BIOLOGICAL NUTRIENT REMOVAL FROM INDUSTRIAL WASTEWATER USING A SEQUENCING BATCH REACTOR

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DECLARATION

I, the undersigned **Siphesihle Mangena Khumalo** hereby declare that,

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As the candidate supervisors we have approved this dissertation for submission

Dr B.F. Bakare

Prof. S. Rathilal

DEDICATION

To

My loving and caring mother, Ntombikayise Dlamini and my aunt Mumcy Dlamini all I am or hope to be, I truly owe to you

And

In memory of my father, Erasmus Khumalo, as well as the ones who were gone before us and the ones who are yet to come

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John Donne, (1572-1631) one of the greatest poets in history wrote that “no man is an island, entire of himself”. I would like to express my heartfelt gratitude to the following persons and organisations for their endless help and support they furnished me during the period I was conducting this research work. Without their passionate participation and input this study could not have been successful.

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ABSTRACT

South Africa is not an exception when it comes to the issue of fresh water scarcity perpetuated by environmental pollution among many other factors. Industrial wastewater particularly emanating from the brewing industry, contains high-strength organic, inorganic, and biological compounds which are toxic to the environment. Due to stringent industrial effluent dewatering standards enforced by both local and international environmental protection entities, industrial wastewater cannot be discharged into receiving water bodies prior to treatment.

The overall aim of this study was to evaluate the performance or treatment efficacy of a laboratory scale sequencing batch reactor on biological nutrient removal using industrial wastewater from brewery. In this study, two laboratory scale sequencing batch reactors (SBRs) operated in a cyclic aerobic-anaerobic configuration inoculated with activated sludge were investigated for their removal of orthophosphates and nitrogen compounds from brewery wastewater. SBR-1 was investigated for nitrogen group pollutant removal and SBR-2 was investigated for orthophosphate removal. The findings of the study are reported based on overall removal efficacies for the following process monitoring parameters: orthophosphates, ammoniacal nitrogen, total Kjeldahl nitrogen, total nitrogen, total organic nitrogen, total inorganic nitrogen and NO_3-N+NO_2-N.

From the investigation, the following overall removal efficacies were obtained: 69% orthophosphates, 69% ammoniacal nitrogen, 59% total Kjeldahl nitrogen, 60% total nitrogen, 64% total organic nitrogen, 67% total inorganic nitrogen and 56% NO_3-N+NO_2-N at an organic loading rate of 3.17 kg Total Chemical Oxygen Demand (TCOD) /m^3.day with a food to microorganism ratio of 2.86 g TCOD/g Volatile Suspended Solids (VSS).day. These removal efficacies were attained for a hydraulic retention time of 18 hours for both SBRs with a solids retention time of 5 days for SBR-1 and 7 days for SBR-2.

Both reactors were operated at a mesophilic temperature range of 23 to 26˚C and a pH range of 5 to 8.5. The temperature was left unadjusted because it was observed that it did not hinder any microbial activities during the biodegradation process. The *Michealis-Menten's* and Monod models were implemented to study the substrate utilisation rate kinetics and microbial growth rate kinetics recording 15 141 g COD/m^3.day; 12 518 g VSS/g VSS.day; 20 343 g

COD/m^3.day and 16 860 g VSS/g VSS.day for SBR-1 and SBR-2, respectively. The Monod model demonstrated a strong correlation fit between the substrate utilisation rate and microbial growth rate recording a polynomial correlation constant of R^2 = 0.947 and 0.9582 for SBR-1 and SBR-2, respectively.

The findings of this study showed that the cyclic aerobic-anaerobic configuration on a laboratory scale SBR inoculated with activated sludge for treatment of brewery wastewater for biological nutrients was feasible.

PREFACE

The work presented in this dissertation was conducted at Mangosuthu University of Technology (MUT), Faculty of Engineering in the Department of Chemical Engineering. All laboratory analyses were done at MUT Wastewater Management Research Laboratory. Furthermore, this project was funded by both the Durban University of Technology and the Environmental Pollution and Remediation Research Group (EPRG) at MUT. From the work presented in this dissertation, so far two journal articles which are currently under review as indicated in the research output section have been written.

RESEARCH OUTPUT

1. **Khumalo, S.M.**, Bakare, B.F. and Rathilal, S. 2018. Biological Nitrogen Removal Processes from Industrial Wastewater with High-strength Ammonium and Low Organic Substrate: A Review. *Chemical Engineering & Technology* (submitted).

2. **Khumalo, S.M.**, Bakare, B.F. and Rathilal, S. 2018. Characterization of Industrial Wastewater Generated from the Brewery with Variation of Oxidation Reduction Potential: Case of South African Breweries. *Journal of Water Reuse and Desalination* (submitted).

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GLOSSARY

Term	Definition
Acclimate	The adaptation of microorganisms to a new environment.
Activated sludge process	Biological treatment process that involves the conversion of organic matter and/or other constituents in the wastewater to gases and cell tissue by a large mass of aerobic microorganisms maintained in suspension mixing and aeration.
Aerobic process	Biological treatment process that occur in the presence of free dissolved oxygen, whereby oxygen is consumed by aerobic microorganisms in oxidation reactions to produce energy for cell growth and cell maintenance.
Anaerobic process	Biological treatment process that occur in the absence of oxygen.
Anoxic process	Biological treatment process that occurs in the absence of free dissolved oxygen where nitrate and nitrite are used as the main electron acceptors in biological oxidation/reduction reactions.
Biomass	The total mass of biosolids in a reactor consisting mainly of organic matter and microorganisms.
Biochemical oxygen demand	Is the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in wastewater sample at certain temperature over a specific time period.
Bioreactor	A vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic, anaerobic, or anoxic.
Chemical oxygen demand	Is a measure of the capacity of wastewater to consume oxygen during the decomposition of organic

	matter and the oxidation of inorganic chemicals such as ammonia and nitrite.
Denitrification	The biological process by which nitrate or nitrite is reduced to nitrogen and other gaseous end products.
Eutrophication	The process by which a body of water becomes enriched in dissolved nutrients (such as phosphates) that stimulate the growth of aquatic plant life usually resulting in the depletion of dissolved oxygen thus, killing fish.
Flow equalization	Is a technique used to consolidate wastewater effluent in holding tanks for "equalizing" before introducing wastewater into downstream brewery treatment processes or for that matter directly into the municipal sewage system.
Grit removal	Is a process in which wastewater is allowed to flow into a grit chamber where sand, grit, and small stones settle to the bottom.
Hydraulic retention time	The average time spent by wastewater inside the reactor during treatment.
Inoculum	The act of introducing microorganisms into a culture medium.
Mesophilic conditions	Temperature range between 20 – 50˚C.
Microorganism	An organism that can be seen only with the aid of a microscope and that typically consists of only a single cell (i.e., bacteria, protozoans, and certain algae and fungi).
Mixed liquor suspended solids	The mixture of bio-solids from activated sludge and wastewater in the bio-reactor.
Nitrification	The two-step biological process by which nitrogen is converted to nitrite and then to nitrate.
Solid retention time	The average time spent by bio-solids inside the reactor.
Substrate	The organic matter or nutrients that are converted during biological treatment.

LIST OF ABBREVIATIONS

ANAMMOX	:	Anaerobic ammonium oxidation
AOB	:	Ammonia Oxidising Bacteria
APHA	:	American Public Health Association
BNR	:	Biological Nutrient Removal
BWW	:	Brewery Wastewater
C	:	Carbon
COD	:	Chemical Oxygen Demand
DEA	:	Department of Environmental Affairs
DWAF	:	Department of Water Affairs
EBPR	:	Enhanced Biological Phosphorus Removal
FVS	:	Fixed Volatile Solids
HRT	:	Hydraulic Retention Time
JLR	:	Jet Loop Reactor
N	:	Nitrogen
P	:	Phosphorus
PAO	:	Polyphosphates Accumulating Organisms
PCOD	:	Particulate Chemical Oxygen Demand
SAB	:	South African Breweries
SCOD	:	Soluble Chemical Oxygen Demand
SD	:	Standard Deviation
SBR	:	Sequencing Batch Reactor
SRT	:	Solids Retention Time
TCOD	:	Total Chemical Oxygen Demand
TDS	:	Total Dissolved Solids
TIN	:	Total Inorganic Nitrogen
TKN	:	Total Kjeldahl Nitrogen
TN	:	Total Nitrogen
TON	:	Total Organic Nitrogen
TP	:	Total Phosphorus
TS	:	Total Solids
VFA	:	Volatile Fatty Acids
VSS	:	Volatile Suspended Solids

TABLE OF NOMENCLATURE

Symbol	Meaning	Units
p	Alpha value	---
r_{NH}	Ammonia-oxidising rate	$g/m^3.day$
X	Biomass concentration	$g\ VSS/m^3$
S_o	Concentration	mg/L
EC	Electrical conductivity	µS/cm
Q	Flowrate	m^3/day
$K_{O,AOB}$	Half-velocity for AOB	mg/L
K_{NH}	Half-velocity for NH_3-N	mg/L
$\mu_{max,AOB}$	Maximum specific growth rate of AOB	g VSS/VSS.day
r_g	Microbial growth rate	$g\ VSS/m^3.day$
S_{NH}	NH_3-N concentration	mg/L
ORP	Oxidation-reduction potential	mV
b_{AOB}	Specific endogenous decay rate of AOB	G VSS lost/g VSS.day
μ_{AOB}	Specific growth rate of AOB	g VSS/g VSS.day
r_{su}	Substrate utilisation rate	$g\ TCOD/m^3.day$
L_{org}	Volumetric organic loading rate	$kg\ TCOD/m^3.day$
V	Volume	m^3

Chemical Formula	Full Name
NH3-N	Ammoniacal nitrogen
NH_4^+	Ammonium ion
Cr^{3+}	Chromic ion
$Cr_2O_7^{2-}$	Dichromate ion
PO_4^{3-}	Orthophosphates
NO_3-N	Nitrate
NO_2-N	Nitrite

CHAPTER ONE
INTRODUCTION

1.1 Background Information and Motivation

The discharge of industrial wastewater with a high-concentration of biological nutrients (i.e. nitrogen and phosphorus compounds) and organic matter pollutants into receiving water bodies stimulates the growth of algae which promotes eutrophication, thus destroying aquatic life and resulting in environmental pollution (Liu and Liptak 1999; Safafar *et al.* 2015). Eutrophic waters are characterised by high concentrations of aquatic weeds and algae, which eventually die, sink to the bottom and decay, thus reducing the levels of dissolved oxygen in water (Liu and Liptak 1999; Sathasivan 2009; Haberman and Haldna 2014). Furthermore, according to Metcalf and Eddy (1991), this phenomena is also perpetuated by the cycling of dissolved oxygen which accompanies the process of photosynthesis and respiration.

The choice of industrial wastewater treatment which could be either biological or chemical methods depends solely on the composition of the pollutants. Industrial wastewater from food and beverage industries contain significant amounts of biodegradable compounds which can be treated by biological methods (Ochieng, Odiyo and Mutsago 2003; Atalay and Ersöz 2016). The brewing industry is said to be one of the largest consumers of fresh water for beer production as well as being amongst the largest producers of industrial effluent which contributes to environmental pollution (Parawira *et al.* 2005; Tansiphorn, Suraphong and Prasert 2009; Simate 2015). Brewery effluent is characterised by its high-concentration of organic, inorganic and biological pollutants in terms of chemical oxygen demand (COD), biochemical oxygen demand (BOD), ammoniacal nitrogen, total Kjeldhal nitrogen (TKN), inorganic nitrogen (i.e. nitrate and nitrite), phosphorus (i.e. orthophosphates) and other pollutants depending on the chemicals that are used during the cleaning process (Goldammer 2008; Abimbola *et al.* 2015; Simate 2015).

The use of only aerobic biological processes for wastewater treatment results in the generation of large amounts of biomass which needs to be handled and disposed, thus increasing treatment costs (Zvauya, Parawira and Wawadza 1994; Parawira *et al.* 2005; Ahn and Logan 2013). Recently, a number of studies have been reported on anaerobic processes for treating brewery wastewater (Shao *et al.* 2008; Wang *et al.* 2010; Hill 2015). The use of anaerobic systems is

associated with advantages such as: less energy is required because there is no aeration needed; methane is produced which can be used for energy production; small amounts of biomass and sludge are formed, thus resulting in lower disposal costs (Parawira *et al.* 2005). However, the anaerobic system comes with its own disadvantages. The common problem is associated with the start-up and operation of the anaerobic treatment process due to the complexity of the process which is carried out by a consortium of unidentified and interdependent microorganisms, which makes the process unstable and difficult to monitor (Lettinga 1995; Jeinson and Chamy 1999; Parawira *et al.* 2005).

The application of biological nutrient removal processes in industrial wastewater treatment using sequencing batch reactors (SBRs) has come with its own benefits such as environmental, economical, and operational benefits. Activated sludge SBR operated under aerobic/anaerobic/aerobic-anaerobic conditions has demonstrated high nutrient removal efficacies which is an environmental benefit. In SBR systems both the reaction and settling phases take place in one vessel which makes the system to be inexpensive to set-up compared to previously adopted conventional systems which require separate settling tanks, and lastly the system has cemented its application due to its easy operation (Arun 2011). To date, there are other technologies besides the SBR which are used in treating industrial wastewater, such as the application of up-flow anaerobic sludge beds, biogas-lift reactors, fluidised bed bioreactors etc., however, they all face difficulties in treating wastewater with high solids content (Gregor, Matej and Milenko 2007; Shu-Guang *et al.* 2007; Fu *et al.* 2013). Therefore, they require solids removal prior to treatment.

In this study, a cyclic aerobic-anaerobic laboratory scale SBR under activated sludge was used to treat brewery wastewater for biological nutrient removal (i.e. nitrogen and phosphorus pollutants). The system performance parameters were measured in terms of removal efficiency of ammoniacal nitrogen, TKN, total nitrogen (TN), total organic nitrogen (TON), total inorganic nitrogen (TIN) and orthophosphates. The controlled and monitored parameters were dissolved oxygen, pH and temperature respectively.

In South Africa, the Department of Water Affairs and Forestry (DWAF) is the custodian of the country's water resources. Part of the DWAF's mission is to ensure that the quality of water resources remains fit for recognised water uses and to protect aquatic life (Holmes 1995). Through the DWAF and the department of environmental affairs (DEA), the Republic of South

Africa regulates industrial effluent discharged into receiving bodies. This is done by putting into practice the National Water Act (NWA), 1999 (Act No. 20526 of 1999) and Integrated Coastal Management Act, 2008 (Act No. 240 of 2008) by setting allowable pollutant limits for wastewater to be discharged into receiving water bodies, however, most industries are still struggling to meet the allowable wastewater discharge limits. According to the South African NWA,1999 (Act No. 20526 of 1999) wastewater discharged to water receiving bodies must not have more than a COD of 75 mg/L, ammonia as nitrogen of 3 mg/L, nitrate or nitrite as nitrogen of 15 mg/L, orthophosphate as phosphorus of 10 mg/L, and a pH range of 5.5 to 9.5. This research study is aligned with both the DWAF and DEA's mission which is to protect aquatic life by reducing biological pollutants from industrial effluent to allowable discharge limits.

1.2 Problem Statement

Many municipal wastewater treatment plants are designed to handle domestic wastewater. Industrial wastewater, specifically brewery wastewater, tends to upset the treatment system due to its high-concentration of organic and inorganic matter content thus compromising the treatment efficacy (Parawira *et al.* 2005; Aguado *et al.* 2009; Matthew *et al.* 2010). Recently new processes for simultaneous nitrogen and phosphorus removal from wastewater have been developed, such as: partial nitrification (nitritation), anaerobic ammonium oxidation (ANAMMOX), autotrophic nitrogen removal over nitrite (CANON), etc. (Khin and Annachhatre 2004; Wei *et al.* 2014; Chan, Guisasola and Baeza 2017). However, these systems are all limited in treating wastewater with high-concentration ammonium and low organic matter, thus presenting a number of drawbacks in terms of treatment capacity and efficiency of wastewater with high organic and biological nutrient pollutants (Gregor, Matej and Milenko 2007; Shao *et al.* 2008). This study focus on reducing biological nutrient pollutants in industrial wastewater from the brewery prior to being discharged into municipal sewer to improve wastewater treatment plants efficacy as well as minimizing nitrogen and phosphorus pollution.

1.3 Aim and objectives of the study

Based on the challenges stated earlier, the aim of the research study is to evaluate the performance/treatment efficacy of a cyclic aerobic-anaerobic laboratory scale SBR under activated sludge microbial population for biological nutrient removal using industrial wastewater from a brewery.

The objectives of the research study are to:

- Characterise wastewater generated from the brewery.
- Determine the sequencing batch reactor treatment efficiency in treating brewery wastewater for biological nutrients in terms of percentage removal.
- Investigate the effect of influent organic pollutant strength/organic volumetric loading rate in terms of chemical oxygen demand, in treating brewery wastewater for biological nutrients.
- Use Monod and *Michaelis-Menten's* models to study the microbiology kinetics in terms of substrate utilisation rate kinetics, and microbial growth rate kinetics.

1.4 Significance of the study

Looking at the objectives described in the previous sub-section, the significance of conducting the study was to successfully treat industrial wastewater from the brewery for biological nutrient removal, using a cyclic aerobic-anaerobic SBR under activated sludge heterotrophic bacteria. The study aims to assist wastewater producing industries in developing in-house wastewater pre-treatment processes to reduce biological pollutants to meet dewatering standards as stipulated by local government entities, thus reducing the load on municipal wastewater treatment plants.

1.5 Dissertation outline

Apart from the introduction which constitutes **Chapter One**, the dissertation is structured as follows:

Chapter Two focus on reviews related to biological nutrients found in wastewater as well as latest removal processes, characteristics of brewery wastewater (BWW), biological processes used in treating BWW as well as previously done studies on BWW treatment using activated sludge systems. The chapter concludes with a brief description of the importance of microbial kinetics growth models specifically unstructured Monod model. **Chapter Three** gives a detailed description of the materials and methods used during experimental runs on biological nutrient removal. This chapter explains the operation of a SBR, sampling methods conducted, as well as statistical methods used for data analysis. **Chapter Four** presents the results obtained during the experimental study and a discussion of results. In this chapter the results are presented in tables and graphs. **Chapter Five** presents conclusions drawn on this study based on the findings presented in **Chapter Four** as well as recommendations.

CHAPTER TWO
REVIEW OF LITERATURE

This chapter presents an organisation of literature relevant to the understanding of biological processes, particularly activated sludge systems, that are used for industrial wastewater treatment for biological nutrient removal, specifically brewery wastewater, prior to discharge into water receiving bodies. Section 2.1 presents an overview of information on biological nutrients which are found in wastewater and methods used in treating wastewater for nutrient removal. Section 2.2 presents a review of literature relevant to the parameters affecting biological systems in nutrient removal from wastewater. Section 2.3 presents a review of literature pertinent to the general characteristics of brewery wastewater. Section 2.4 presents brewery wastewater treatment methods as well as previously done research work related to each method. Section 2.5 provides information on microbial growth kinetics, the importance of studying microbial growth as well as empirical models which are widely used in microbial growth studies. A summary of the reviewed literature is presented as the last section of this chapter.

2.1 Biological Nutrients in Wastewater

Biological nutrients in industrial wastewater are characterised by both nitrogen and phosphorus pollutants, which perpetuates the process of eutrophication in water bodies (Liu and Liptak 1999; Henze *et al.* 2008; Safafar *et al.* 2015). Nitrogen pollutants in wastewater exist in two different forms i.e. organic nitrogen which is estimated as TKN and inorganic nitrogen (ammonia, nitrate and nitrite). Ammonia is considered a toxic nitrogen compound in wastewater, moreover, ammonia is associated with a high solubility in water, thus it exists as an ammonium ion (NH_4^+) (Philips *et al.* 2002). This biological nutrient is imperative for plant growth, therefore, enrichment of water bodies with ammonium ions perpetuates the growth of algae which results in the reduction of dissolved oxygen in water, thus killing aquatic life e.g. fish (Mulkerrins, Dobson and Colleran 2004; Safafar *et al.* 2015).

On the other hand, phosphorus pollutants in industrial wastewater are made up of dissolved forms, which are characterised as orthophosphates, inorganic forms characterised as polyphosphates, and organically bonded phosphorus compounds. The combination of these different forms of phosphorus is characterised as total phosphorus. However, in wastewater treatment processes the quantity of phosphorus is measured in terms of orthophosphates since

both the inorganic phosphorus and organically bonded phosphorus during analysis are dissolved and converted to orthophosphates. According to Henze *et al.* (2008) and Safafar *et al.* (2015), phosphorus is the key element which promotes the growth of aquatic plants and algae thus promoting eutrophication.

2.1.1 Biological Nutrient Removal

According to Metcalf and Eddy (2014), biological nutrient removal (BNR) is defined as the removal of nitrogen and phosphorus by means of biological treatment processes. Biological processes which are used for nutrient removal in wastewater are divided into two main categories i.e. suspended growth and attached growth processes (Metcalf and Eddy 2014). In suspended growth processes, the microorganisms responsible for treatment are maintained in liquid suspension and the activated sludge process is commonly used in wastewater treatment processes as discussed in section 2.4 of this chapter. Moreover, in attached growth processes the microorganisms responsible for the conversion of biological nutrients and organic material are attached to an inert packing material. In attached growth processes, wastewater treatment is done by the flowing of wastewater through the attached growth also known as biofilm. For the attached growth processes the trickling filter technology has cemented its application in wastewater treatment plants. Both the suspended growth and attached growth processes can be operated as aerobic or anaerobic or anoxic processes (Arun 2011; Metcalf and Eddy 2014). The biochemistry of nitrogen and phosphorus removal is discussed in detail in the consequent sub-sections.

2.1.2 Biological Nitrogen Removal

Nitrogen removal from wastewater is mostly done through the process of nitrification and denitrification (Carrera *et al.* 2003; Jeyanayagam 2005; Wei *et al.* 2014). According to Metcalf and Eddy (2014) the process of nitrification is a two step process which takes place in the presence of oxygen involving two groups of autotrophic bacteria i.e. *Nitrosomonas* and *Nitrobacteria*. Metcalf and Eddy (2014) and Wei *et al.* (2014) indicated that during the first stage of the nitrification process, ammonia is oxidised into nitrite by *Nitrosomonas* bacteria, and the second nitrification stage involves the oxidation of nitrite into nitrate by *Nitrobacteria*. The biochemical path for the nitrification process is presented by equations [2.1],[2.2] and [2.3] below (Metcalf and Eddy 2014):

$$2NH_4^+ + 3O_2 \xrightarrow{Nitrosomonas} 2NO_2^- + 4H^+ + 2H_2O \quad [2.1]$$

$$2NO_2^- + O_2 \xrightarrow{Nitrobacteria} 2NO_3 \quad [2.2]$$

Total oxidation reaction:

$$NH_4^+ + 2O_2 \xrightarrow{Autotrophic\ bacteria} NO_3^- + 2H^+ + H_2O \quad [2.3]$$

Moreover, according to Wei *et al.* (2014), the denitrification process is the biological reduction of nitrate to nitric oxide, nitrous oxide, and nitrogen gas in the absence of oxygen. During the process of denitrification, the reduction of nitrate can occur in two different modes i.e. assimilating and dissimilating nitrate reduction (Metcalf and Eddy 2014). Assimilating nitrate reduction is associated with conversion or reduction of nitrate to ammonia for use in cell synthesis. Whereas, dissimilating nitrate reduction is imperative for the respiratory electron transport chain, whereby nitrate or nitrite is used as an electron acceptor for the oxidation of a variety of organic or inorganic electron donors (Peng and Zhu 2006; Metcalf and Eddy 2014). The biochemical path of the denitrification process is given by equation [2.4] below (Peng and Zhu 2006; Metcalf and Eddy 2014; Wei *et al.* 2014):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad [2.4]$$

With the latest technology improvement in biological wastewater treatment systems, the process of nitrification and denitrification can take place simultaneously in one vessel by using the activated sludge sequencing batch reactor technology (Lee, Jeon and Park 2001; Daverey *et al.* 2013; Wei *et al.* 2014). Recent studies have indicated that the paradigm that the only way to biologically convert ammonium from wastewater to nitrogen gas (N_2) requires complete oxidation to nitrate followed by heterotrophic denitrification has been antiquated (Khin and Annachhatre 2004; Wei *et al.* 2014). There are a number of novel biological nitrogen removal processes which are used and they are summarised in the subsequent sub-section.

