap water,
- The slide was dabbed dry and then viewed under a microscope.

Appendix A2: Vitek biochemical test results of E1 (*E. coli*)

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	(-)	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Appendix B: Effect of bioflocculants on TSS

Appendix B1: Zeta potential results of Kaolin Clay and Kaolin clay/$CaCl_2$

Physico-chemical conditions		Zeta potential (mV)		
pH	**Bio Concentration (%v/v)**	**KC/CaCl/Bio**	**KC**	**KC/$CaCl_2$**
2.96	2	-17.3	-36	-11.3
4	1	-16.5	-40.4	-14.5
4	3	-17.9		
6.5	0.59	-18.8	-44.6	-18.8
6.5	2	-20.4		
6.5	3.41	-18.7		
9	1	-19.1	-50.4	-17.8
9	3	-20.4		
10.04	2	-18.2	-49.1	-16.3

KC = Kaolin clay   Bio = Bioflocculant
NB: in KC and KC/$CaCl_2$ no bioflocculant was added.

Appendix B2: Flocculation mechanism (bonding type) results

Appendix C: Analytical methods

Appendix C1: DAF system operations

Conventional DAF BioDAF

Fig C1: Photographic representation of Con DAF vs BioDAF during a pre-treatment process

Appendix C2: Average wastewater parameters of PSW before pre-treatment compared to the limit allowed as stipulated in the City of Cape Town wastewater and industrial effluent bylaw.

Parameter	Values not to be exceeded	PSW Average values
pH	5.5-12	7
TSS (mg/L)	1000	1245*
Turbidity (NTU)	-	855
tCOD (mg/L)	5000	3244
sCOD (mg/L)	-	1715
tProtein (mg/L)	-	475
sProtein (mg/L)	-	234
FOG (mg/L)	400	537*

**out of specification*

Appendix C3: Toxicity test

- Samples are collected into sample vials,
- 7 drops of the sample is transferred using pasteur pipettes provided into an eppindorf that contains the dried reagents,
- The solution is mixed and incubated at room temperature for 20min,

- After incubation the test strip is dipped into the solution and further incubated at room temperature for 10 minutes,
- The strips are then put on a flat surface and left to stand for 5 minutes and qualitative observations are then made.

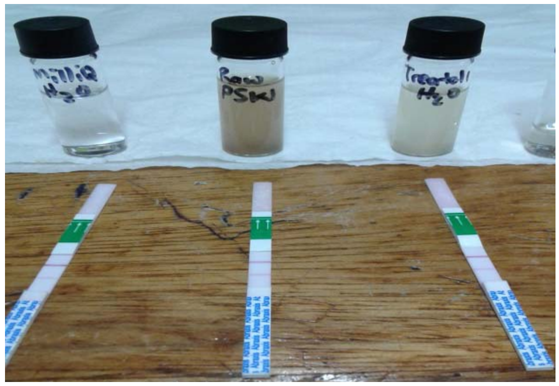

Fig C2: photographic illustration of toxicity test results

Appendix C4: COD Analysis

- The sprectroquant thermo reactor which was pre-set to 148℃ for two hours was switched on and was allowed to heat up to the desired temperature,
    - o Using the high range COD solution A and B 2.2mls of solution A and 1.8mls of solution B was pipetted into a cell,
    - o 1ml of the sample was then pipetted into the cell with the mixture of solution A and B,
    - o Using low range test kit 3mls of the sample was added to the cell with the premixed test solution,
- The caps were tightly screwed on and the mixtures were vigorously mixed with a shaker,
- The cells were then heated in the spectroquant thermo reactor at 148℃ for two hours,
- Thereafter the cells were placed in a rack to cool down and after 10mins they were vigorously mixed with a shaker and were left to cool off at room temperature for at least 30 minutes,
- The COD concentration was read after cooling off in a Spectroquant Nova 60 with the input of 0.24 for high range (500-10000) and 0.23 for low range,
- This was the method for tCOD with the only difference for sCOD that the sample was filtered through suction and using a 0.7μm glass fibre filter.

Appendix C5: Total Suspended Solids

- Glass fibre disk was inserted onto the base and a clamp funnel, all connected to a suction flask,
- Vacuum was applied and the filter was washed with 3 successive 20ml volumes of milli-Q water,
- The filter was removed using a twizzer and was placed in an aluminium dish which was then ignited in the muffle furnace at 550℃ for 30minutes,
- The filter was then rewashed with 3 successive 20mls of milli-Q water and was dried in an oven for 1 hour at 103℃,
- Thereafter the filter was put in a desiccator to cool down and then weighed,
- The filer paper was placed between the base and clamp funnel connected to a suction flask and a small volume of milli-Q water was added so as to attach the filter paper onto the base,
- The sample was vigorously mixed and a certain volume of the sample-usually less than 200mls was transferred onto the filter paper and vacuum was applied even after water has passed through,
- The filter paper was then put in the aluminium dish and was heated at 103℃ in a drying oven for 1hour,
- Thereafter it was put in a desiccator to cool off and was then weighed afterwards,
- The equation below was used to calculate TSS concentration,

$$TSS\left(\frac{mg}{L}\right) = (A - B) * 1000/C$$

- Where: A= Filter and dish+ residue weight in mg
  - B=Filter and dish weight in mg
  - C= Sample volume in mL

Appendix C6: Protein determination using Bradford assay

- The Bradford reagents (1X dye) was removed from 4 storage and left at room temperature to warm up and it was then inverted a few times so as to mix,
- 2mg/ml BSA was diluted to different standard concentrations (2000, 1500, 1000, 750, 500, 250, and 125µg/ml),
- 60 µl of each standard, water (blank) and unknown sample were pipetted into separate cuvette and 3ml of 1X reagent dye was added into the cuvettes and was mixed,

- The mixtures were incubated at room temperature for 5 minutes and thereafter the absorbance were read using a spectrophotometer at 595nm,
- A standard curve was made by plotting absorbance vs concentration and the unknown sample concentration was determined using the standard curve.