Partial Nitrification

The removal of nitrogen pollutants from wastewater by the process of partial nitrification is defined by She *et al.* (2016) and Schmidt *et al.* (2003) as the oxidation of ammonium into nitrite as the end product. The process is known as nitritation. Conventional nitrogen removal processes use heterotrophic bacteria to facilitate the process of nitrification and denitrification which require a carbon source for metabolic processes (Yongzhen *et al.* 2007; She *et al.* 2016). Whereas, the nitritation process use aerobic chemoautotrophs as ammonium and nitrite oxidising bacteria producing nitrogen gas, which does not require organic matter as a carbon source for metabolic processes, however, these bacteria use carbon dioxide as a carbon source to oxidise inorganic compounds (i.e. NH_4^+ and NO_2^-) to nitrogen gas (Metcalf and Eddy 2014). This process has cemented its application in industrial wastewater treatment with a low carbon : nitrogen ratio. Wei *et al.* (2014) and Yongzhen *et al.* (2007) with practical evidence reported that under temperature conditions above 30˚C, pH levels of above 7.5 and DO concentration between 0.5 and 1 mg/L, the nitritation process is favoured. Moreover, under the above mentioned operating conditions, Wei *et al.* (2014) reported a sludge volume index from 115.6 to 56.6 mL/g.

Anaerobic Ammonium Oxidation (ANAMMOX) process

The ANAMMOX process is basically the anaerobic oxidation of ammonium with nitrite as an electron acceptor and producing nitrogen gas as a final product (Metcalf and Eddy 2014; She *et al.* 2016). Mulder *et al.* (1995) conducted a study on the ANAMMOX process and in their study they observed high ammonium consumption rate with nitrate being an electron acceptor as presented by equation [2.5]. However, van de Graaf *et al.* (1995) and Bock *et al.* (1995) investigated the ANAMMOX process and in their findings they reported that nitrite was the preferred electron acceptor for the process as presented by equation [2.6], which contradicted with what was reported by Mulder *et al.* (1995).

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \qquad [2.5]$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad [2.6]$$

According to Schmidt and Bock (1997), Puyol *et al.* (2014) and She *et al.* (2016), nitrogen removal in the ANAMMOX process is stimulated by ammonium oxidising microbial

community which are able to oxidise ammonium in the presence of gaseous nitrogen dioxide under anoxic conditions. The ANAMMOX microbial community was investigated by Strous *et al.* (1997), who reported that it was an autotrophic bacterium under the *Planctomycetales*, and it required no organic carbon for cell growth, however, it uses carbon dioxide as a carbon source. The ANAMMOX bacteria is characterised by a slow growth-rate, Schmidt *et al.* (2003) reported that it can take 100 – 150 days before an ANAMMOX reactor seeded with activated sludge can reach full capacity.

Completely autotrophic nitrogen removal over nitrite (CANON) process

Zhu *et al.* (2008) and Zhang *et al.* (2015b), reported that the CANON process is a novel biological nitrogen removal process from wastewater with low organic loads. Furthermore, the process is based on partial nitrification and anoxic oxidation of ammonia. This process was motivated by wastewater characterised with high-strength ammonium load and low carbon : nitrogen ratios which has demonstrated a high amount of nitrogen pollutant loss in the form of element nitrogen (Hippen *et al.* 1997; Koch *et al.* 2000; Khin and Annachhatre 2004). This phenomenon is explained by Daverey *et al.* (2013) and Khin and Annachhatre (2004) where they reported that it is as a result of the microorganisms responsible which are characterised as autotrophic microbial population which is able to denitrify under low dissolved oxygen conditions.

Furthermore, in the CANON process, ammonia in wastewater is converted directly to nitrogen gas with nitrite being produced as an intermediate. Hanaki, Wantawin and Ohgaki (1990) investigated the biochemical path of the CANON process and reported that under limited oxygen environment, ammonium is oxidised to nitrite by aerobic nitrifiers as presented in equation [2.7] below:

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad [2.7]$$

Furthermore, with the produced nitrite, the anaerobic ammonium oxidisers convert ammonium directly into nitrogen gas with traces of nitrate being produced. The biochemical path is presented in equation [2.8] below (Strous 2000):

$$NH_4^+ + 1.3NO_2^- \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \qquad [2.8]$$

The wastewater treatment efficacy in the application of the CANON process is highly dependent on the interaction of both the aerobic and anaerobic ammonium-oxidising bacteria. Moreover, the removal of nitrogen from wastewater can be achieved within a single reactor vessel.

The novel biological nitrogen removal processes discussed in this section have demonstrated a number of advantages over the conventional biological nitrogen removal from wastewater namely;

- They require less energy for aeration, thus they are characterised by low sludge production rate which is expensive to handle and dispose,
- These phenomenal processes use a microbial population which does not require an organic carbon source nor chemicals to provide a carbon source, thus making the process easy to operate, and
- The processes discussed in this section can take place in a single reactor tank which associates them with low start-up costs.

However, these novel biological nitrogen removal processes focus on nitrogen removal from wastewater with high-strength ammonium and low organic loads. This study focuses on removal of nitrogen from industrial wastewater with high organic loads since brewery wastewater is characterised by high levels of COD and BOD (Alvarado-Lassman *et al.* 2008; Goldammer 2008; Abimbola *et al.* 2015).

2.1.3 Biological Phosphorus Removal

The conventional biological phosphorus removal results from biomass produced by cell growth by heterotrophic bacteria from biochemical oxygen demand (BOD) removal which contain about 0.015 g P/g VSS. This system is reported to achieve a phosphorus removal efficiency of about 10 to 20% from wastewater (Metcalf and Eddy 2014). However, since the late 1970's a lot of research work has been done on advanced technologies for phosphorus removal. This has led to the design of treatment plant configurations favouring the phosphorus storing bacteria well known as *Polyphosphorus Accumulating Organisms* (PAOs), which have been used to provide a biological phosphorus removal efficiency of more than 80% (McLaren and Wood 1976; Barnard 1998; Liu and Liptak 1999; Metcalf and Eddy 2014). To date, this process has cemented its application in wastewater treatment plants because it is associated with less

sludge production and reduced chemical costs as compared to chemical precipitation (Metcalf and Eddy 2014). This process is now referred as the enhanced biological phosphorus removal (EBPR).

The enhanced biological phosphorus removal process is the biological uptake and removal of phosphorus by activated sludge systems in excess of the amount that is removed by complete aerobic activated sludge systems (Henze *et al.* 2008; Metcalf and Eddy 2014; Saad *et al.* 2016). This phosphorus removal process is characterised by circulation of activated sludge through the anaerobic and aerobic environment, coupled with the introduction of influent wastewater in the anaerobic phase (Mulkerrins, Dobson and Colleran 2004; Saad *et al.* 2016).

According to Grady Jr *et al.* (2011), the enhanced biological phosphorus removal process is a two-step process in which an anaerobic environment or phase is followed by an aerobic phase. During the anaerobic phase polyphosphorus accumulating organisms transport and consume influent that is readily biodegradable in the form of volatile fatty acids by using energy made available from stored phosphorus as polyphosphate, thus enabling the polyphosphorus accumulating organisms to become dominant (Metcalf and Eddy 2014; Saad *et al.* 2016). The anaerobic phase favours the polyphosphorus accumulating organisms because the other heterotrophic bacteria under anaerobic conditions require an electron acceptor such as oxygen, nitrite or nitrate for oxidation reduction reactions to provide energy for substrate utilization organisms (Metcalf and Eddy 2014).

Furthermore, during the aerobic phase or environment in which microorganisms grow new biomass and take up phosphorus, typically more than the amount they released in the anaerobic phase. **Figure 2.1** presents a typical concentration profile of BOD as a substrate and orthophosphorus.

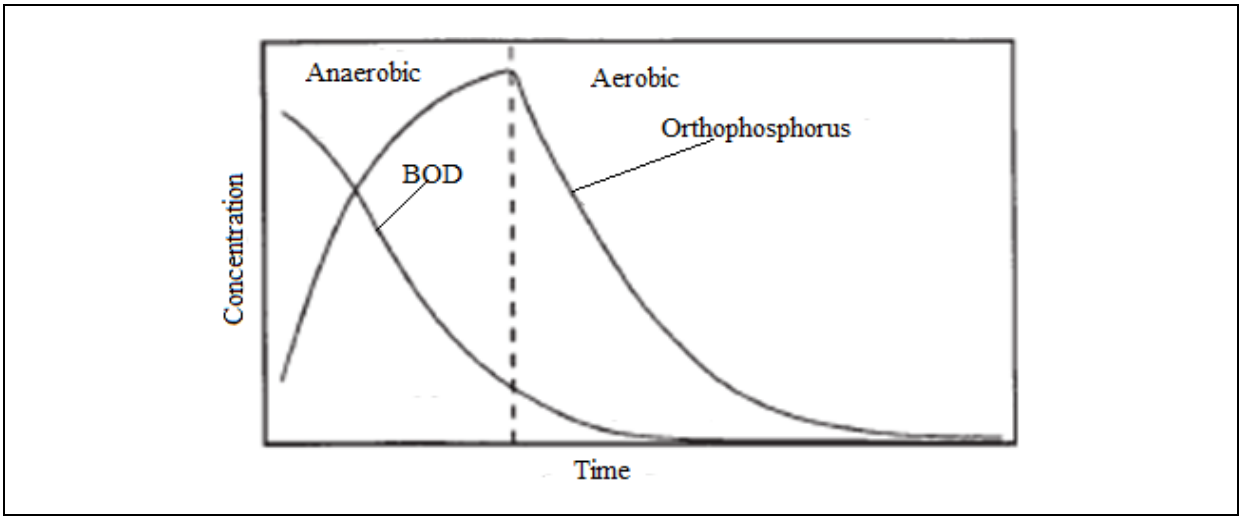


Figure 2.1: Concentration profile of BOD and phosphorus in EBPR process (Liu and Liptak 1999).

The concentration profile presented in **Figure 2.1** above explicitly indicates that during the anaerobic phase, the substrate utilization rate increases rapidly with an increase in orthophosphorus release. However, the orthophosphorus release rate is normally faster than the subsequent orthophosphorus uptake rate in the aerobic phase. Thus the aerobic phase lasts longer than the anaerobic phase for maximum orthophosphates removal (Liu and Liptak 1999; Metcalf and Eddy 2014).

The removal of phosphorus by activated sludge was first noted in the 19th century by Srinath, Sastry and Pillai (1959) and Alarcon (1961). However, in their discoveries, they could not give a clear understanding on the biochemical path which defines phosphorus uptake under aerobic conditions (Henze *et al.* 2008). This wonderful observation opened a door in research on the enhanced biological phosphorus removal process, and over the past decades new processes have been studied and applied for phosphorus removal from wastewater. The subsequent section presents in detail, enhanced biological phosphorus removal systems.

Anaerobic/Oxic (A/O) Process

The anaerobic/oxic process is an activated sludge, two step system which involves both the anaerobic and aerobic treatment phases, as presented in the schematic drawing in **Figure 2.2** below. The anaerobic/oxic treatment mechanism require wastewater influent to be mixed with return activated sludge from the aerobic vessel prior to being fed into the anaerobic tank (McLaren and Wood 1976; Metcalf and Eddy 2014). Both the anaerobic and aerobic vessels

consist of mixers to maintain uniform mixed liquor suspended solids. To meet the oxygen demands in the oxic zone, different aeration methods are used such as fine bubble diffusers, surface mechanical aerators, and oxygen aeration (Liu and Liptak 1999).

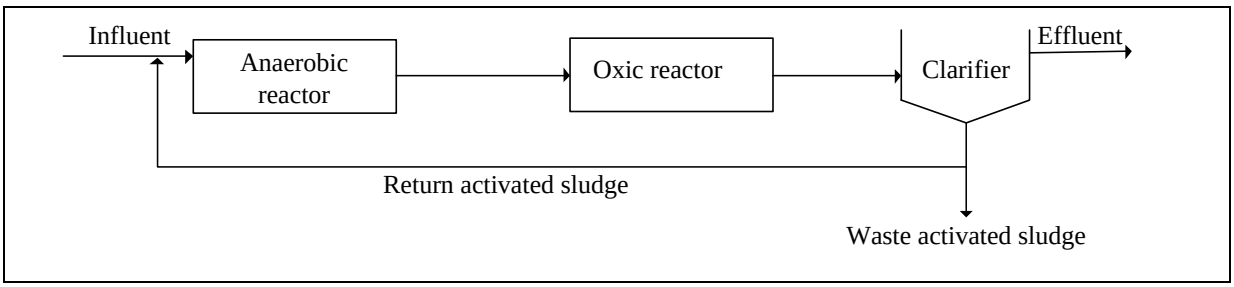


Figure 2.2: Anaerobic/Oxic phosphorus removal process (Liu and Liptak 1999).

The anaerobic/oxic process follows the same biochemical path as presented in **Figure 2.1**. The anaerobic/oxic process application in wastewater treatment plants has demonstrated to be effective for enhanced biological phosphorus removal with a very low hydraulic retention time of about 2.5 to 3.5 hours and a food to microorganisms ratio of about 0.5 to 0.9 L/day (Liu and Liptak 1999). Furthermore, studies have indicated that the process can also be adopted for simultaneous phosphorus removal and nitrification, which could be met by adjusting the sludge age and hydraulic retention time in the aerobic zone (Liu and Liptak 1999; Henze *et al.* 2008). However, there are concerns in the anaerobic zone of excessive nitrate concentrations in return activated sludge from the aerobic zone, therefore the system will require some modification before it can be fully adopted for simultaneous phosphorus removal and nitrification.

PhoStrip Process

According to Liu and Liptak (1999), Henze *et al.* (2008) and Barnard and Comeau (2014), the PhoStrip process is similar to any other enhanced biological phosphorus removal processes, however, this particular process combines both biological and chemical phosphorus removal processes. The PhoStrip process is based on the findings by Levin *et al.* (1972) that aeration of mixed liquor favour a microbial population which take up dissolved phosphorus in excess of the amount required for growth. Furthermore, during the anaerobic environment microorganisms consume all of the dissolved oxygen and release phosphorus which was taken up during aerobic conditions, and during aerobic conditions microorganisms take up phosphorus.

According to Henze *et al.* (2008) and Barnard and Comeau (2014), the PhoStrip process consists of a single aeration vessel with a clarifier. The process diverts from other enhanced biological phosphorus removal processes because it consists of a side-stream enriched with phosphorus (with a flow rate of about 10 to 30% of the influent flow rate) from the underflow of the clarifier to an anaerobic stripper which promotes the release of intracellular phosphate. The phosphorus enriched supernatant is then treated with lime in a precipitator tank to chemically precipitate phosphorus which is settled and wasted. The precipitator tank supernatant is either returned to the influent for further phosphorus removal or taken to the effluent stream. The application of the PhoStrip in wastewater treatment has demonstrated higher phosphorus removal efficiencies. Jin *et al.* (2014) conducted a study on treating domestic sewage using the Phostrip process and lime as the phosphorus removal chemical. They reported overall removal efficiencies on COD, TN and TP of 86, 62.8 and 98.1% respectively.

Other Enhanced Biological Phosphorus Removal Processes

With practical research evidence presented by Fuhs and Chen (1975) and Barnard (1947), the enhanced biological phosphorus removal process from waste streams follow the same phosphorus removal mechanism which is basically subjecting activated sludge microbial population to a sequence of anaerobic and aerobic conditions. Furthermore, Fuhs and Chen (1975) studied the microbiological aspect of the enhanced biological phosphorus removal process and they reported that the *Aeinetobacter* is the main organism genus which stimulates phosphorus removal. There are other enhanced biological phosphorus removal processes which are used, however, they all follow the same phosphorus removal mechanism processes like the Phoredox method which was introduced by Barnard (1976).

For the Phoredox phosphorus removal process Barnard (1976), explained the phosphorus removal mechanism from a different point of scientific view. In the Phoredox method Barnard (1976) states that it is not the phosphorus release which stimulates phosphrus removal, however, the release of phosphorus indicates the establishment of a low redox in the anaerobic environment, therefore phosphorus removal is stimulated by the low redox potential. In further studying the Phoredox process, Barnard (1976) experienced some challenges in measuring the redox potential which led to a conclusion that by measuring phosphorus release in the anaerobic zone could serve as a parameter to indicate that conditions necessary for enhanced biological phosphorus removal prevailed (Henze *et al.* 2008). In preventing nitrate from affecting the

phosphorus removal process in the Phoredox system, the retention time of the anaerobic reactor is increased to one hour which is suggested to be the nominal time (Liu and Liptak 1999; Henze *et al.* 2008).

To date, studies are still being conducted in improving the phosphorus removal processes from waste streams, and the sequencing batch reactor system has been investigated for biological nutrient removal, not just phosphorus removal (Mittal 2011). Enhanced biological phosphorus removal can be achieved by employing the sequencing batch reactor system. This phosphorus removal system has cemented its application in wastewater treatment plants due to its flexible operation configuration (Mittal 2011; Metcalf and Eddy 2014). Phosphorus removal in the sequencing batch reactor system is met by peforming a sequence of operations which are basically anaerobic, mixing, aeration and clarification with all steps taking place in a single reactor (Liu and Liptak 1999; Mittal 2011; Metcalf and Eddy 2014).

The sequencing batch reactor system application in wastewater treatment for phosphorus removal has demonstrated some advantages over other enhanced biological phosphorus removal systems. The sequencing batch reactor system can accomplish biological phosphorus removal alone or it can be modified to accomplish phosphorus removal with nitrification (Manning and Irvine 1985; Metcalf and Eddy 2014).

2.2 Parameters Affecting Biological Nutrient Removal

There are a number of factors which can limit the performance of any biological nutrient removal system from wastewater, thus they need to be taken into consideration. The subsequent sub-sections discuss some of these factors.

2.2.1 Wastewater composition

The composition of industrial wastewater fluctuates significantly depending on the activities taking place inside. Biological nutrient removal processes are sensitive to disturbances caused by pollutant fluctuations in wastewater which result in sudden drastic changes to the system (Shehab *et al.* 1996; Mulkerrins, Dobson and Colleran 2004). Fluctuations of organic loads in influent streams compromise the treatment efficiency of biological nutrients removal, in particular phosphates which can increase about 60% in the effluent stream (Carucci *et al.* 1999).

Furthermore, biological nutrient removal processes involve the use of microorganisms which are sensitive to disturbances. Changes in influent organic pollutants composition, such as from volatile fatty acids to sugars, stimulate the accumulation of glycogen accumulating organisms (Satoh, Mino and Matsuo 1994). Moreover, it is imperative to determine the optimal COD loading rate since excessive COD loading rates can lead to deterioration of the nutrient removal process (Satoh, Mino and Matsuo 1994). According to Randall, Barnard and Stensel (1992), for optimal removal of phosphorus the influent in the anaerobic zone of the biological phosphorus removal process should have a BOD : TP ratio of > 20 : 1 or COD : P ratio of > 40 : 1. Based on the above discussed parameters it is clear that wastewater composition or pollutant concentration can have a direct effect on the treatment efficiency for biological nutrient removal.

2.2.2 Temperature

According to Metcalf and Eddy (2003) and Gou *et al.* (2014), the performance or efficiency of any biological process can be greatly affected by temperature, since it has a direct effect on the metabolic activities of the microbial population, the gas transfer rate as well as the settling characteristics of bio-solids. Furthermore, Mulkerrins, Dobson and Colleran (2004) reported that biological nutrient removal higher effeciencies are achieved at a temperature range of 20 to 37˚C. However, Mulkerrins, Dobson and Colleran (2004) also indicated that there is available literature in contrast, which state that better nutrient removal particularly phosphorus removal is observed at a lower temperature range of 10 to 15˚C. The light in understanding the contrast in optimum temperature range is given by Metcalf and Eddy (2003), where it was indicated that microorganisms are classified according to certain temperature ranges in which they function best. Therefore, bacteria is classified as *chrophilic, mesophilic,* or *thermophilic* with an optimum temperature range of 12 to 18˚C, 25 to 40˚C and 55 to 65˚C respectively. Furthermore, the different bacteria categories growth rate doubles with approximately every 10˚C increase in temperature until the optimum temperature is reached (Metcalf and Eddy 2003).

Brdjonovic *et al.* (1997) investigated the effect of temperature on dissolved oxygen consumption rate for biological phosphorus removal. In their findings, they reported that incomplete phosphorus uptake was observed at temperatures between 5 and 10˚C in the aerobic environment. Moreover, at temperatures between 20 and 30˚C complete phosphorus uptake

was observed. Similar observations were reported for the nitrification process (Mulkerrins, Dobson and Colleran 2004). Gou *et al.* (2014) reported that temperature has a significant effect on metabolic activities of microbial population compared to the organic loading rate.

2.2.3 pH control

From basic chemistry knowledge, lower pH values indicate acidic environment and higher pH values indicate alkaline environment. According to Carrera *et al.* (2003), the optimum pH range is from 7.5 to 8.5 for effective microbial population activity for the nitrification process. Furthermore, Carrera *et al.* (2003) indicates that higher pH values are associated with the equilibrium ammonium-ammonia being displaced to ammonia, thus inhibiting the nitrification process. Metcalf and Eddy (2014), explains that the pH of an environment is a major factor in the growth rate of organisms, moreover, at pH levels of above 9.5 or below 4.0, microbial population metabolic activities are inhibited. Furthermore, Surampalli *et al.* (1997), with practical evidence, reported that the nitrification rate decreases with pH. At pH levels of 6 and below the nitrification process ceases.

2.2.4 Dissolved Oxygen (DO)

In a combined biological nutrient removal process it is imperative for the process to be designed such that it satisfies the oxygen demand for all microbial communities present in the system (Mulkerrins, Dobson and Colleran 2004). Louzeiro *et al.* (2002) investigated the potential for denitrification and phosphorus removal of a full-scale sequencing batch reactor, with the use of methanol as an external carbon source. In their findings, they reported that for biological nutrient removal, DO levels of at least 2 mg/L are required and the optimum concentration range is 3 to 4 mg/L for nitrification. Moreover, DO levels greater than 4 mg/L do not stimulate any biological nutrient removal, thus they are associated with a waste of energy in terms of aeration (Mulkerrins, Dobson and Colleran 2004).

2.2.5 Sludge quality and settleability

Zhang *et al.* (2015a) defines sludge settling as the separation from water by gravitational means of suspended particles which are heavier than water. A good settling sludge is imperative for a solid free effluent, thus improving the treatment efficacy of wastewater treatment processes. According to Metcalf and Eddy (2003), the characteristics of activated sludge is determined by the sludge volume index (SVI), which is basically the volume of 1 g of sludge after 30 minutes

of settling. Furthermore, SVI values below 100 are desired and values above 150 are normally associated with filamentous growth.

Moreover, according to Mulkerrins, Dobson and Colleran (2004), activated sludge treatment facilities experience filamentous bulking which result when "filamentous organisms proliferate to such an extent as to interfere with the proper compaction of settling sludge." Andreasen and Sigvardsen (1996) investigated the settling properties of activated sludge. In their findings, they reported that phosphorus removal processes have demonstrated good sludge settling characteristics, while plants performing simultaneous denitrification have demonstrated otherwise. Chang, Chiou and Ouyang (1996) investigated the sludge settleability for a process treating wastewater with high COD : P ratio. In their study they reported SVI values fluctuating between 69 and 370 mL/g with phosphorus removal of 75% on average. Krishna and van Loosdrecht (1999) with practical evidence, reported that for the sequencing batch reactor system, the SVI increased with temperature from 15 to 35˚C.

2.2.6 Volatile Fatty Acids (VFA)

According to Comeau *et al.* (1996), one of the factors considered for process optimisation in biological nutrient removal processes is maximasing VFA production, particularly in phosphorus removal systems. The effect of VFA on nutrient removal was investigated by Pitman (1999) and Ruel *et al.* (2002) where they reported that VFA is essential for effective biological phosphorus removal. For every 1 mg of phosphorus removed, about 7 – 9 mg of VFA is needed. Moreover, effluent phosphorus levels of 0.2 – 0.3 mg/L has been reported by Oldham *et al.* (1994) where they used VFA to stimulate phosphorus removal. Apart from VFA there are other organic compounds which can be used by PAOs under anaerobic environment compounds such as carboxylic acids, sugars and amino acids. Moreover, the maximum rate of phosphorus release under anaerobic conditions can be achieve when acetate and propionate are utilised as the carbon source (Satoh *et al.* 1996).

2.2.7 Solids Retention Time (SRT)

The SRT is defined as the average period of time that sludge has remained in the system. For activated sludge process design the SRT is considered as a critical parameter since it affects the process performance, reactor volume, sludge production, and oxygen requirements. The SRT may vary from 3 to 5 days depending on the mixed-liquor temperature. Under mesophilic

conditions a SRT of 3 days is desired. However, under low temperature conditions the SRT tends to be longer since under operation conditions of 10˚C a SRT of 5 to 6 days is recommended by Metcalf and Eddy (2003).

A number of studies have been conducted on SRT variation for optimum biological nutrient removal, in particular for phosphorus removal. Mamais and Jenkins (1992) obtained higher nutrient removal at SRT greater than 2.9 days. Moreover, Chuang *et al.* (1998) achieved higher phosphorus removal at a SRT of 10 days. Chang, Chiou and Ouyang (1996) investigated SRT variation on phosphorus removal using a SRT of 5, 10 and 15 days and in their findings they reported a higher phosphorus removal efficiency at a SRT of 10 days. On the other hand, Ge, Batstone and Keller (2015) investigated the performance of an SBR for enhanced phosphorus removal under very low SRT between 0.5 to 2 days using abattoir wastewater with a high organic loading rate. The findings of their study reported a stable EBPR system with SRT of 2 to 2.5 days with 80 % COD and phosphorus removal.

2.3 Brewery Wastewater

The brewing industry is considered to be one of the industries which use voluminous quantities of fresh water and generate enormous amounts of wastewater during beer production. Industrial wastewater generated from the brewery is characterised by large quantities of toxic chemicals, biological nutrients and organic matter pollutants. These pollutants may pose serious risk to the environment, particularly to water receiving bodies if not properly treated prior to being disposed (Goldammer 2008; Geoffrey *et al.* 2011; Abimbola *et al.* 2015).

The subsequent sub-sections of this chapter focus on brewery wastewater characteristics, biological methods that are used for brewery wastewater treatment as well as previously done studies on wastewater.

2.3.1 Characteristics of brewery wastewater

Wastewater from the brewery contains high concentrations of biodegradable organic and inorganic pollutants which are characterised by the 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), ammonia, and total suspended solids (TSS) (Gregor, Matej and Milenko 2007; Fu *et al.* 2013; Bakare, Shabangu and Chetty 2017). The organic pollutants

which are found in brewery wastewater are mainly sugars, soluble starch, ethanol, volatile fatty acids and many more (Goldammer 2008).

Previously done studies on brewery wastewater indicate that pollutant composition in brewery wastewater fluctuates greatly depending on the activities taking place inside the brewing house (Cronin and Lo 1998; Carrera *et al.* 2003; Goldammer 2008; Fu *et al.* 2013). The brewing process involves a number of batch operations which require voluminous water usage when processing raw material to the final beer product (Parawira *et al.* 2005; Geoffrey *et al.* 2011; Abimbola *et al.* 2015). Moreover, after each batch large volumes of water are used for general washing of floors, cleaning the brewing-house, cellars and cleaning-in-place, thus resulting in the production of brewery effluent high in organic and inorganic pollutants as well as being acidic (Parawira *et al.* 2005; Tansiphorn, Suraphong and Prasert 2009).

According to a research study conducted by Goldammer (2008), brewery wastewater have temperatures ranging from 25 to 38˚C, but occasionally reaching much higher temperatures. pH levels range from 2 to 12 and are influenced by the amount and type of chemicals used in the cleaning and sanitizing process which are normally caustic soda, phosphoric acid and nitric acid. Furthermore, besides the organic pollutant characteristics of brewery wastewater mentioned above which are BOD_5, COD and TSS, brewery wastewater is also characterized by the concentration of biological nutrients, particularly nitrogen as nitrates and phosphorus as orthophosphates (Ochieng, Odiyo and Mutsago 2003; Goldammer 2008). Wastewater from the brewery requires treatment prior to being discharged into receiving bodies since it contains a high-strength of organic and inorganic pollutants which require oxygen for degradation (Geoffrey *et al.* 2011). **Table 2.1**, which was formulated from previously done research work on brewery wastewater, presents pollutant composition in brewery wastewater.

Table 2.1: Characteristics of brewery wastewater before treatment (Young-Ho, Kyung-Sok and Richard 2001; Parawira *et al.* 2005; Alvarado-Lassman *et al.* 2008; Abimbola *et al.* 2015).

Parameter	**Range** (Abimbola *et al.* 2015)	**Average** (Alvarado-Lassman *et al.* 2008)	**Range** (Parawira *et al.* 2005)	**Range** (Young-Ho, Kyung-Sok and Richard 2001)
pH	4.6 – 7.3	10.0	3.30 – 6.30	6.3 – 7.0
TCOD	1 096 – 8 926	2 083	8 240 – 20 000	920 – 1 910
SCOD	1 179 – 5 848	1 726	---	680 – 1 560
BOD_5	1 609 – 3 980	1 375	---	730 – 1 470
COD : BOD	---	1.51	---	---
TSS	---	750	2 901 – 3 00	61 - 378
TS	1 289- 12 248	---	5 100 – 8 750	---
TDS	---	---	2 020 – 5 940	---
VSS	961 – 1 483	---	---	43 - 200
TP	---	4.8	16 - 124	5.3 – 12.5
TN	---	---	0.019 – 0.033	---
TKN	---	116	---	16.4 – 36.4
$N\text{-}NH_4$	0.48 – 13.05	13.3	---	3 – 11.5
Temp., ˚C	24 – 30.5	---	25 - 35	---
Nitrate	1.14 – 11.55	---	---	---
Nitrite,	0 – 0.24	---	---	---

*All parameters presented in **Table 2.1** above are in mg/L except otherwise stated.

The information presented in **Table 2.1** above on brewery wastewater characteristics explicitly indicate that pollutant composition in brewery wastewater fluctuates greatly. Furthermore, the data presented in **Table 2.1** above indicates that there is less reporting or work done on characterising brewery wastewater for nitrogen in the form of nitrates, nitrite, and total nitrogen. Therefore, brewery wastewater needs to be treated for the above mentioned organic and inorganic pollutants before being discharged to receiving bodies, thus minimising the environmental pollution.

2.4 Brewery wastewater treatment methods

Brewery wastewater requires treatment before being discharged into water receiving bodies i.e. lakes, rivers, streams, ocean and municipal sewer. However, in the case whereby brewery

wastewater is discharged into municipal sewer, it requires to undergo a pre-treatment stage to lessen organic loads to allowable limits imposed by municipalities thus improving treatment efficiency (Geoffrey *et al.* 2011).

There are basically three main modes of operation used to treat not only brewery wastewater but wastewater in general, namely: 1) primary treatment, which is the first treatment stage of wastewater which involves the application of physical forces to remove contaminants particularly course and suspended solid matter. The physical operations include screening, flow equalization, grift removal and gravity sedimentation; 2) secondary treatment, is designed for further reduction of organic pollutants by means of applying both biological and chemical operations (i.e. trickling filter, granular filtration, activated sludge processes, aerated lagoons, chlorination and flocculation); 3) tertiary treatment, is considered to be the final treatment stage of wastewater for reuse. It involves both biological and chemical operations i.e. membrane filtration, membrane bio-reactor, reverse osmosis and ion exchange (Goldammer 2008; Geoffrey *et al.* 2011). It is imperative for brewery wastewater to undergo at least the first two treatment stages (i.e. primary and secondary treatment) prior to discharge into water receiving bodies to minimise organic pollutants thus avoiding severe environmental pollution (Fu *et al.* 2013).

2.4.1 Brewery wastewater biological treatment methods

Wastewater from either municipalities or industries is characterised by having soluble organic pollutants. Biological treatment has been considered important and an integral part of any wastewater treatment plant that treats wastewater from the above mentioned wastewater sources (Arun 2011). Biological treatment processes for brewery wastewater involves the use of micro-organisms particularly bacteria which convert dissolved and particulate carbonaceous organic matter into simple end-products and additional biomass (Metcalf and Eddy 2003; Geoffrey *et al.* 2011).

Based on available literature, biological methods are considered to be appropriate for brewery wastewater treatment since it is characterised by a high-strength of microbial contaminants which are generally treated by biological methods (Gregor, Matej and Milenko 2007; Shao *et al.* 2008). This technology has cemented its place in wastewater treatment plants particularly brewery wastewater treatment plants over other processes such as chemical oxidation, thermal oxidation etc. due to its obvious economic advantage, both in terms of capital investment and

operation costs (Arun 2011). Biological treatment methods of brewery wastewater can be either aerobic, anaerobic, or anoxic (Goldammer 2008; Shao *et al.* 2008) as discussed in more detail in the subsequent sections.

2.4.1.1 Aerobic biological treatment processes for brewery wastewater

Aerobic biological treatment can be defined as a process which takes place in the presence of oxygen using micro-organisms called aerobes particularly bacteria that metabolize organic pollutants in wastewater thereby producing biomass and inorganic end-products (Arun 2011; Geoffrey *et al.* 2011). Traditionally brewery wastewater is treated by employing aerobic processes (Shao *et al.* 2008). In practice there are multitudes of aerobic and anaerobic biological treatment processes, however, in this dissertation three processes which are widely applicable in industrial wastewater treatment, specifically brewery wastewater are discussed namely; 1) conventional activated sludge process system; 2) cyclic activated sludge process system; 3) membrane bioreactor system. However, research studies conducted on brewery wastewater treatment indicates that aerobic treatments require an intensive amount of energy for aeration. Furthermore, aerobic treatment processes produce large amounts of wasted sludge, thus making the process to be costly since large capital is required for the handling and disposal of sludge (Parawira *et al.* 2005; Shao *et al.* 2008; Matthew *et al.* 2010).

Conventional activated sludge process system

This technology is considered to be the ancient and most common biological treatment process used to treat both municipal and industrial wastewater. After the primary treatment stage of wastewater i.e. the removal of suspended solids particularly in brewery wastewater, the conventional activated sludge process which is basically a biological process is then employed as the next treatment stage for wastewater. The system consists of an aeration vessel/tank which could be absolute mixed or a plug flow bioreactor operating under specific concentration of biomass measured as mixed liquor suspended solids (MLSS) maintained with sufficient dissolved oxygen for effective biodegradation of solute organic impurities. The MLSS from the aerated vessel is allowed to overflow by gravity to a secondary clarifier unit to separate out biomass from clarified treated water (Metcalf and Eddy 2003; Arun 2011; Geoffrey *et al.* 2011). **Figure 2.3** is a schematic presentation of a conventional activated sludge process giving an overview summary of the process.

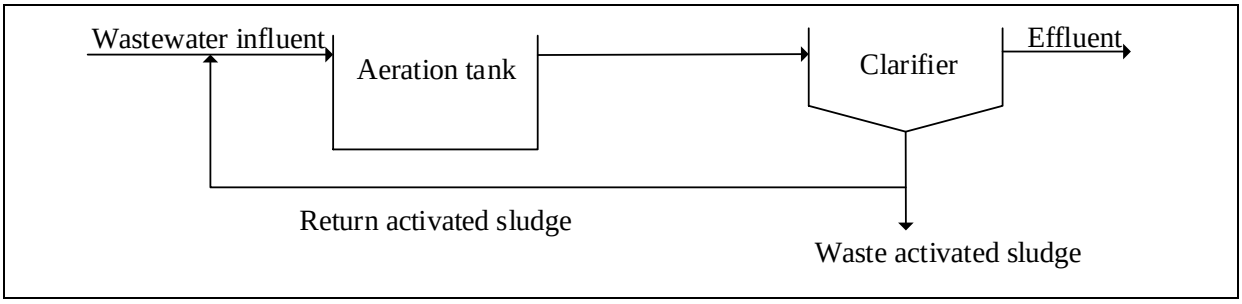


Figure 2.3: Conventional activated sludge process diagram (Arun 2011).

The Jet Loop Reactor (JLR) is considered to be widely employed in the conventional activated sludge process system for wastewater treatment (James, Anderson and Willey 1995). Yildiza *et al.* (2005), investigated the suitability of an aerobic JLR for biological treatment of wastewater with a high strength of organic pollutants, and brewery wastewater is characterised with a high strength of organic pollutants as mentioned in **section 2.3** of this chapter. In their study a treatment efficiency of 97% in terms of COD removal was attained, for an organic load of 68 kg COD/m^3.day over a period of 10 weeks. However, in their study, the treatment efficiency decreased to 60% for higher organic loads and it was concluded that the decrease in treatment efficiency was as a result of insufficient oxygen intake. Aerobic activated sludge processes are associated with biomass or sludge formation. Yildiza *et al.* (2005), in their study at a food/microorganism ratio of above 17/day sludge with unsatisfactory settling characteristics formed under turbulent conditions.

Furthermore, the performance of a high rate aerobic JLR activated sludge process for biological treatment of brewery wastewater was investigated by James, Anderson and Willey (1995). In their study, a treatment efficiency of 97% in terms of COD removal was attained with a loading rate of 50 kg COD/m^3.day over a period of 5 weeks. The study also gave acceptable sludge settleability with a maximum growth rate of 12.2/day and a yield of 0.4 kg VSS produced/kg COD removed. Eusebio *et al.* (2004) also investigated the performance of an aerobic JLR for biological treatment of winery wastewater. In their study an overall treatment efficiency of 80% in terms of COD removal was achieved at a retention time range of 0.8 – 1 day. Based on the studies discussed above which were conducted by (James, Anderson and Willey 1995; Eusebio *et al.* 2004; Yildiza *et al.* 2005). The aerobic JLR activated sludge system under the mentioned operating conditions demonstrated higher treatment efficiencies which makes it suitable for brewery wastewater pre-treatment with a 97% treatment efficiency. Moreover, based on the unsatisfactory sludge settling obtained by Yildiza *et al.* (2005), in their study a

recommendation can be made that further investigation needs to be done in the reactor aeration system to be able to handle higher organic loading rates.

Cyclic activated sludge system (CASS) or Sequencing Batch Reactor (SBR) System

To date, wastewater from municipal sewers and a variety of industries including food processing plants, brewery wastewater treatment plants, refineries and petroleum wastewater treatment plants use the cyclic activated sludge treatment system (Arun 2011). The CASS is basically one of the most popular sequencing batch reactor (SBR) systems employed in treating wastewater from the above mentioned wastewater sources. This biological treatment system of wastewater has cemented its application in wastewater treatment plants because it offers several operational and performance advantages over conventional ASP systems namely; 1) performs all the functions of a conventional activated sludge process in a single basin under alternating mode of operation; 2) offers a methodology that has operational simplicity, flexibility and reliability which is not available in conventionally configured activated sludge systems (Metcalf and Eddy 2003; Arun 2011).

To date, studies are conducted on biological nutrient removal from industrial wastewater particularly brewery wastewater for further improvement of the SBR technology which has cemented its application in most wastewater treatment plants (Arun 2011). Rodrigues, Brito and Melo (2001) investigated the performance of a SBR for biological post-treatment of brewery wastewater rejected by an upflow anaerobic sludge blanket reactor. The main objective of their research study was the removal of biological nutrients, specifically the nitrogen group pollutants from brewery wastewater to achieve the required wastewater quality discharge to surface water. In their study, the SBR was operated in an aerobic-anoxic sequence to allow simultaneous nitrification and denitrification processes to take place. An ammonium ($NH_4 – N$) removal efficiency of 97% was achieved at a maximum rate of 0.175 kg $NH_4 – N$/kg VSS.day, however, it was reported that the denitrification process was supressed when the bulk liquid oxygen concerntration was increased above 6 mg O_2/L.

Based on the above mentioned findings of the study, it can be concluded that the SBR technology demonstrated a high treatment efficiency on biological nutrient removal from brewery wastewater when operated under the above mentioned conditions. Moreover, in their study, they did not experience any sludge settling challenges which is a fundamental factor to

be observed when employing any activated sludge treatment process particularly the SBR system for wastewater treatment.

Ab Halim *et al.* (2015) conducted a study using an aerobic SBR under inoculum sludge from a conventional activated sludge wastewater treatment system. The main objective of their research was to study the formation of aerobic granular sludge for simultaneous organic and nutrient removal with a 3 h complete cycle. The SBR was operated at 50 ± 1˚C. The findings of their study reported 85% COD removal efficiency while ammonia nitrogen and total phosphorus removal efficiencies were up to 88% and 70% respectively, at a loading rate of 1.6 kg COD/m^3.day. Moreover, aerobic granular sludge was successfully cultivated with excellent settling ability. The study demonstrated that aerobic SBR has a high treatment efficiency of wastewater in terms of biological nutrient removal when operated under the mentioned conditions. However, from a practical point of view, the process seems to be expensive to be implemented because it requires energy to elevate temperatures within the reactor since brewery wastewater/effluent temperature ranges between 24 and 30.5˚C as confirmed by Abimbola *et al.* (2015).

Membrane bioreactor (MBR) process system

Membrane bioreactor (MBR) process systems have been extensively used for various industrial wastewater treatment plants due to its distinct advantages over conventional technologies (Hongjun *et al.* 2012). Moreover, the MBR system is considered to be the latest technology for biological degradation of soluble impurities in wastewater (Metcalf and Eddy 2003; Arun 2011). This technology is similar to the conventional activated sludge process. Both systems have mixed liquor solids in suspension in an aeration vessel. However, in a MBR process the bio-solids are separated by means of a polymeric membrane based on micro-filtration, ultrafiltration, nanofiltration or hyper-filtration as presented by the enhanced membrane bioreactor schematic diagram in **Figure 2.4** which is the most adopted system in industries (Metcalf and Eddy 2003; Arun 2011; Geoffrey *et al.* 2011). The MBR system in its simplest form consists of two primary parts which are the biological unit responsible for the biodegradation of waste compounds and the membrane module for the physical separation of treated water from mixed liquor (Cicek 2003).

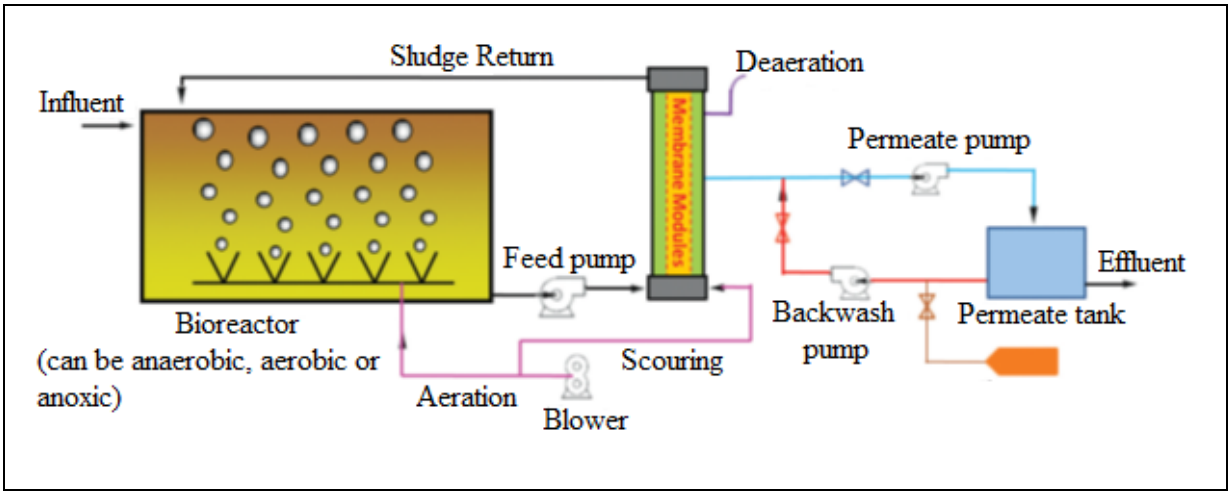


Figure 2.4: Enhanced membrane bioreactor schematic diagram (Arun 2011).

The MBR wastewater treatment process is mostly used at the tertiary treatment stage of wastewater, particularly industrial wastewater for reuse (Geoffrey *et al.* 2011). A research study was conducted by Ahna *et al.* (2006) to investigate the treatment efficiency of an aerobic membrane bioreactor in treating wastewater for organic and nitrogen compound pollutants. In their study, the aerobic membrane bioreactor was operated under mesophilic temperature and pressure conditions. Average removal efficiencies of organic pollutants and nitrogen were reported to be 99 and 46% respectively at a hydraulic retention time (HRT) of 24 h. Organic and nitrogen concentrations in the influent stream ranged from 6 000 to 14 500 mg/L and 300 to 1 000 mg/L respectively. Based on the findings of the study, the nitrogen removal efficiency was found to be less than 50%, therefore from a scientific point of view, Ahna *et al.* (2006) needs to do more work on the process before being implemented probably investigate its performance when using a higher HRT to achieve higher nitrogen removal.

2.4.1.2 Anaerobic biological treatment processes for brewery wastewater

Anaerobic wastewater treatment is the biological treatment of wastewater without the use of oxygen (Geoffrey *et al.* 2011). The anaerobic treatment process involves the use of micro-organisms called anaerobes, which does not require the element oxygen to assimilate organic impurities, thus producing methane, carbon dioxide and biomass as end-products (Arun 2011). These anaerobic micro-organisms include fermentative bacteria, acetogenic bacteria and methanogens, which are responsible for digestion of organic pollutants through multiple degradation steps such as hydrolysis, fermentation, acetogenesis and methanogenesis (Parawira *et al.* 2005; Hill 2015). The biochemical path for the anaerobic process particularly for brewery wastewater treatment is shown in **Figure 2.5** below.

This biological treatment has cemented its application in most wastewater treatment plants particularly wastewater that is characterised by a high-strength of organic/biodegradable pollutants, over the aerobic treatment process for a number of advantages, namely: 1) the anaerobic process produces less biomass, thus requiring less biomass disposal costs which makes the process cost effective; 2) less energy is required because no aeration is needed; 3) produce methane which can be used for energy generation (Metcalf and Eddy 2003; Arun 2011; Geoffrey *et al.* 2011).

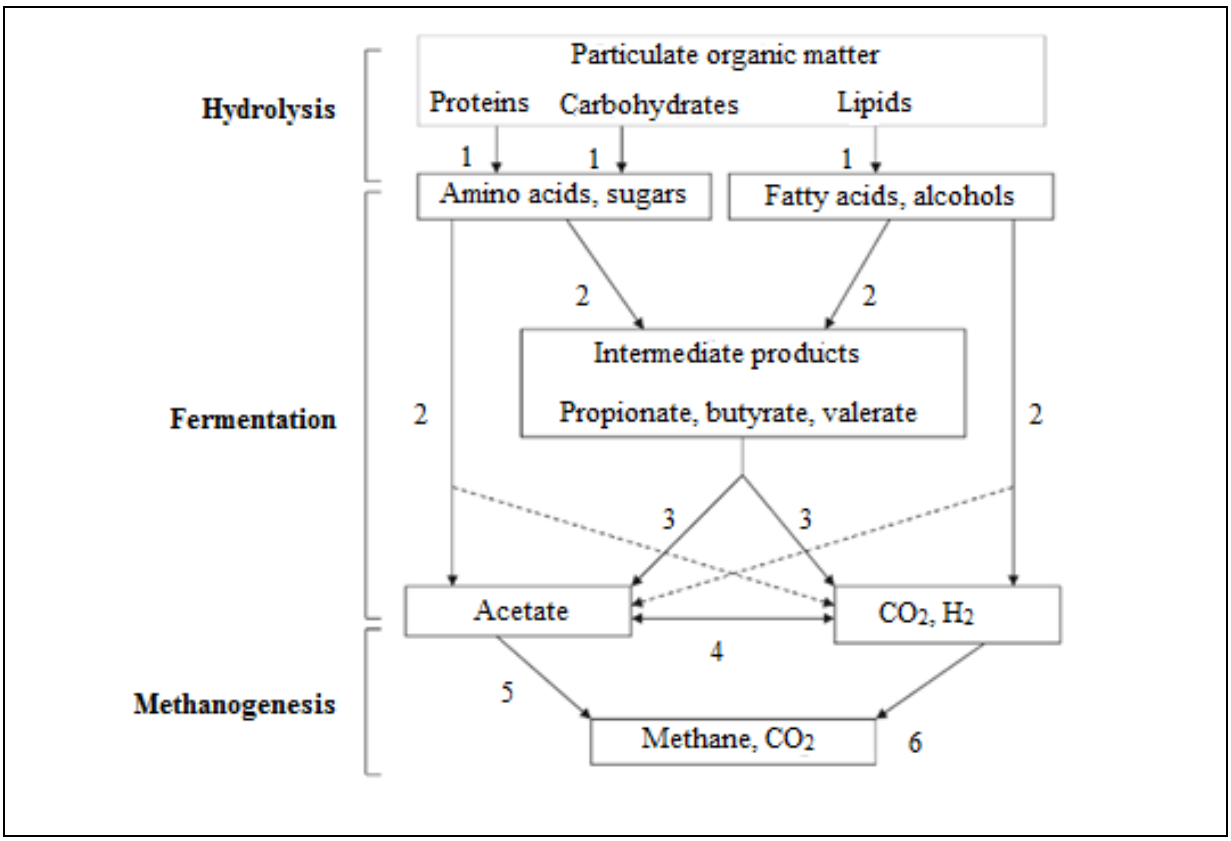


Figure 2.5: Schematic diagram of different reactions involved in anaerobic digestion of complex organic matter in wastewater (Hill 2015).

According to Hill (2015), the anaerobic process biochemical path presented in **Figure 2.5** can be explained as follows:

1. Hydrolysis of complex polymers by extracellular enzymes to simpler soluble products.
2. Fermentative or acidogenic bacteria convert simpler compounds to short-chain fatty acids, alcohols, ammonia, hydrogen and carbon dioxide.
3. Break down of short-chain fatty acids to acetate, hydrogen and carbon dioxide, which act as substrates for methanogenic bacteria.

4. Reaction carried out by acetogenic bacteria.
5. About 70% of methane is produced by aceticlastic methanogens using acetate as a substrate.
6. Methane production by hydrogenophillic methanogens using carbon dioxide and hydrogen.

Application of Anaerobic Sequencing Batch Reactors in Wastewater Treatment

From available documented literature, in recent years there has been an increasing interest in the application of anaerobic treatment for brewery wastewater since the nature and strength of brewery wastewater often provides ideal conditions for anaerobic operations (Parawira *et al.* 2005). Shao *et al.* (2008) investigated the performance of a pilot-scale SBR in treating brewery wastewater for organic pollutants. The reactor was operated under mesophilic conditions at a controlled temperature of ±33˚C, with a working reactor volume of 45 L. The findings of their study reported a COD removal of more than 90% for a controlled organic loading rate between 1.5 kg COD/m^3.d and 5.0 kg COD/m^3.d at a HRT of one (1) day. Furthermore, sludge granulation was achieved in the reactor at approximately 60 days. Moreover, in their study a high treatment efficiency of more than 90% was achieved at a HRT of one day which is less than the reported HRT by Kim *et al.* (2006) of 8 to 12 days under mesophilic conditions. Their work demonstrated that the anaerobic SBR technology can be implemented for brewery wastewater treatment operating under the mentioned organic loading rate. However, nothing was reported on the amount of methane produced, therefore, futher investigation has to be conducted in determining the quantity of methane produced by the system.

Wang *et al.* (2010) investigated the effect of high-strength ammonia nitrogen acclimation on sludge activity in a SBR system. In their study, two batch experiments were conducted to treat a series of wastewater influent with ammonia nitrogen concerntration ranging from 59 to 1152 mg/L, one with activated sludge acclimated to higher ammonia nitrogen concerntration and the other with unacclimated activated sludge. The findings of their study reported a COD removal greater than 83.81% and ammonia nitrogen removal of 99.83% for all 5 experimental runs respectively, for activated sludge acclimated to higher ammonia nitrogen. Furthermore, a COD removal of 54.16% and ammonia nitrogen removal of 61.98% was reported for the unacclimated sludge treatment process. In their research study, it was found that activated sludge acclimated to higher ammonia nitrogen concerntrations revealed higher COD and ammonia nitrogen removal efficiencies. The findings of their study indicated that the activities

of bacteria in activated sludge were inhibited by high-strength ammonia nitrogen influent, whereas the activated sludge acclimated to high-strength ammonia nitrogen showed substantial resistance to inhibition by influents containing high levels of ammonia nitrogen. Based on the findings of their study it is imperative for activated sludge to be acclimated depending on the nauture of influent to be treated, to allow micro-organisms in activated sludge to adapt to the new environment, thus improving influent treatment efficiency.

2.5 Microbial Growth Kinetics

Metcalf and Eddy (2014) wrote that "the performance of biological processes used for wastewater treatment depends on the dynamics of substrate utilization and microbial growth," therefore it is imperative to understand the biological reactions involved in the process for effective process design and operation. The study of microbiology kinetics focus on the dynamic manifestations of microbial life i.e. growth itself, survival and death, adaptations, mutations, product formation, cell cycles, interaction with environment and other organisms (Panikov 2014). The kinetics of microbial growth focus on the oxidation of substrate and the production of new biomass which is considered to contribute in concentration of total suspended solids and volatile suspended solids. In the case of industrial wastewater, particularly brewery wastewater, the concentration of organic compounds is given by the biodegradable chemical oxygen demand (COD) or biochemical oxygen demand (BOD), since they comprise of dissolved, colloidal, and particulate biodegradeable components.

In the field of science and technology, the ability to understand and describe a process in mathematical terms is central to our paradigms for analysis and control. The application of mathematical models permits the advantage of designing model-based processes, monitoring, control and optimisation. In biological processes, modelling is imperative to have clear understanding of the relationship between the rate of microbial population growth, substrate consumption, and product formation, therefore, it is fundamental to properly model the specific growth rate as a function of substrate. A traditional and widely adopted approach is the modelling of bioprocesses with the assumption that only one substrate is limiting (Neeleman 2002; Metcalf and Eddy 2014). Furthermore, models used in biological processes can be classified as either structured or unstructured (Zeng and Deckwer 1995).

2.5.1 Structured versus unstructured models

Most biological processes are modelled using the unstructured models since they seem to be suitable for microbial growth kinetics. Unstructured models do not involve the internal dynamics of microbial cells. They treat the system in a uniform quantity, in which the reaction rates depend only upon the macroscopic conditions of the mixed-liquor inside the reactor. Such models have cemented their application in bioprocesses because they define kinetics growth, substrate uptake, and product formation. The Monod empirical model is considered to be an unstructured model and is the most commonly used model to relate the microbial growth with substrate utilization. Structured models incorporate genetic conditions of biomass cells, which makes it possible for the models to describe microbial growth phenomena since trends and responses can be recognised. These models are structured on the basis of biomass components such as concentration of metabolites, enzymes, DNA or RNA (Neeleman 2002; Kayombo *et al.* 2003).

Substrate utilization rate

According to Metcalf and Eddy (2014), the substrate utilization rate for soluble substrates in a biological wastewater treatment system can be modelled by adopting the *Michaelis-Menten* and the Monod models, presented by equations [2.9] and [2.10] respectively. These empirical models treat the system on the bases in which the rate of substrate utilization increases as the reactor substrate concentration increases for a given biomass concentration.

$$r_{su} = \frac{kXS}{K_s + S} \qquad [2.9]$$

Where r_{su} = substrate utilization rate per unit of reactor volume, g/m^3.d

k = maximum specific substrate utilization rate, g substrate/g microorganisms.d

X = biomass concentration, g/m^3

S = growth-limiting substrate concentration in solution, g/m^3

K_s = half-velocity constant, substrate concentration at one-half the maximum specific substrate utilization rate, g/m^3

Ammonia-oxidising rate (Monod model)

$$r_{NH} = \mu_{max,AOB}\left(\frac{S_{NH}}{S_{NH}+K_{NH}}\right)\left(\frac{S_o}{S_o+K_{o,AOB}}\right)\left(\frac{QN_{ox}SRT}{V[1+b_{AOB}SRT]}\right) \quad [2.10]$$

Where, r_{NH} = Ammonia oxidation rate, g/m^3.d

Q = Average daily influent flowrate, m^3/d

N_{ox} = NH_4-N oxidized by AOB from influent, g/m^3

V = Volume of reactor containing AOB, m^3

Both the *Michaelis-Menten* and the Monod models were formulated from an enzymatic-substrate model. These models are based on using coefficients derived from biological reactor data and is interpreted graphically by plotting r_{su} or r_{NH} versus the substrate concentration.

Bacteria growth rate

The bacteria growth rate from substrate utilization can be studied using empirical models proposed by Monod (1942) as presented in equations [2.11] and [2.12], for the specific growth rate of bacteria in which the limiting substrate is available to the microbial population in a dissolved form.

$$r_g = \frac{\mu_m XS}{K_s+S} \quad [2.11]$$

Where r_g = bacteria growth rate from substrate utilization, g/m^3.d

μ_m = maximum specific bacteria growth rate, g biomass/g biomass.d

$X, S,$ and K_s as defined in equation [2.9]

$$\mu_{AOB} = \mu_{max,AOB}\left(\frac{S_{NH}}{S_{NH}+K_{NH}}\right)\left(\frac{S_o}{S_o+K_{o,AOB}}\right) - b_{AOB} \quad [2.12]$$

Where, μ_{AOB} = Specific growth rate of ammonia-oxidising bacteria, g VSS/g VSS.d

$\mu_{max,AOB}$ = Maximum specific growth rate of ammonia-oxidising bacteria, g VSS/g VSS.d

b_{AOB} = Specific endogenous decay rate of ammonia-oxidising bacteria, gVSS lost/g VSS.d

S_{NH} = NH_4-N concentration, mg/L

K_{NH} = Half-velocity coefficient for NH_4-N, mg/L

S_o = DO concentration, mg/L

$K_{o,AOB}$ = Half-velocity coefficient for AOB, mg/L

The maximum specific growth rate and specific endogenous decay rate coefficients are known to be a function of temperature and are modelled as per the following equations.

$$\mu_{max,T} = \mu_{max,20}(\theta^{T-20}) \quad [2.13]$$

Where, $\mu_{max,T}$ = Maximum specific growth rate coefficient at temperature T, (˚C)

$\mu_{max,20}$ = Maximum specific growth rate coefficient at 20˚C

$$b_T = b_{20}(\theta^{T-20}) \quad [2.14]$$

Where, b_T = Endogenous decay coefficient at temperature T, (˚C)

b_{20} = Endogenous decay coefficient at 20˚C

According to (Metcalf and Eddy 2014) the Monod models for bacteria growth rate are based on the fact that as the substrate is being consumed by the bacteria, the energy produced from the substrate oxidation is used to process carbon and nutrients to produce biomass, thus making the new growth being directly proportional to the substrate being consumed.

2.6 Summary of literature review

The reviewed literature on brewery wastewater composition indicates that wastewater from the brewery is generally characterised by its high-strength of organic pollutants and biological processes are widely used to reduce organic pollutant concentrations. Furthermore, previously done work on brewery wastewater treatment highlighted that the activated sludge sequencing batch reactor has the ability to reduce organic pollutant compositions in brewery wastewater.

However, most of the work done on brewery wastewater treatment focused on reducing organic pollutants in terms of COD removal under anaerobic conditions since the system produces less biomass compared to aerobic operating conditions. Moreover, the reviewed literature indicates that it is imperative to study microbial growth kinetics for process optimisation and design.

Based on the reviewed literature, there is less reported work done on reducing brewery wastewater pollutants in terms of nitrogen and phosphorus removal. A lot of studies focused on COD removal. Furthermore, there is a gap in designing systems to handle wasted sludge in a cost effective way. This research is driven by the necessity to reduce organic pollutant concentrations, particularly biological nutrients i.e. nitrogen and phosphorus compounds in industrial effluent which promotes eutrophication in receiving water bodies.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Introduction

This chapter presents the experimental approach and materials used in this study, which covers the aspect related to sample collection and preparation; detailed description of the equipment design and the operational approach that was adopted in executing the study; the analytical techniques which covers the type of sample analyses which were conducted and the methods which were used. The chapter closes with a section of data analyses which explicitly discuss the kind of statistical data analyses done and the tools which were used.

3.2 Experimental Materials

3.2.1 Raw Brewery Wastewater

In achieving the overall objective of the study, industrial wastewater samples were collected from a local brewery wastewater treatment plant in Durban, South Africa. Samples were collected mainly for characterisation and operation of the two laboratory scale SBRs to investigate the performnace of the SBR system on orthophosphates, ammoniacal nitrogen, nitrate and nitrite, total Kjeldhal nitrogen, total nitrogen, total inorganic nitrogen and total organic nitrogen removal from industrial wastewater generated from the brewery. Collected samples were transported to the laboratory in a cooler box full of ice to avoid any biological activities, thus maintaining the sample's biological condition from the sampling point to the laboratory. Upon arrival at the laboratory, samples were allowed to warm up to room temperature to conduct physicochemical analyses, thereafter charged to the reactors (i.e. SBR-1 for nitrogen removal and SBR-2 for orthophosphates removal) to commence treatment immediately. In cases whereby it was not possible to conduct all analyses immediately, samples were stored in a refrigerator at 4˚C and analyses were conducted within 48 hours from the time of sampling.

3.2.2 Sludge sampling and preparation

Activated sludge (microorganisms) was harvested from an anaerobic digester at a local brewery wastewater treatment plant. Sludge was harvested using a 10 L bucket and then transported to the laboratory as presented in **Figure 3.1** below. In preparing the harvested sludge for treatment, no chemicals were added in brewery wastewater nor to the microorganisms to

balance the N: C: P ratio as recommended by Randall, Barnard and Stensel (1992), the sludge was not acclimated. Only the condensed, almost granular sludge was used for treatment since granular sludge is associated with good settleability, which is imperative for optimum treatment efficacy.

Figure 3.1: Activated sludge sample harvested from an anaerobic digester.

3.3 Experimental Methods

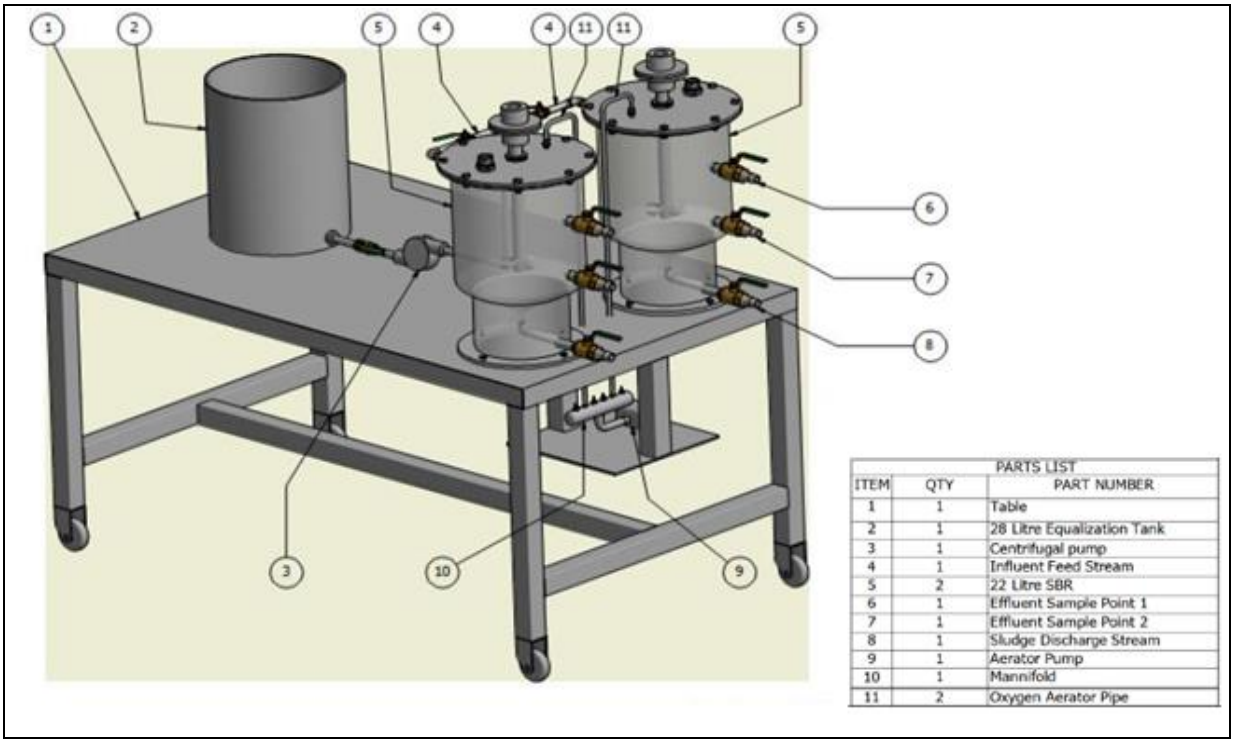
This section presents in detail the description of equipment and experimental methodological approach used in this study.

3.3.1 Equipment Design

The laboratory scale sequencing batch reactor consists of two identical reactor tanks made of transparent polyvinyl chloride, each calibrated to 18 L with a conical bottom having a slope of 60˚ for easy drainage of bio-solids. Each reactor had a diameter of 35 cm and a height of 45 cm, with a theoretical total volume of 22 L. For experimental runs, the working volume was set at 13 L with the microbial population occupying 4 L and raw brewery wastewater occupying 9 L. This working volume was based on the selected hydraulic retention time and solids retention time since they are both affected by the reactor working volume. Furthermore, both

reactors were not utilised to their maximum working volume to accommodate sludge bulking since the bacteria growth rate is directly proportional to the substrate utilisation rate (Monod 1942; Metcalf and Eddy 2014). The conical bottom of the reactor tanks allowed a quiescent and easy gravitational settling mechanism.

Each reactor tank had three spigot valves as shown in **Figure 3.2** for sample collection as well as for mixed liquor suspended solids drainage. Furthermore, both reactor tanks had a portable shaft mixer which was operated continuously to keep bio-solids suspended inside the reactor, thus allowing perfect mixing. Both the mixer shaft and impeller blades were made of stainless steel, with a drive motor mounted at the top of the reactor tanks in a rubber gasket operating at 10 W and 350 rpm. The impeller blade design reduced the shear mechanism during mixing, thus avoiding sludge bulking, since microorganisms in nature are very sensitive to shear mechanism. Moreover, the mixing shafts were positioned at about 15 cm above the bottom of the reactor tanks to allow the dispersion of large solids as presented in **Figure 3.2**. The sequencing batch reactor had an aeration pump, which provided oxygen in the form of air by means of a 5.0 mm diameter pipeline connecting from the top down to the central height of the reactor tanks.



PARTS LIST		
ITEM	QTY	PART NUMBER
1	1	Table
2	1	28 Litre Equalization Tank
3	1	Centrifugal pump
4	1	Influent Feed Stream
5	2	22 Litre SBR
6	1	Effluent Sample Point 1
7	1	Effluent Sample Point 2
8	1	Sludge Discharge Stream
9	1	Aerator Pump
10	1	Mannifold
11	2	Oxygen Aerator Pipe

Figure 3.2: Isometric view of the sequencing batch reactor (Shabangu 2016).

3.3.2 Experimental Approach

Cyclic aerobic-anaerobic sequencing batch reactor operation

Wastewater treatment in sequencing batch reactor systems is accomplished over a series of steps all taking place in a single reactor vessel which operates in time rather than space (Patil *et al.* 2013; Metcalf and Eddy 2014). The sequencing batch reactor system has cemented its application in wastewater treatment plants because of its unique set-up which does not require a return activated sludge system, because both the reaction and settling phases occur in the same tank (Metcalf and Eddy 2014). The following procedure, which includes a sequence of operational steps was adopted.

Filling phase

The filling phase was considered as the first operational phase of the sequencing batch reactor system. Both reactors were first seeded with 4 litres of activated sludge under anaerobic conditions. Raw brewery wastewater was fed into the reactor holding tank where suspended solids were allowed to settle by gravitational force for a period of 2 hours, as presented in **Figure 3.3**. After the settling phase, 9.0 L of the raw brewery wastewater supernatant was pumped to each reactor. The filling phase took place under anaerobic conditions, however, the

stirrer was switched on and set to operate at 350 rpm to allow mixing. According to Metcalf and Eddy (2014), a mix only during the filling stage promotes filamentous growth control and improves settling and thickening. The agitation speed of the stirrer was set to be 350 rpm, because it was observed that higher agitation speed resulted in sludge bulking thus compromising solids settleability. The filling period on average for all experimental runs lasted for 5 minutes.

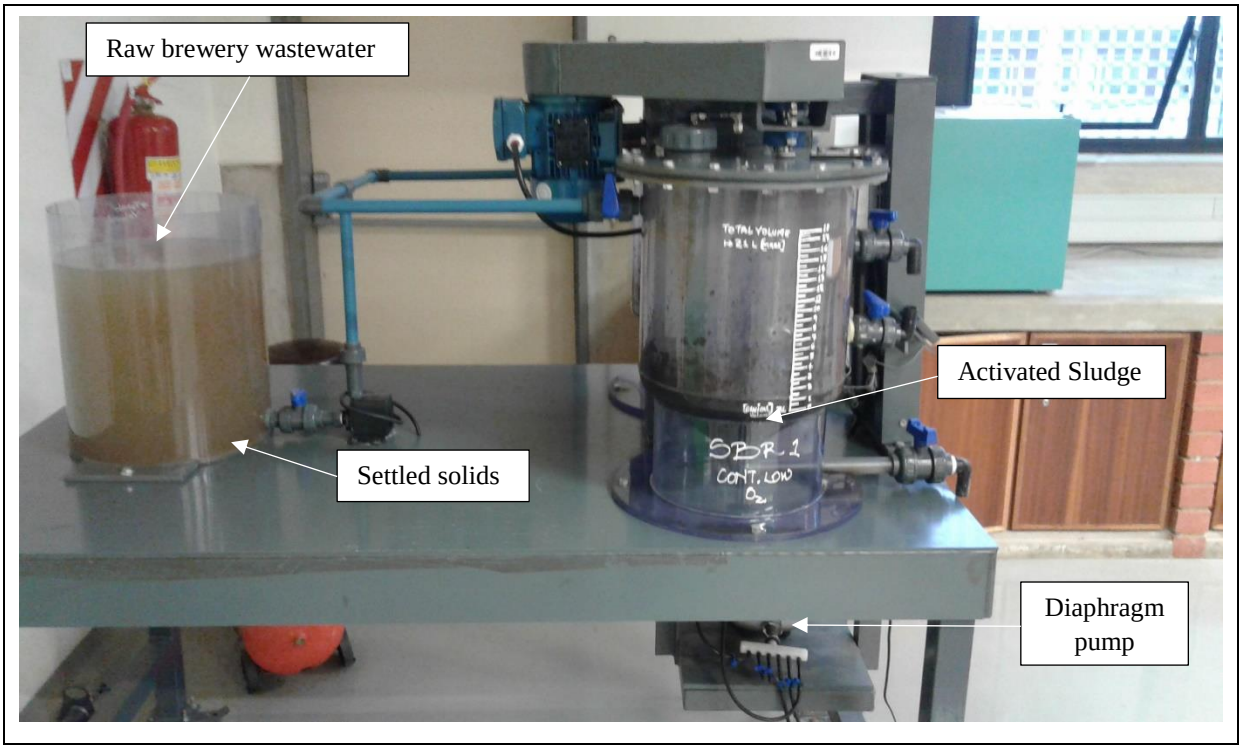


Figure 3.3: Laboratory scale sequencing batch reactor in operation.

Reaction/Aeration Phase

After filling both reactors (i.e. SBR-1 and SBR-2) to their maximum working volume, the reaction/aeration phase was instigated for SBR-1. The reaction phase was done by adopting cyclic aeration and continuous mixing to promote biological nitrification and denitrification for ammoniacal nitrogen, nitrates and nitrites removal. In the case of SBR-2, the aeration phase was initiated after the system had undergone the anaerobic stage in which polyphosphorus accumulating organisms are favoured. During the reaction/aeration phase, microorganisms consume substrates (i.e. ammonia and orthophosphates) under controlled environmental conditions (Metcalf and Eddy 2014). Therefore, the pH inside the reactor was monitored and maintained between the range of 4.0 and 9.0 and the temperature inside the reactor was also

monitored and left unadjusted at mesophillic temperature conditions. Hydrochloric acid was added into the reactor if pH levels were above 9, and sodium hydroxide pellets were added into the reactor at pH levels below 4.

Aeration was carried out by means of a diaphram air pump mounted on the SBR frame beneath the reactor tanks (see **Figures 3.2 and 3.3**). Air from the diaphram pump was transported by means of rubber pipelines connected to a copper pipeline which descended from the top of each reactor tank down to the central height for optimum reaction and mixing. The aeration phase lasted for 4 hours for SBR-1 and 14 hours for SBR-2. The aeration duration and anaerobic phase duration were determined experimentally for SBR-2 and for SBR-1 it was selected based on literature.

Settling phase

During the settling phase, bio-solids were allowed to separate gravitationally from the treated liquid under quiescent conditions resulting in a clear clarified supernatant. During the settling phase, the stirrer was switched off as well as the aeration system and no influent was charged into the reactor tanks nor effluent drawn. The settling period lasted for 2 hours to enhance optimum settling of bio-solids containing biodegradable organic and biological pollutants, thus resulting in a clear clarified supernatant with minimum suspended solids.

Decanting/Drawing phase

The decanting/drawing phase was considered as the final treatment operational stage for the sequencing batch reactor system. During this phase, the clarified supernatant was sampled as the treated reactor effluent by tapping the reactor effluent into a 500 mL sterile glass bottle for laboratory analysis. Sampling was done at the mid-sampling point, **valve 7** (**see Figures: 3.2**) to avoid sampling of effluent with floating matter. The drawing period lasted for 3 minutes on average for all experimental runs.

After each run an observation was made on the increase in quantity of biomass inside the reactor tanks, since the substrate utilization rate is directly proportional to the microbial specific growth rate (Monod 1942). In preparation for the next run, treated brewery wastewater was drawn from each reactor tank together with “new biomass” to avoid further sludge bulking and improve bio-solids settlebility, thus improving the system treatment efficacy. This process is called the idling phase, which lasted for 1 hour on average for all batches.

3.3.3 Experimental Design

A summary of biological nutrients, organic load as well as physicochemical properties tested and number of replicates for each analysis conducted in this study is presented in **Table 3.1**.

Table 3.1: Experimental design.

Sample Tested	Parameter	Replicates
Influent and effluent	PO^{3-}_4	3
	NH_3-N	
	TKN	
	NO_3-N + NO_2-N	
	TN	
	TCOD	
Inffluent	SCOD	
Online	Temperature	
Inffluent and online	pH	
Inffluent and effluent	ORP	
	Conductivity	
	Turbidity	
	TD	
	TDS	
	VSS	

3.5 Analytical Techniques

This section describes the analytical techniques used in analysing raw brewery wastewater and treated brewery wastewater. Analysis were conducted in accordance with the *Standard Methods for the Examination of Water and Wastewater* (APHA 2012) standard method.

Ammoniacal-Nitrogen (NH_3-N)

Nitrogen pollutants exist in different forms in wastewater. In this study, ammoniacal ntrogen (ammonia) was used to evaluate the treatment efficacy of the SBR system in treating industrial wastewater for nitrogen pollutants (i.e. ammonia). Ammonia, a toxic pollutant in wastewater, is considered to be highly soluble in water and exists as ammonium ion (Philips *et al.* 2002), thus this parameter was measured to give an approximate concentration of nitrogen pollutants in the form of ammonia contained in brewery wastewater. During experimental runs both the reactor influent and effluent were analysed for NH_3-N and the difference between influent stream and effluent stream in NH_3-N concentration gave the percentage removal which was used to evaluate the SBR treatment efficacy.

Measurement: NH_3-N concentration was measured colorimerically using a Hach DR 3900 spectrophotometer. Ammonium ions reacted with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol. The amount of colour fromed is directly proportional to the ammoniacal nitrogen present in the sample. Samples were measured at a wavelenght of 694 nm, using test vials. For data credibility all samples were analysed in triplicates.

Total Kjeldahl Nitrogen (TKN)

Wastewater is characterised by a variety of organic compounds containing nitrogen which can not be analysed by a single test which can allow each compound to respond in an equal manner. TKN is basically the combination of organically bound nitrogen and ammonia in wastewater. In this study, TKN anlysis were conducted to quantify the amount of nitrogen contained in the organic form. Nitrogen pollutants in wastewater exist as both organic and inorganic forms. The reactor influent and effluent was analysed for TKN and the difference between the reactor influent and effluent TKN concentration was used in evaluating the SBR system for organic nitrogen removal.

Measurement: TKN was measured colorimetrically using a Hach DR 3900 spectrophotometer. In this test method inorganic and organic nitrogen was oxidized to nitrate by digestion with peroxodisulfate. The nitrate ions reacted with 2,3-dimethylphenol in a solution of sulfuirc and phosphoric acid to form a nitrophenol. Oxidized froms of nitrogen i.e. nitrite and nitrate are also determined. Samples were measured at a wavelenght of 345 nm. For data credibility, samples were analysed in triplicates. Total organic nitrogen (TON) and total inorganic nitrogen (TIN) concentrations were estimated using equations [3.1] and [3.2] respectively.

$$TON = TKN - (NH_3N + NH_4N) \quad [3.1]$$

$$TIN = (NH_3N + NH_4N) + (NO_3N + NO_2N) \quad [3.2]$$

Orthophosphates (PO_4^{3-})

Phosphorus in wastewater is found in different forms, including the dissolved (orthophosphates), inorganic (reactive plus condensed or acid hydrolyzable phosphate) and organically bound forms. In this study, phosphorus concentration was measured as orthophosphates since industrial wastewater from the brewery is characterised by a high fraction of inorganic orthophosphates (Rossle and Pretorius 2001; Abimbola *et al.* 2015). All analyses were done in triplicates for data credibilty.

Measurement: orthophosphates concentration was measured colorimetrically using a Hach DR 3900 spectrophotometer by adopting the molybdovanadate method. In the molybdovanadate method, orthophosphate reacts with molubdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, a yellow molybdovanadophosphiric acid is formed. The intensity of the yellow colour is proportional to the phosphate concentration. Samples were measured at a wavelength of 430 nm. All data obtained was validated at 95% confidence level.

Chemical Oxygen Demand

The measure of capacity of water to consume dissolved oxygen during the decomposition of organic matter and the oxidation of inorganic chemicals such as ammonia and nitrite, is known as chemical oxygen demand (COD). In this study, the COD application was used to quantify organic pollutants concentration in industrial wastewater generated from the brewery, which was considered to be a quick indicator of organic pollutant in brewery wastewater. The COD was expressed in milligrams of oxygen per litre (mg O_2/L) which is the amount of oxygen consumed per litre of solution. In this case it was the amount of oxygen consumed per litre of brewery wastewater. Both the reactor influent and treated effluent were characterised for COD. No analyses on COD were conducted during treatment.

Measurement: COD was measured spetrophotometrically (DR 3900) using the colorimetric method. Samples were heated for 2 hours at 150˚C in the presence of sulfuric acid and a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds reacted, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}). The amount of chromic ion produced was measured spectophotometrically at a wavelength of 620 nm to give COD concentration.

Moreover, the COD reagent also contains silver and mercury ions. Silver act as a catalyst and mercury is used to complex chloride interferences.

pH and oxidation reduction potential (ORP)

The pH is an important parameter in biological wastewater treatment systems because it affects the microbial metabolic process, thus compromising wastewater treatment efficacy as well as the environment and aquatic life when disposed. Microorganisms in their nature are pH sensitive, such that at pH levels less than 4 and pH levels more than 9.5 they do not perform well (Metcalf and Eddy 2014). The pH was analysed during characterisation of reactor influent and treated effluent in accordance with the APHA (2012) standard methods. Furthermore, samples were analysed in triplicates to ensure data credibility.

Measurement: during treatment process, pH monitoring inside the reactor tanks was done every 2 hours, by directly measuring the pH inside the reactor tanks using a calibrated Thermo Scientific Orion Star A215 pH/conductivity meter.

Conductivity

Conductivity is a measure of the ability of water to pass an electric current. In water treatment systems, conductivity is greatly affected by the presence of inorganic dissolved solids such as nitrate, phosphates anions etc. Organic compounds like alcohol and sugars do not conduct electrical current very well, therefore they have low conductivity in water (Cintron 2016). In this research study, conductivity was used to give a quantitative measure of the total dissolved solids in water.

Measurement: conductivity was measured using a calibrated Thermo Scienctific Orion Star A215 pH/conductivity in micro Siemens per centimeter. Conductivity data was statistically validated at 95% confidence level.

Dissolved Oxyegn (DO)

Biological treatment is defined as an aerobic activated-sludge process in the aeration zone for treating wastewater. For this study, DO with the units of mg O_2/L was considered as an important parameter to make certain that the microbial community in the aerobic zone has enough DO to remain alive. During experimental runs air was supplied to both reactors by

means of a diaphram air pump to maintain a DO minimum concentration of 2 mg O_2/L for the survival of the microbial community in the aerobic zone. The DO concentration was measured directly in the reactors using an online calibrated DO probe Orbeco Hellige Series 150. The dissolved oxygen was measured during the aerobic phase for process monitoring purposes.

Total Solids (TS) and Total Dissolved Solids (TDS)

According to the *Standard methods for the Examination of Water and Wastewater* (APHA 2012), TS are total dissolved solids plus suspended and settleable solids in water. In the case of brewery wastewater dissolved solids consist of nitrate, phosphorus and other particles. On the other hand suspended solids include fine organic debris and other particulate matter. The difference between dissolved solids and suspened solids is that dissolved solids can pass through a filter with pores around 2 microns and suspended solids can not pass through a 2 micron filter. This parameter was measured to quantify the amount of solids in both the inffluent and effluent streams.

Mesurement: total solids and total dissolved solids were mearured gravimetrically in mg TS/L and mg TDS/L respectively. A well-mixed sample was dryed at 105˚C for 24 hours. The TS fraction was given by the wight of the residue after drying.

Volatile Suspended Solids (VSS) and Fixed Volatile Solids (VFS)

In this study, the VSS was measured to quantify the amount of organic matter and the VFS was used as a meaurement for the inorganic solids fraction in both the reactor influent and effluent steams.

Measurement: the VSS was measured gravimetrically in mg VSS/ L by igniting samples in a muffle furnace at 550℃. The portion lost during the ignition process was taken to be equavalent to the organic fraction, and the residue after ignition gave the FVS fraction.

3.6 Microbiology Kinetics

The Monod and *Michaelis-Menten's* empirical equations, as discussed in sub-section 2.5 of **Chapter Two**, were used to study the substrate (i.e. TCOD) concentration in relation to the substrate utilisation rate kinetics and microbial growth rate kinetics. Reference should be made

to **Appendix D**, for detailed kinetics equations and constants that were used in this research study.

3.7 Data Analysis

Data obtained in this research study was statistically analysed using Minitab 15 and Microsoft Office Excel as statistics tools. The data was first checked for normality distribution through the skewness and kurtosis value, and was analysed using descriptive statistics to obtain the mean, standard deviation and range. The *two tail Student's t-Test* with unequal variances at a 95% confidence level was implemented. Furthermore, the data was used to study the relationship between output variables and tested parameters, as discussed in **Chapter Four**.

CHAPTER FOUR
RESULTS AND DISCUSSION

This chapter presents results obtained in this study as well as the discussion of results. Section 4.1 presents the findings of the study and discussion on characterisation of brewery wastewater composition. Section 4.2 presents the results obtained and their discussion on investigating the effect of sludge retention time and hydraulic retention time on orthophosphates removal. Section 4.3 presents results and discussion on total chemical oxygen demand removal as well as orthophosphate removal with variation in organic volumetric loading rates. Section 4.4 presents results and discussion on orthophosphates and total chemical oxygen demand overall percentage removal as well as orthophosphate material balance results. Section 4.5 presents the findings of the study as well as discussion on the effect of sludge retention time on ammoniacal-N removal, effect of ammoniacal-N concentration on the sequencing batch reactor treatment efficacy, effect of organic volumetric loading rate on nitrogen pollutant removal, as well as temperature and pH effects on biological nutrient removal. Section 4.6 presents the overall results and discussion on the sequencing batch reactor treatment efficacy on biological nutrient removal. The chapter ends with section 4.7 which presents the findings of the study and discussion on substrate utilisation rate kinetics and microbial growth rate kinetics.

4.1 Results on Characterisation of Brewery Wastewater Composition

Table 4.1 presents a summary of results on the characterisation of brewery wastewater composition used in conducting this experimental research study. The findings of the study on brewery wastewater characterisation are presented in terms of the mean expressed in standard deviation and the range, which were statistically analysed at a 95% confidence level (reference should be made on **Table A.1** from **APPENDIX A**).

Table 4.1: Summary of results for the characteristics of brewery wastewater composition.

Parameter	**Mean(±SD)**	**Range**
Temperature (˚C)	31±3.7	25.3 – 37
pH	6.5±2.4	4.4 – 12.2
ORP (mV)	13.7±139	-305 to 135
Conductivity (µS/cm)	2718±1020	1893 – 6017
Turbidity (NTU)	570±164	303 – 1039
TCOD (mg/L)	7687±2030	3447 – 11813
SCOD (mg/L)	6323±1542	2287 – 8627
PCOD (mg/L)	1454±917	127 – 3693
PO^{3-}_4 (mg/L)	343±64	229 – 424
NH3-N (mg/L)	12.2±7.5	2.21 – 27.8
TKN (mg/L)	29.3±25.6	6.24 – 94.7
Total Nitrogen (mg/L)	38.6±29.0	13.7 – 106
NO3-N + NO2-N (mg/L)	10±11	2.87 – 49.4
Total Organic Nitrogen (mg/L)	8.92±11.1	0 – 39.1
Total Inorganic Nitrogen (mg/L)	34.4±22	7.78 – 93
TS (mg/L)	5951±3387	2942 – 14981
TDS (mg/L)	4121±1503	2198 – 7400
FVS (mg/L)	2327±1118	825 – 4975
VSS (mg/L)	1799±571	1043 – 2572

The findings of the study presented in **Table 4.1** above for the tested parameters explicitly indicate that brewery wastewater composition fluctuates significantly. As indicated in the literature, the sudden changes in brewery effluent composition results from the activities taking place inside the brewing plant (i.e. washing of floors, cleaning the brewing house, cellars and cleaning in place) as well as the chemicals used during the cleaning process (Parawira *et al.* 2005; Goldammer 2008; Fu *et al.* 2013). Furthermore, the data presented in **Table 4.1** showed similar characteristics of brewery wastewater composition as to the ones presented in the literature regarding similar studies in **Table 2.1**. The results also showed that brewery wastewater comprises of a high fraction of organic pollutants in terms of TCOD in which a large fraction is given by soluble COD compared to particulate COD.

4.2 The Effect of Sludge Retention Time (STR) on Orthophosphate Removal Efficiency

The findings of the study on orthophosphate removal profile with SRT variation are presented in **Figure 4.1**.

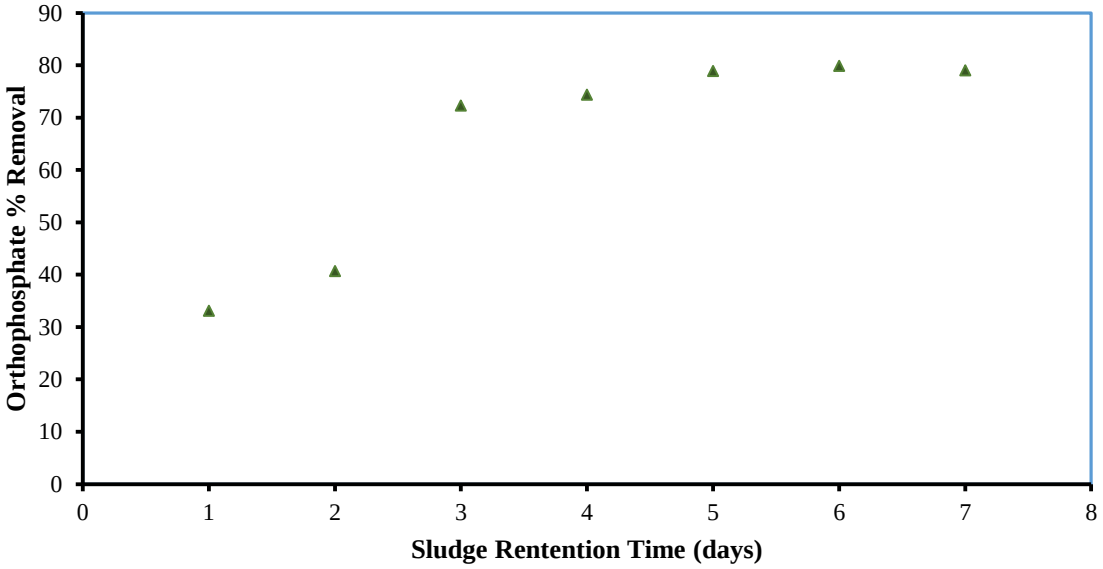


Figure 4.1: Orthophosphate removal profile with SRT variation.

From **Figure 4.1** it is shown that the orthophosphate removal percentage increased with an increase in SRT. The process of enhanced biological phosphorus removal is facilitated by polyphosphate accumulating organisms. The microbial population was exposed to alternating anaerobic and aerobic environments in order to enrich the sludge with polyphosphate accumulating organisms, which are responsible for the up-take of orthophosphates and store it as polyphosphate in the aerobic environment, thus resulting in net orthophosphate removal. It was reported that at SRT of 3 days and above, the sludge was enriched with polyphosphate accumulating organisms recording an orthophosphate removal of more than 60%. Moreover, as presented in **Figure 4.1**, a stable enhanced biological phosphorus removal system was achieved after a SRT of 5 days corresponding to an orthophosphate removal of 80%. The findings of the study on orthophosphate removal showed similarities with previous studies reported by Mamais and Jenkins (1992) and Ge, Batstone and Keller (2015) on wastewater with high organic loading rates. A SRT of 7 days was adopted since the system indicated to be stable at a SRT of 5 days and more. Furthermore, according to Chan, Guisasola and Baeza (2017), longer SRT of more than 10 days have the advantage of promoting the growth of glycogen accumulating organisms which results in a decrease in polyphosphate accumulating organisms, however, short SRT known to be between 2 to 10 days, is highly recommended.

4.2.1 The Effect of Hydraulic Retention Time on Orthophosphate Removal

As mentioned in **Chapter Three**, the HRT for the orthophosphate removal was determined experimentally. **Figure 4.2** presents the orthophosphate concentration profile with a variation of the HRT.

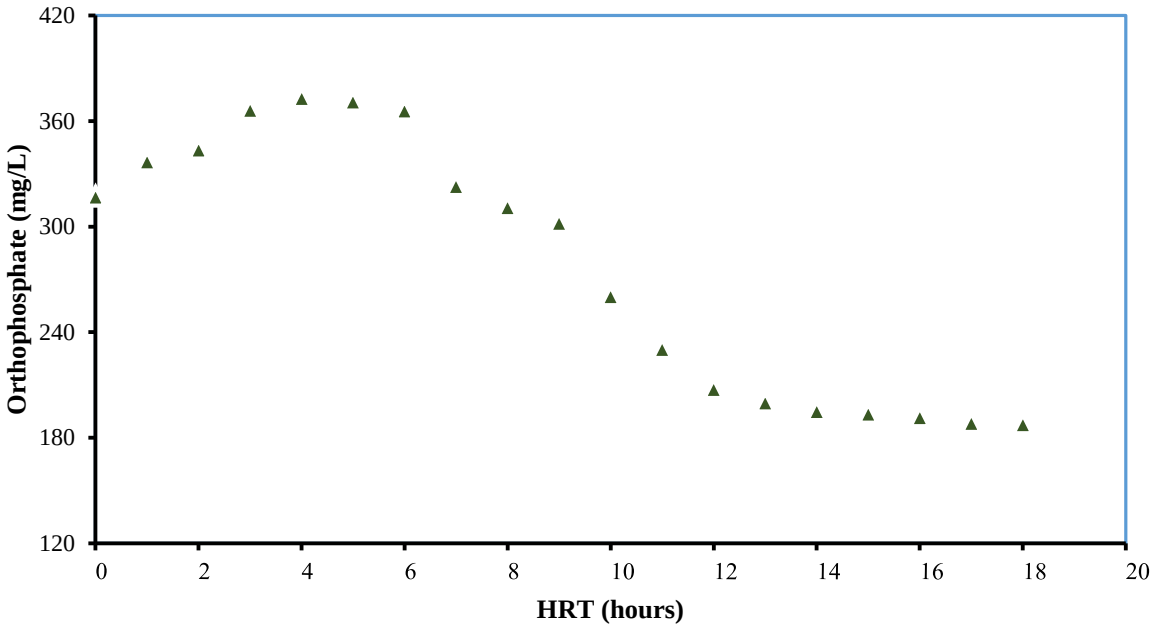


Figure 4.2: Orthophosphate concentration profile with variation of HRT.

When analysing **Figure 4.2** above, it can be seen that there was an increase in orthophosphates during the first 4 hours of the anaerobic phase. This was an indication of polyphosphate accumulating organisms presence in the sludge. According to Saad *et al.* (2016) and Metcalf and Eddy (2014) polyphosphate accumulating organisms are favoured in the anaerobic phase because they do not require oxygen as an electron donor, however, they consume readily biodegradable substrate in wastewater thus accumulating phosphorus as orthophosphates. In this study, it was observed that orthophosphate release was significant during the first 4 hours of operation under anaerobic conditions. Furthermore, during the aerobic phase, at a HRT of 6 to 12 hours, the orthophosphate up-take rate was high and the was insignificant reduction at a HRT of between 12 and 18 hours as presented in **Figure 4.2**. This was indicated by the sharp slope representing high up-take of orthophosphates and flat slope of the graph representing insignificant removal efficiencies of orthophosphates. Furthermore, the findings of the study presented in **Figure 4.2** indicate strong congruence with the orthophosphates removal profile presented in **Figure 2.1** from the reviewed literature.

4.3 Total Chemical Oxygen Demand (TCOD) Removal Results

The results obtained from this experimental research study on TCOD removal in SBR-2 are presented in **Figure 4.3**.

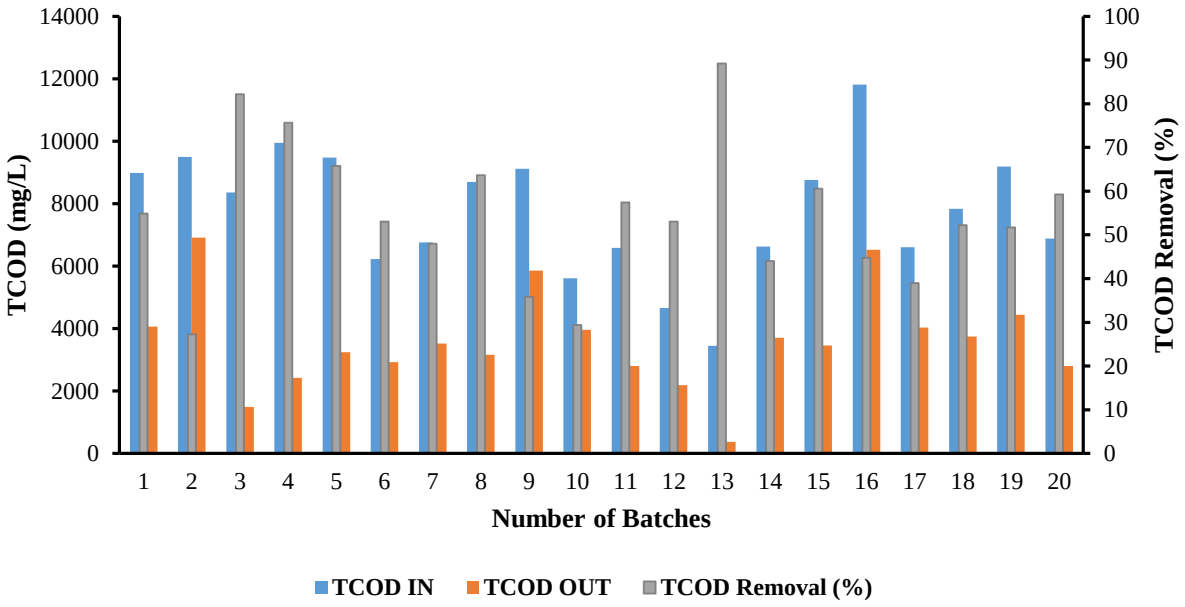


Figure 4.3: TCOD Removal Results (SRT of 7 days and HRT of 18 hours).

From the analysis of **Figure 4.3**, it can be stated that the reduction in TCOD concentration in the reactor effluent stream was as a result of microbial activities taking place in the system during treatment. This is supported by Metcalf and Eddy (2014) who confirmed that during the anaerobic phase, polyphosphate accumulating organisms consume readily biodegradable organic substrate (i.e. COD) with the aid of energy made available from stored phosphorus, thus enriching the sludge with the polyphosphate accumulating organisms microbial population. However, when analysing **Figure 4.3**, it can be seen that the TCOD removal varied with different batches, therefore, the system did not show an indication of stability regarding TCOD removal. Furthermore, the TCOD removal was recorded at 55% on average, this was less than the findings reported from previous studies (Shao *et al.* 2008; Wang *et al.* 2010; Ab Halim *et al.* 2015). Generally, the low TCOD removal was caused by the variation in terms of TCOD loading rates as presented in **Figure 4.3**, microorganisms are very sensitive to sudden changes in wastewater composition, thus compromising the treatment efficiency (Mulkerrins, Dobson and Colleran 2004). Moreover, the low TCOD removal was an indication confirming that for the brewery wastewater used in this study, only 55% of the TCOD fraction was readily biodegradable COD and 45% slowly biodegradable COD. It is imperative to stress on the fact

that high removal efficacies on slowly biodegradable COD can be achieved under long HRT operation. This justifies the lower TCOD removal efficiency because of lower HRT. Higher TCOD removals can be achieved at a HRT of 3 to 5 days (Metcalf and Eddy 2014; Bakare, Shabangu and Chetty 2017).

4.3.1 Orthophosphates and TCOD Removal Results

Figures 4.4 and **4.5** present a summary of results obtained from this study on orthophosphates and TCOD removal efficiencies.

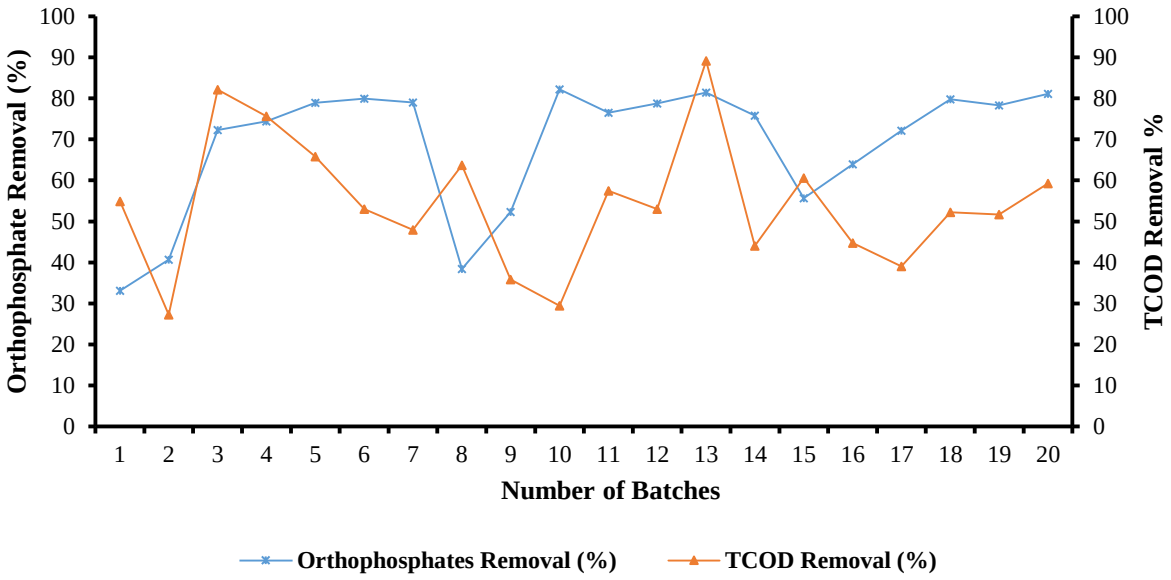


Figure 4.4: Orthophosphates and TCOD removal results (SRT of 7 days and HRT of 18 hours).

As presented in **Figure 4.4** above, the orthophosphate removal efficiencies were higher compared to TCOD removal for most batches. It can be stated that the system was stable for most of the batches i.e. from batches 3 to 7, followed by batches ranging from 10 to 14 and finally with batches ranging from 18 to 20 with orthophosphate removal of not less than 75%. However, for TCOD removal the system did not show any stability. The system was unstable because TCOD concentration in the influent stream was fluctuating greatly for all batches. Moreover, the findings of the study on orthophosphate removal with a variation in the Organic Volumetric Loading Rate (OVLR) measured in COD kg/m^3.day are presented in **Figure 4.5**.

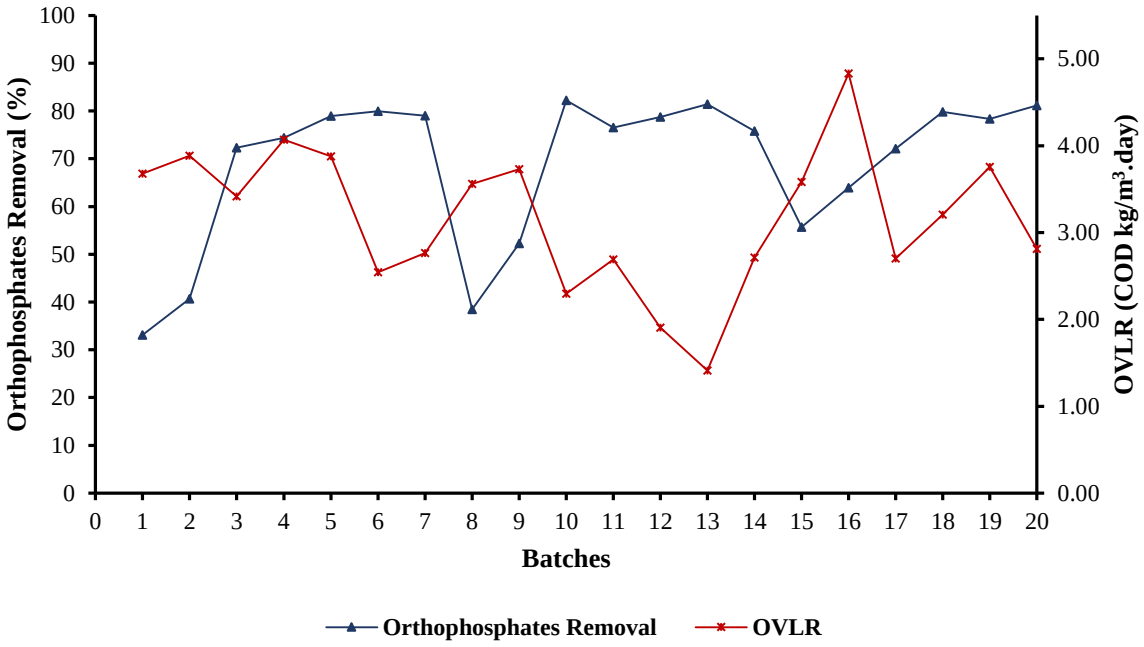


Figure 4.5: Orthophosphate removal with variation in OVLR (SRT of 7 days and HRT 18 hours).

As indicated in **Figure 4.5**, it was found that the OVLR fluctuated significantly for all experimental batches. The system showed higher orthophosphate removal efficiencies when operating at a OVLR less than 3.5kg COD/m^3.day with the highest orthophosphate removal of 81% achieved at a OVLR of 1.41kg COD/m^3.day. However, looking at the overall performance, it can be stated that the variation in OVLR did not have a significant effect on the system performance. This was because the microbial population and wastewater used in this study was harvested from the same brewery wastewater treatment plant, therefore, the microbial population was adapted to the variation in OVLR.

Figure 4.6 below presents the overall findings of the study related to orthophosphates and TCOD removal efficiencies of the SBR obtained during the experimental work.

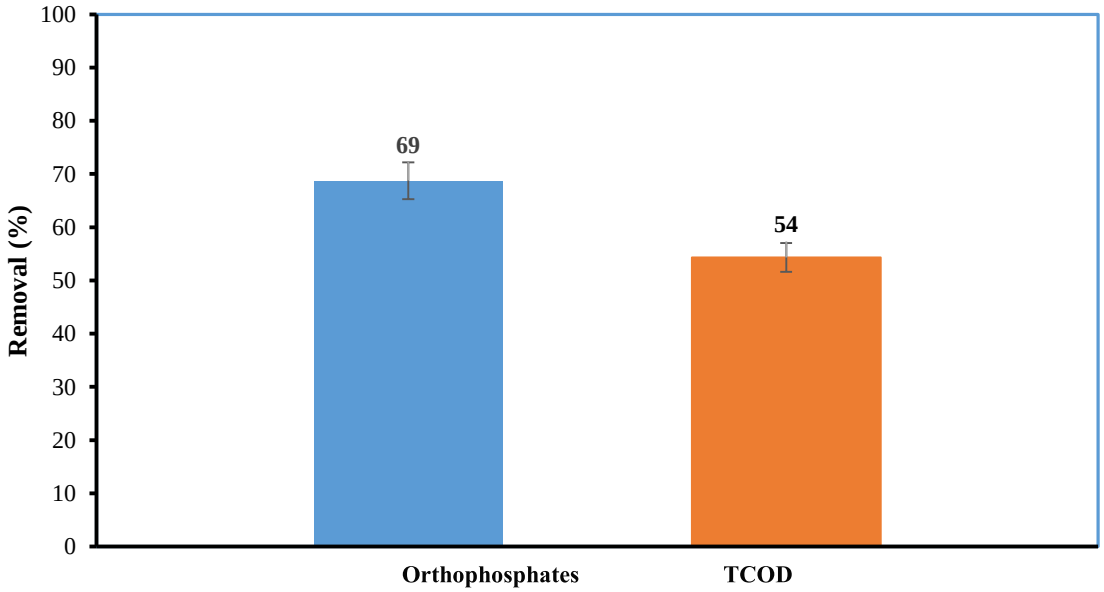


Figure 4.6: Overall removal on orthophosphates and TCOD.

The findings of the study indicate that the SBR operated under activated sludge had an overall removal efficiency of 69% for orthophosphate removal and 54% removal efficiency for TCOD. Furthermore, the findings of the study presented in **Figure 4.6** showed strong congruence with previously done work on wastewater treatment (Yildiza *et al.* 2005; Wang *et al.* 2010; Ab Halim *et al.* 2015; Ge, Batstone and Keller 2015). Moreover, from the *two tail Student's t-Test* results (see **Tables B.1** and **B.2 from Appendix B**) at a significant level of p = 0.05, a p-value of less than 0.05 was attained.

4.4 Summary of Results on Orthophosphates Material Balance

Figure 4.7 and **Table 4.2** presents a summary of results for this research study on orthophosphates material balance. **Table 4.2** presents material balance for all experimental batches. Material balance calculations presented in **Figure 4.7** were conducted on the basis that the influent stream had an orthophosphate of 0.34 PO_4^{3-} kg/day on average.

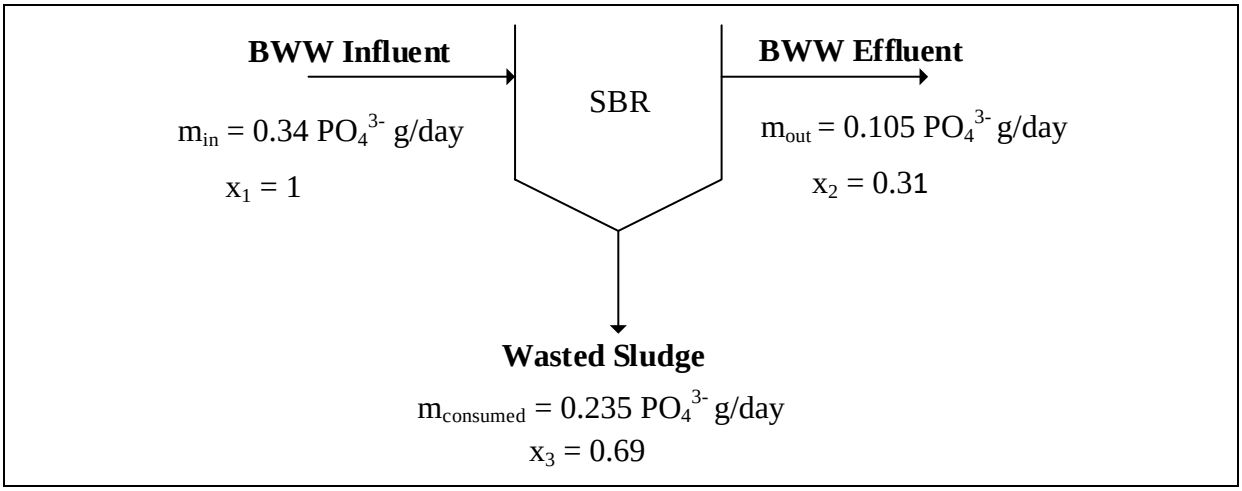


Figure 4.7: Overall material balance on orthophosphate.

As presented in **Figure 4.7**, the microbial population in the system was able to take-up 0.235 PO_4^{3-} kg/day from brewery wastewater, and the reactor effluent had an average of 0.105 PO_4^{3-} kg/day. The findings on material balance calculations showed that 0.235 PO_4^{3-} kg/day on average representing 69% of orthophosphate was retained by the sludge and the remaining 31% was taken by the effluent. Based on these findings, it could be said that the removal of orthophosphates was achieved through solid retention and biological activities in the sludge. From the *two tail Student's t-Test* results a p-value of less than 0.05 was obtained. The results demonstrated that there was a significant orthophosphate reduction between the influent and effluent streams of the reactor. Reference should be made to **Table B.3** in **APPENDIX B**.

Overall, from the analysis of the mass balance, it explicitly shows that the removal of orthophosphates was mainly achieved through solid retention than the anaerobic and aerobic phase. This is explained from the mass balance presented in **Figure 4.7** above where 69% of the orthophosphate was absorbed by the sludge and only 31% was taken by the effluent.

Table 4.2 below, presents a summary of results on material balance calculations on orthophosphate removal for each experimental run. The findings of the study presented in **Table 4.2** also give the SBR percentage removal efficiency for orthophosphates for each experimental batch.

Table 2.2: Orthophosphates material balance results for all experimental runs.

Batch No.	**SBR influent (PO_4^{3-} g/day)**	**SBR consumption (PO_4^{3-} g/day)**	**SBR effluent (PO_4^{3-} g/day)**	**SBR Removal Efficiency (%)**
1	0.275	0.091	0.184	33
2	0.317	0.130	0.187	41
3	0.235	0.169	0.066	72
4	0.229	0.169	0.060	74
5	0.285	0.225	0.060	79
6	0.274	0.219	0.055	80
7	0.348	0.275	0.073	79
8	0.398	0.151	0.247	38
9	0.308	0.160	0.148	52
10	0.365	0.299	0.066	82
11	0.405	0.312	0.093	77
12	0.259	0.205	0.054	79
13	0.323	0.262	0.061	81
14	0.372	0.283	0.089	76
15	0.424	0.237	0.187	56
16	0.369	0.236	0.133	64
17	0.423	0.305	0.118	72
18	0.396	0.317	0.079	80
19	0.392	0.306	0.086	78
20	0.403	0.326	0.077	81

4.5 The Effect of Sludge Retention Time on Ammoniacal Nitrogen Removal

The SRT for nitrogen pollutant removal was determined experimentally by measuring the ammoniacal-N concentration with a variation in SRT. Ammoniacal-N is highly soluble and toxic in water and it exists as an ammonium ion. The findings of the study on ammoniacal-N removal with SRT variation are presented in **Figure 4.8**.

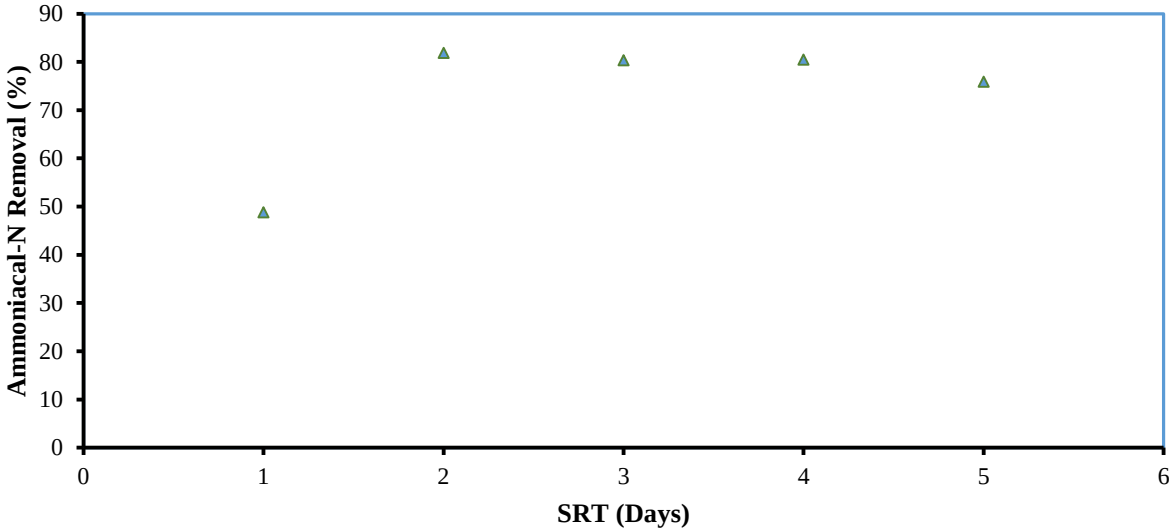
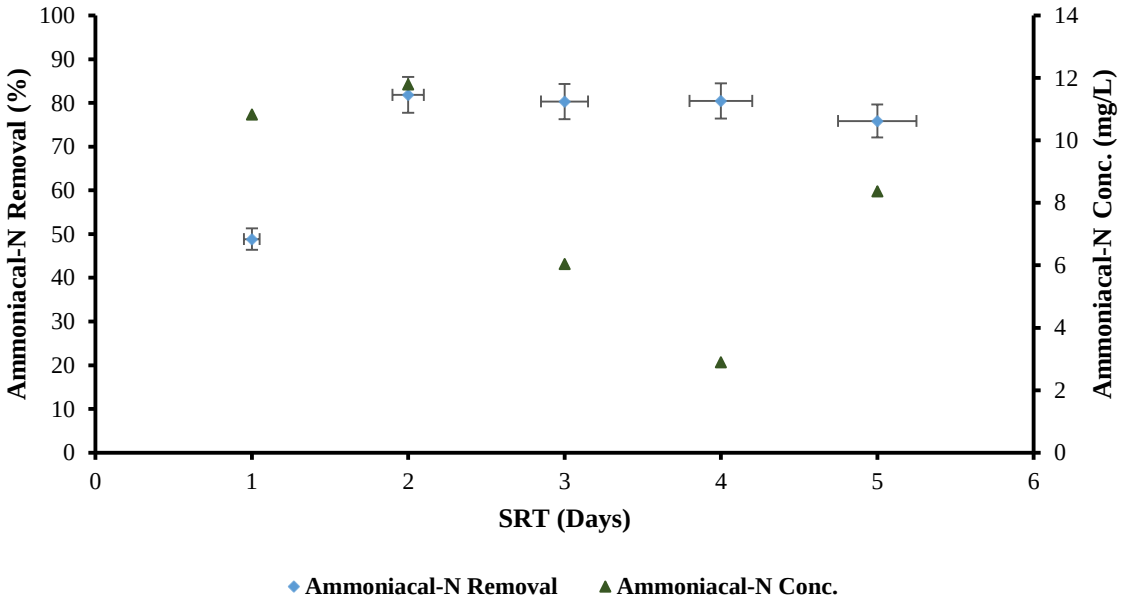


Figure 4.8: Ammoniacal-N removal profile with SRT variation.

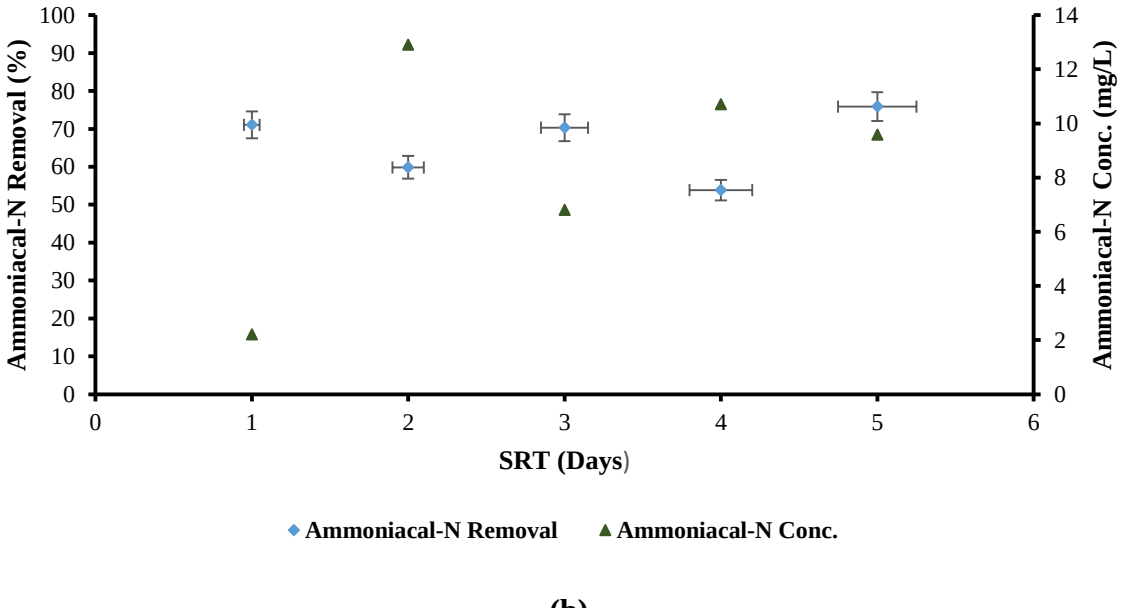
According to Metcalf and Eddy (2014), the optimum SRT for biological nutrient removal range from 3 to 5 days under mesophilic temperature. As presented in **Figure 4.8**, the SBR showed to be stable at a SRT of 3 to 5 days reaching an ammoniacal-N removal efficiency of 80%. This confirmed that the biodegradation of orthophosphates was achieved through solids retention time. The SRT of 5 days was considered to be the maximum since longer SRT is associated with the promotion of the glycogen accumulating bacteria, thus compromising the system treatment efficacy. Furthermore, the findings of the study demonstrated that the conditions under which the SBR was operated favoured the growth of ammonia-oxidising-bacteria (AOB). The AOB presence in the microbial population facilitated the biodegradation process of ammoniacal-N in brewery wastewater.

4.5.1 The Effect of Ammoniacal-N concentration on the SBR Treatment Efficacy

It was observed that ammoniacal-N concentration in the influent stream had no significant impact on the system treatment efficacy with a variation in SRT. **Figures 4.9 (a)** and **(b)** presents the findings of the study on investigating the effect of this parameter.



(a)



(b)

Figure 4.9: The effect of ammoniacal-N concentration on the SBR efficacy with a variation in SRT, (a) = week 1 and (b) = week 2.

According to Mulkerrins, Dobson and Colleran (2004) and Shehab *et al.* (1996), biological nutrient removal systems are very sensitive to disturbances triggered by fluctuations in wastewater composition. In this research study, it was noted that fluctuations in ammoniacal-N concentration in the influent stream did not have a significant effect on the SBR treatment efficacy. When studying **Figure 4.9(a)** above, the system showed stability in terms of ammoniacal-N percentage removal. This could be as a result of minimal fluctuations on ammoniacal-N in the reactor influent stream between the SRT of 1 and 2 days i.e. 10.8 and 11.8 NH_3-N mg/L respectively. However, between the SRT of 3 to 4 days, the ammoniacal-N concentration decreased in a linear fashion i.e. 50 and 49% respectively and it increased to 62% at a SRT of 5 days. The findings of the study presented in **Figure 4.9(a)** explicitly indicates that gradual changes in wastewater composition at a fixed ratio had a minimal effect on the SBR treatment efficacy for ammoniacal-N.

Moreover, from the findings of the study presented in **Figure 4.9(b)**, it can be seen that the SBR system was able to remove more than 50 % of ammoniacal-N from wastewater regardless of the fluctuations in ammoniacal-N concentration. However, the system did not show any stability under such conditions in terms of treatment efficacy. The treatment efficacy of the SBR system varied with varying ammoniacal-N concentration. The behaviour of the microbial population used in this study could be attributed to the fact that the activated sludge and brewery wastewater samples which were used in this study were harvested from the same brewery. The findings of the study presented in **Figures 4.9 (a)** and **(b)** explicitly indicate that the microbial population used in this research study was already adapted to the fluctuations in ammonical-N concentration in the incoming stream. Furthermore, to the results presented in **Figures 4.9 (a)** and **(b)** it can be seen from the *two tail Student's t-Test* results (**see Table B.4** from **Appendix B**) at a significance level of 0.05 a p-value of 0.0003 was obtained. The p-value of less than 0.05 statistically indicating that there was strong biodegradation of ammoniacal-N between the SBR influent and effluent streams.

4.5.2 SBR Results on (NO_3-N + NO_2-N) Removal

Brewery wastewater samples used in this research study were also analysed for nitrates and nitrites. **Figure 4.10** below presents the results of the SBR on nitrites and nitrates removal efficiencies.

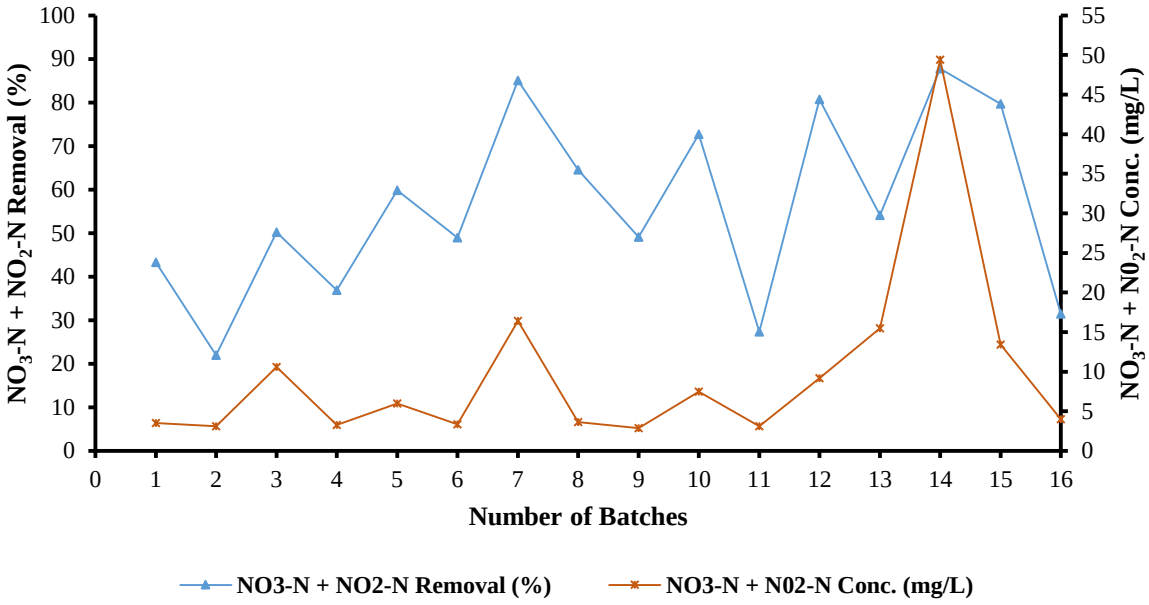


Figure 4.10: Results for the SBR-1 on (NO3-N + NO2-N) removal (SRT of 5 days and 18 hours).

From **Figure 4.10**, it can be seen that the brewery wastewater used in this research study contained NO_3-N+NO_2-N ranging from 2.87 to 49.4 mg/L. When studying **Figure 4.10** the SBR treatment efficiency was less than 50 % for reactor influent with a NO_3-N+NO_2-N concentration of less than 4.0 (NO_3-N+NO_2-N) mg/L. The low SBR removal efficiencies could be attributed to the fact that during the biodegradation process of ammonia in wastewater i.e. nitrification process, both NO_3-N and NO_2-N are produced, thus enriching the microbial population with NO_3-N and NO_2-N. However, the up-take rate of NO_3-N and NO_2-N seemed to be slower during the anaerobic phase which favours the denitrification process (Metcalf and Eddy 2014). Moreover, from the *Student's t-Test two tailed* results (**see Table B.5 in APPENDIX B**) a p-value of 0.03 was obtained. A p-value of less than 0.05 was statistically considered being significant.

4.5.3 The Effect of Organic Volumetric Loading Rate (OVLR) on Biological Nutrient Removal

For the findings of this study presented in **Figures 4.11** to **4.16**, reference should be made to the two tail Student's t-Test results presented in **Tables B.6** to **B.9** in **APPENDIX B**. The findings of the study on the effect of OVLR measured in TCOD kg/m^3.day on biological nutrient removal are discussed in this sub-section.

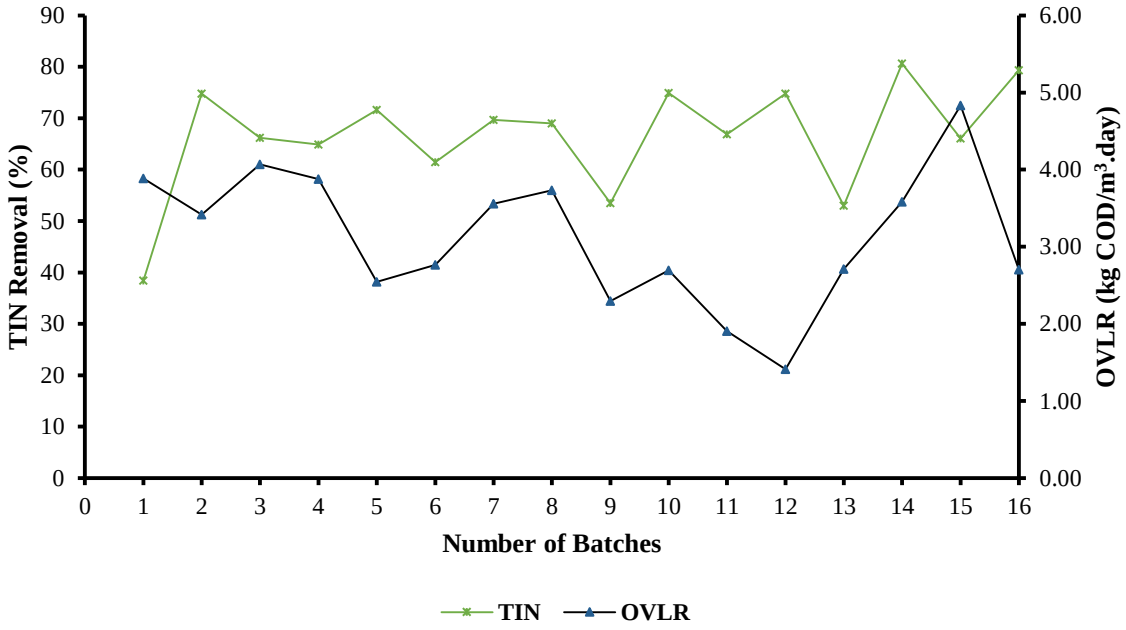


Figure 4.11: Effect of OVLR on TIN removal (SRT of 5 days and HRT of 18 hours).

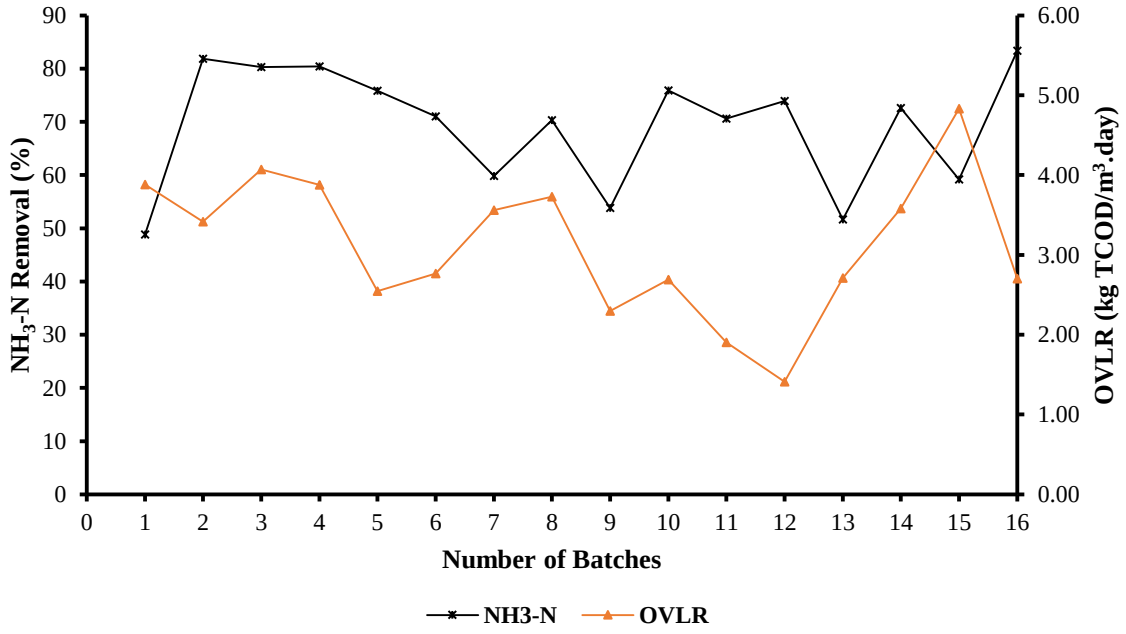


Figure 4.12: Effect of OVLR on ammoniacal-N removal (SRT of 5 days and HRT of 18 hours).

As shown in **Figures 4.11** and **4.12**, the OVLR was fluctuating in all batches ranging from 1.41 to 4.83 kg TCOD/m^3.day. Moreover, it was seen that the fluctuation in OVLR had a minimal effect on both TIN and NH_3-N removal, with a SBR treatment efficacy ranging from 38 to 79%

and 49 to 83% respectively. Experimental data for both TIN and NH_3N were analysed using the *two tail Student's t-Test* p-values of 0.0006 and 0.0003 were attained for TIN and NH_3-N respectively. A p-value of less than 0.05 explicitly indicates that there was significant biodegradation on both TIN and NH_3N between the reactor influent and effluent streams.

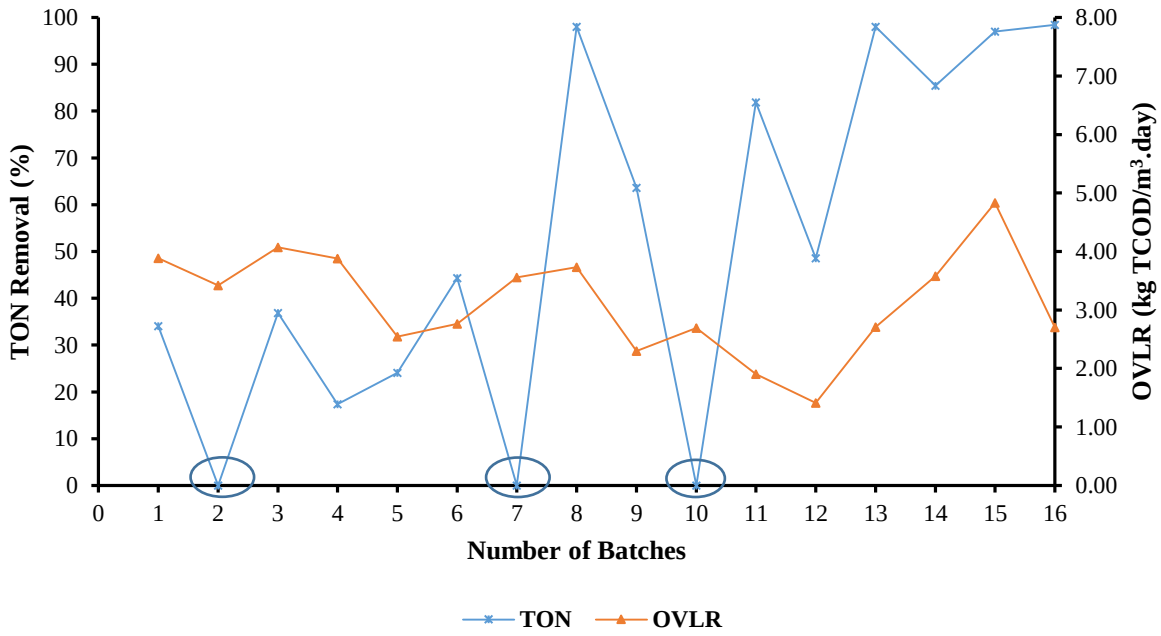


Figure 4.13: The effect of OVLR on TON removal (SRT of 5 days and HRT of 18 hours).

The SBR results on total organic nitrogen (TON), which is given by the sum of dissolved organic nitrogen fraction, particulate nitrogen fraction, and colloidal organic nitrogen are presented in **Figure 4.13** above. As shown, the SBR treatment efficiency varied for all experimental batches with fluctuations in OVLR, which had minimal effect on TON removal. When studying **Figure 4.13** and **Table 4.3**, low TON removal was obtained for the first 6 batches excluding batch number 2, since the influent composition characterisation results gave 0 mg TON/L for batch number 2, 7 and 10, thus recording 0 % TON removal. The low TON removal in the first 6 batches could be attributed to the fact that the TON in the influent stream had a higher fraction of colloidal organic nitrogen which was reluctant to the biodegradation process. Higher TON percentage removals ranging from 49 to 98 % were achieved from batch number 8 to 16. The high TON percentage removals were not affected by the OVLR fluctuations.

Table 4.3: TON removal results with variation in OVLR for experimental runs.

Batch No.	Influent (mg/L)	Effluent (mg/L)	SBR η (%)	OVLR(kg TCOD/m^3.day)
1	4.73	3.12	34	3.88
2	0	0	0	3.42
3	1.03	0.65	37	4.07
4	24.2	20	17	3.88
5	5.40	4.10	24	2.54
6	6.29	3.50	44	2.77
7	0	0	0	3.56
8	3.60	0.072	98	3.73
9	4.59	1.67	64	2.30
10	0	0	0	2.69
11	16.7	3.03	82	1.90
12	39.1	20.1	49	1.41
13	3.30	0.066	98	2.71
14	11.6	1.69	85	3.58
15	1.77	0.053	97	4.83
16	16.2	0.25	98	2.70

As shown from the findings of the study presented in **Figures 4.14, 4.15** and **4.16** on the SBR treatment efficacy on TKN, TN and NO_3-N + NO_2-N respectively, fluctuations in OVLR had minimal effect on the nutrient removal. The SBR treatment efficiency for TKN, TN and NO_3-N+NO_2-N ranged from 41 – 87%, 30 – 84% and 22 – 88% respectively. The low removal efficacies could be related to the fact that in cases whereby the nutrient loading rate in the influent stream was composed of a high fraction of particulate or colloidal biological nutrients which were reluctant to the biodegradation process, thus compromising the SBR treatment efficiency. Experimental data obtained for TKN, TN and NO_3-N+NO_2-N were statistically analysed using *two tail Student's t-Test* and alpha values of 0.01, 0.003 and 0.03 respectively were obtained. The alpha values of less than 0.05 statistically indicates that there was significant reduction on biological nutrients between the reactor influent and effluent.

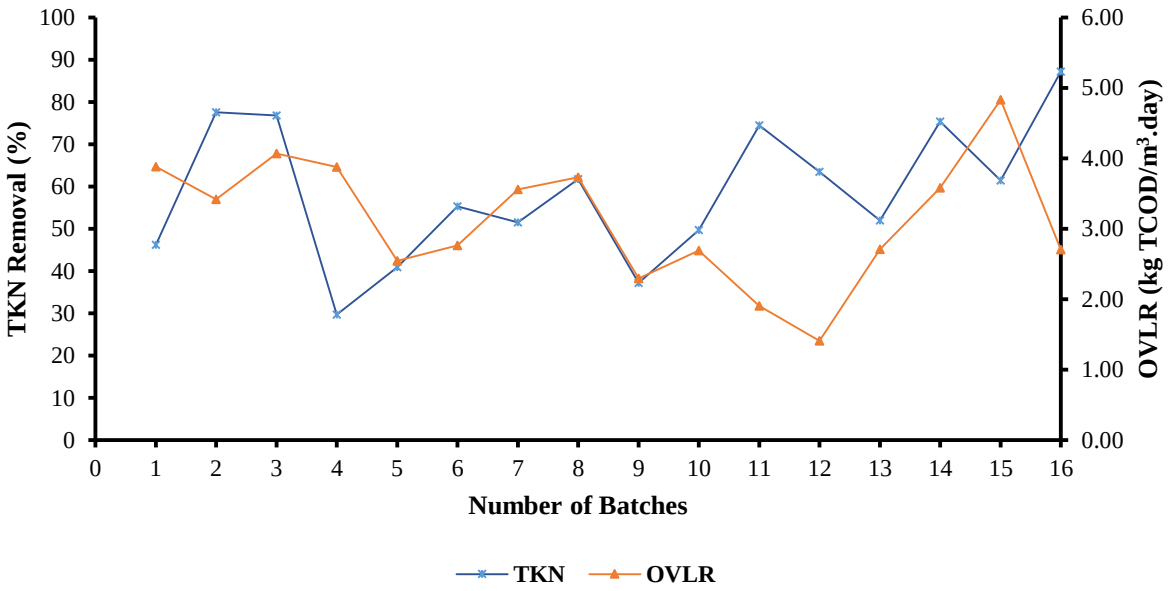


Figure 4.14: SBR-1 removal efficacies on TKN with variation on OVLR (SRT of 5 days and HRT of 18 hours).

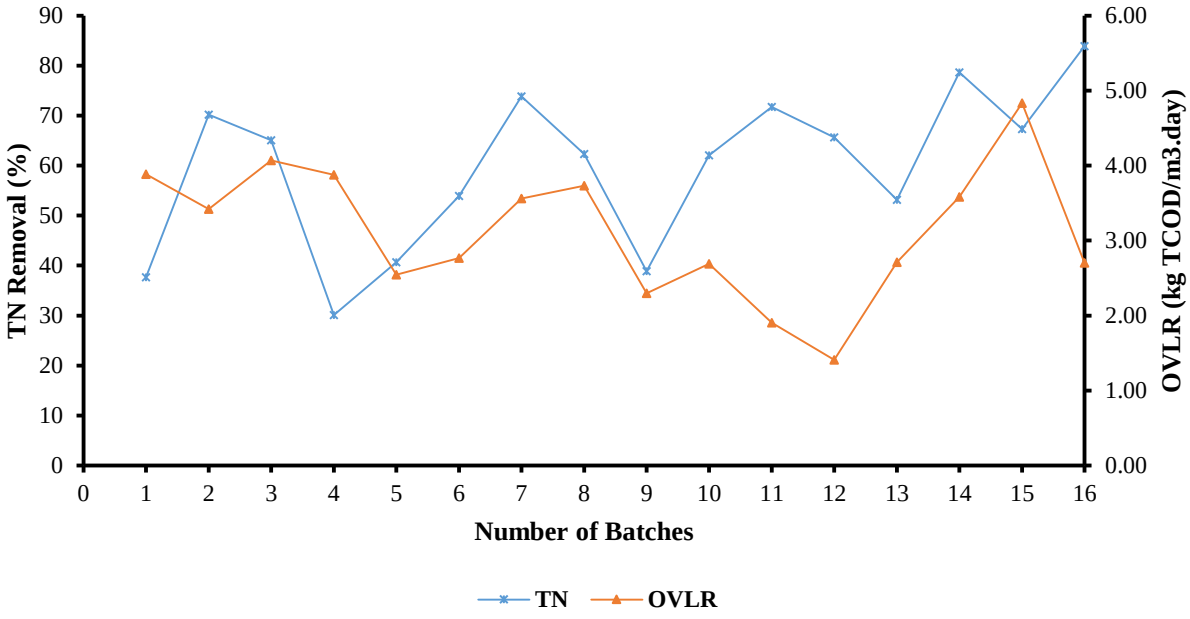


Figure 4.15: SBR-1 treatment efficacies on TN removal with variation on OVLR (SRT of 5 days and HRT of 18 hours).

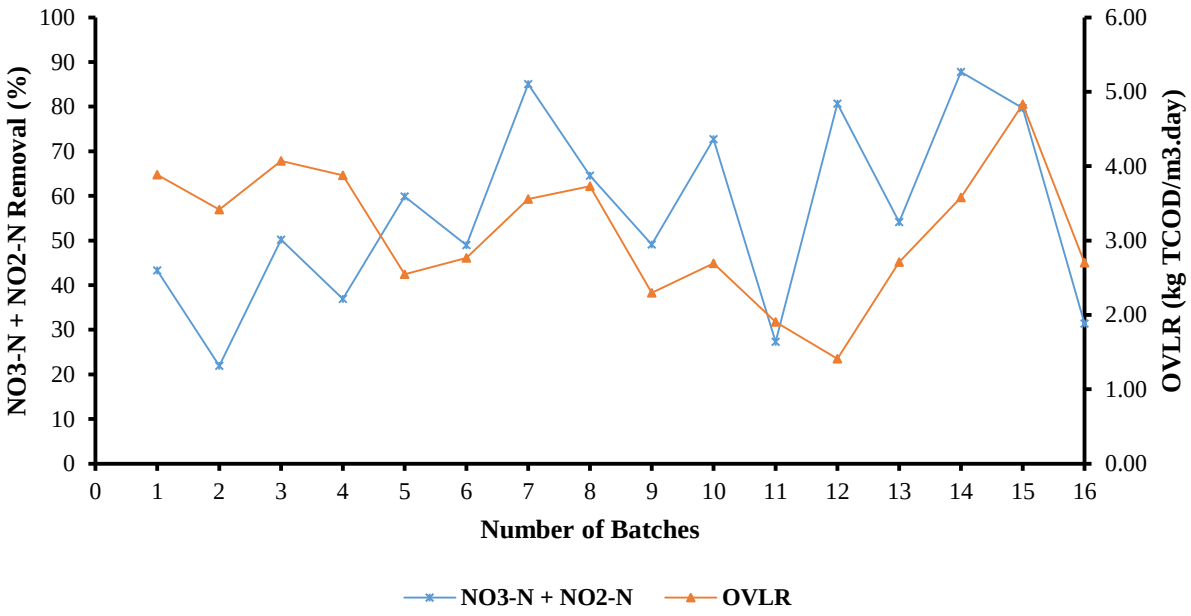


Figure 4.16: SBR-1 treatment efficacy on NO3-N + NO2-N removal with variation on OVLR (SRT of 5 days and HRT of 18 hours).

4.5.4 Effect of Reactor Temperature

According to Mulkerrins, Dobson and Colleran (2004) and Peng and Zhu (2006), temperature has a direct effect on the microbial population metabolic activities, thus it can affect the process of biological nutrient removal. **Figure 4.17** below presents the temperature profile obtained for this research study for both SBR-1 and SBR-2.

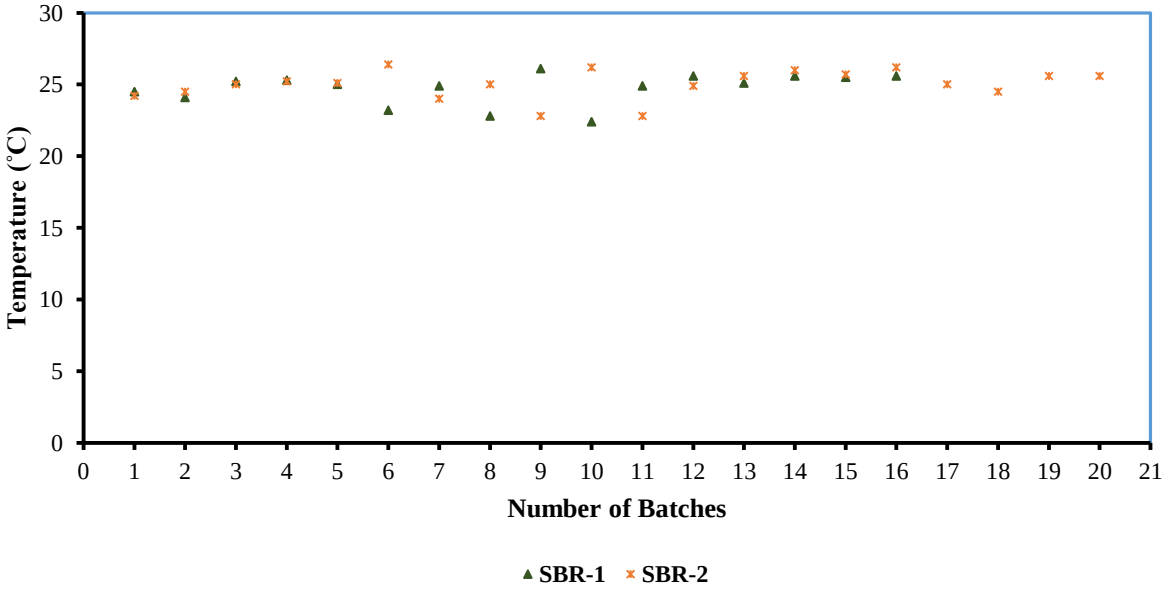


Figure 4.17: Temperature profile for SBR-1 and SBR-2.

The temperature inside the reactors was measured as a process monitoring parameter to ensure that microbial population metabolic activities were not compromised by temperature fluctuations within the reactors. As shown in **Figure 4.17**, it is observed that the temperature inside both reactors ranged between 23 and 26˚C. This temperature range was found to be in congruent with the findings reported by Mulkerrins, Dobson and Colleran (2004) on biological nutrient removal under mesophilic temperature conditions. It was observed that the temperature recorded from this study did not have a negative impact on the microbial activities inside the reactors.

4.5.5 The Effect of pH on Biological Nutrient Removal

According to Metcalf and Eddy (2014), microbial population metabolic activities are inhibited at pH levels of 9.5 and above or below 4.0. **Figure 4.18** below presents the pH profile attained for this research study for both SBR-1 and SBR-2.

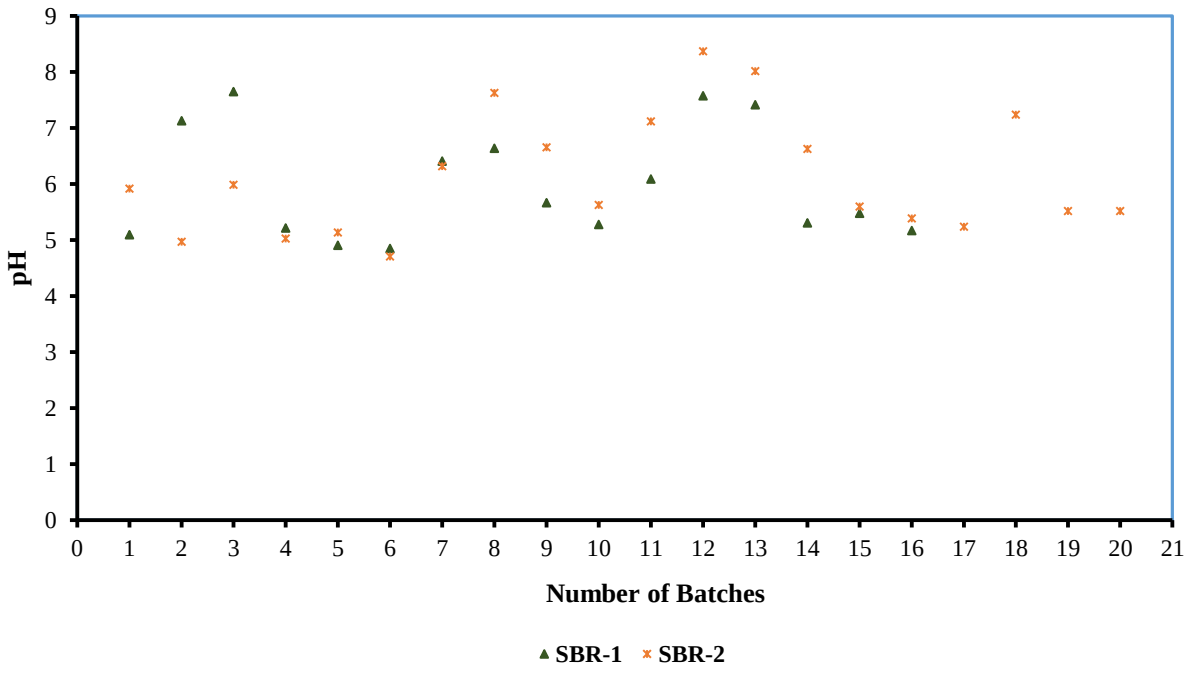


Figure 4.18: pH profile for both SBR-1 and SBR-2.

The pH is a very important parameter which affects microbial metabolic activities. It can be seen from Figure 4.18 above, that the pH was maintained within the range of 4.9 – 8.4 for both SBR-1 and SBR-2. From the pH values obtained in this study it is observed that they did not

inhibit any microbial activities. For all experimental runs, the pH was adjusted by adding NaOH in cases of low pH levels and HCl in cases of high pH levels from the influent stream.

4.6 Summary of results for an aerobic-anaerobic SBR treatment efficacy on biological nutrients removal

Figure 4.19 presents the findings of the study on overall biological nutrients percentage removal obtained in this experimental research study. The results obtained in this study indicated a SBR overall nutrients removal efficacies of 69% NH_3-N, 59% TKN, 56% NO_3-N+NO_2-N, 60% TN, 64% TON and 67% TIN. Furthermore, the findings of the study presented in **Figure 4.19** show strong congruence with previous studies conducted by Ab Halim *et al.* (2015) and Wang *et al.* (2010) on biological nutrient removal using a SBR seeded with unacclimated activated sludge. Furthermore, from the *two tail Student's t-Test* results for all biological nutrients, an alpha value of less than 0.05 was obtained. Thus, an observation was made that there was a statistically significant reduction on biological nutrients in the SBR when comparing the reactor influent and effluent reactor streams.

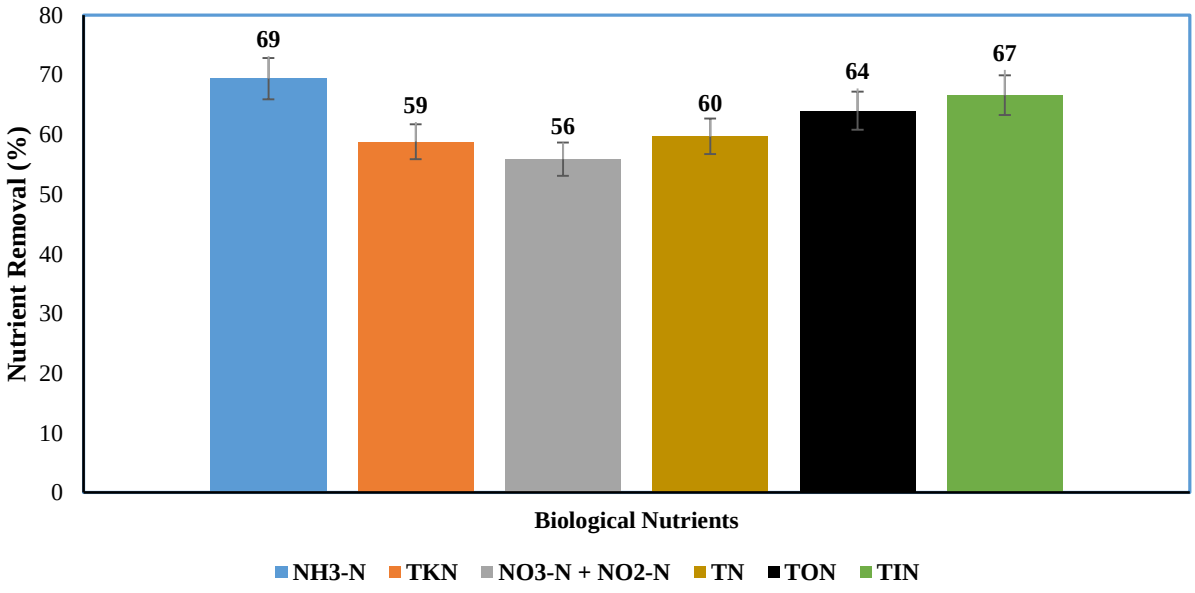


Figure 4.19: SBR-1 overall treatment efficacy for all biological nutrients investigated.

4.7 Substrate utilization rate kinetics and microbial growth rate kinetics

For the results discussed in this subsection, reference should be made to the microbiology kinetics and OVLR descriptive statistics, and microbiology kinetics model constants presented in **APPENDIX C** and **D** respectively.

For the substrate utilisation rate kinetics, the *Michaelis-Menten's* model was implemented as presented by equation [2.9] below, and for the microbial growth rate kinetics, the Monod model was implemented as presented by equation [2.11]. All symbols are defined as per sub-section 2.5.1.

$$r_{su} = \frac{kXS}{K_s+S} \qquad [2.9]$$

$$r_g = \frac{\mu_m XS}{K_s+S} \qquad [2.11]$$

The values k, K_s and μ_m presented in **Table D.1** and **Table D.2** in **Appendix D** generated from previously done studies, were selected as per recommendation by Monod (1942) and Bailey and Ollis (1986).

4.7.1 Results on Substrate Utilization Rate with COD Variation

The findings of this study presented in **Figures 4.20** and **4.21** demonstrated that an increase in the reactor substrate concentration (i.e. g COD/m^3) results in an increase in the substrate utilization rate. The findings of this research study were in line with the *Michaelis-Menten's* empirical model for substrate utilization rate. However, when studying **Figures 4.20** and **4.21** there were cases in which low substrate concentrations gave high substrate utilization rates and visa-versa. In the case of **Figure 4.20**, the high substrate utilization rate with low substrate concentration could be attributed to the fact that the reactor influent was composed of a higher organic matter fraction which gave a higher biomass concentration for the effluent stream in the form of VSS. Furthermore, the substrate utilization rate is a function of VSS. In the case of **Figure 4.21**, the low substrate utilization with high substrate concentration could be attributed to the fact that the reactor effluent stream had a higher fraction of inorganic bio-solids compared to the organic bio-solids fraction which resulted in a lower VSS fraction, thus giving low substrate utilization rates. However, a good correlation between the substrate concentration

and substrate utilization rate was statistically observed giving a polynomial fit constant of R^2 = 0.8179 and 0.8923 for SBR-1 and SBR-2 respectively.

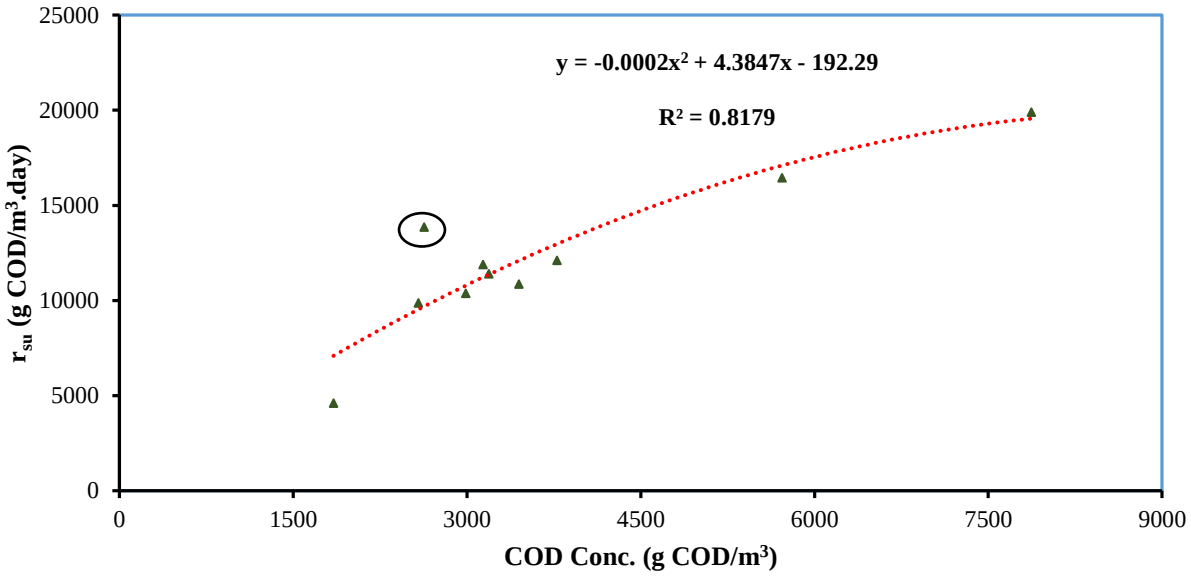


Figure 4.20: Findings of the study on substrate utilization rate for SBR-1.

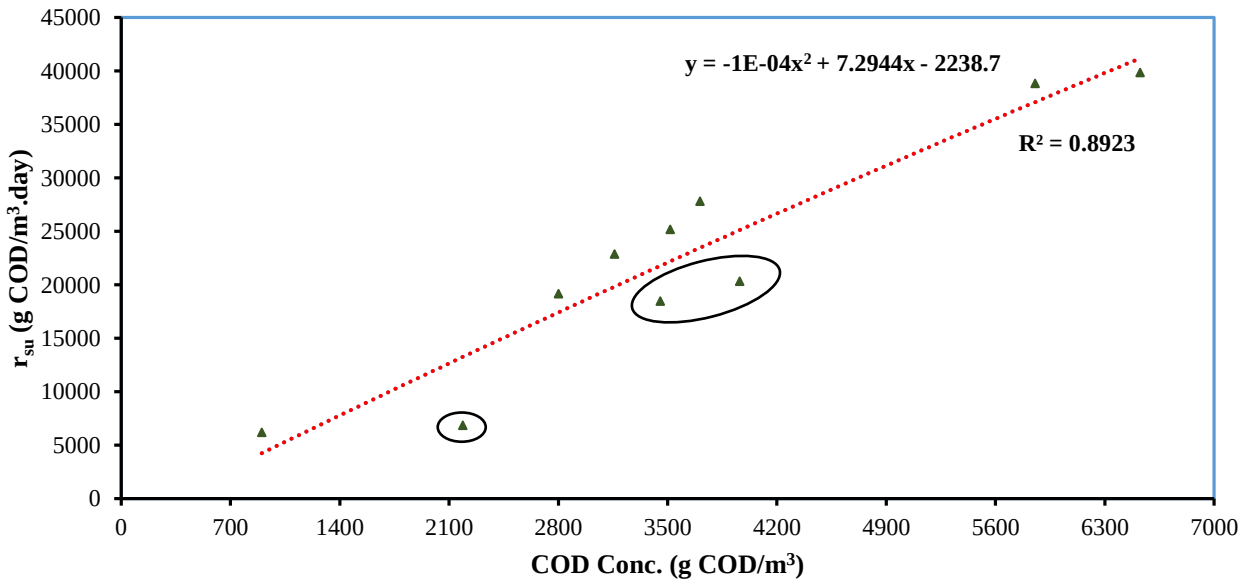


Figure 4.21: Findings of the study on substrate utilisation rate for SBR-2.

4.7.2 Results on Microbial Population Growth Rate from Substrate Utilization Rate Kinetics

From **Figures 4.22** and **4.23** below, it can be seen that an increase in the substrate utilization rate resulted in an increase in microbial growth rate in both SBR-1 and SBR-2 at a growth rate ranging from 4075 to 18121 g/m^3.day and 5465 to 25158 g/m^3.day respectively. Furthermore, an average microbial growth rate of 12518 and 16860 g/m^3.day was obtained for both SBR-1 and SBR-2 respectively. The relationship of the substrate utilization rate and microbial growth rate discussed in this sub-section could be attributed to the fact that the microbial population growth rate is directly proportional to the substrate utilization rate (Monod 1942; Metcalf and Eddy 2014). Moreover, a strong correlation between the substrate utilization rate and microbial growth rate was statistically observed for both SBR-1 and SBR-2 with polynomial fit correlation constants of R^2 = 0.9512 and 0.9745 respectively.

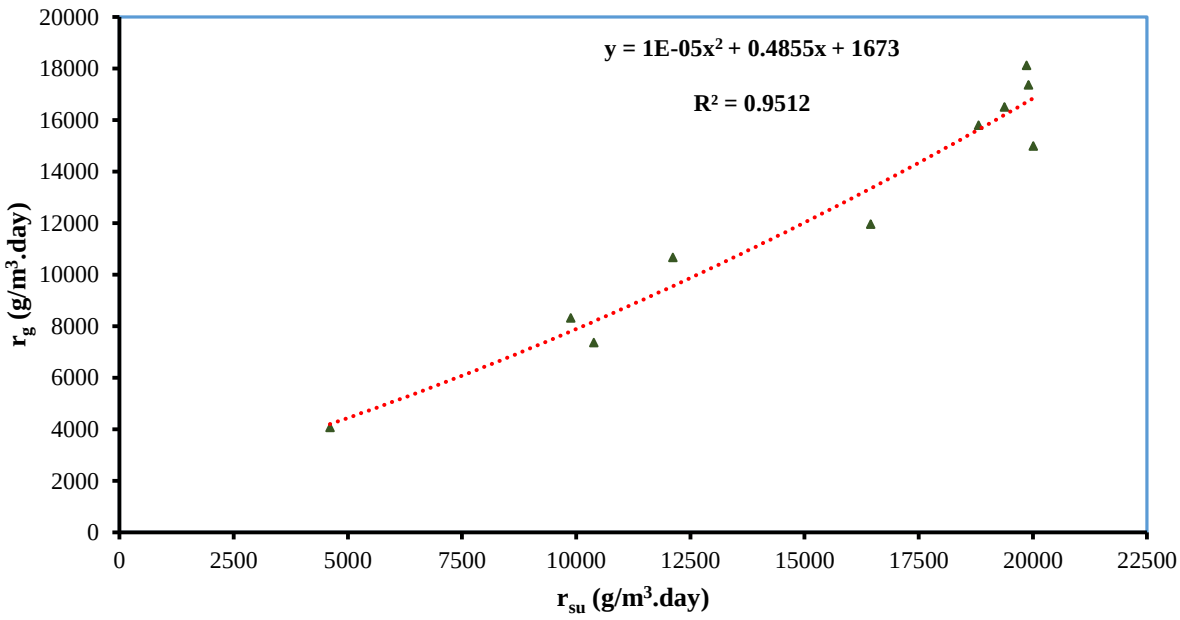


Figure 4.22: Microbial population growth-rate (SBR-1) results.

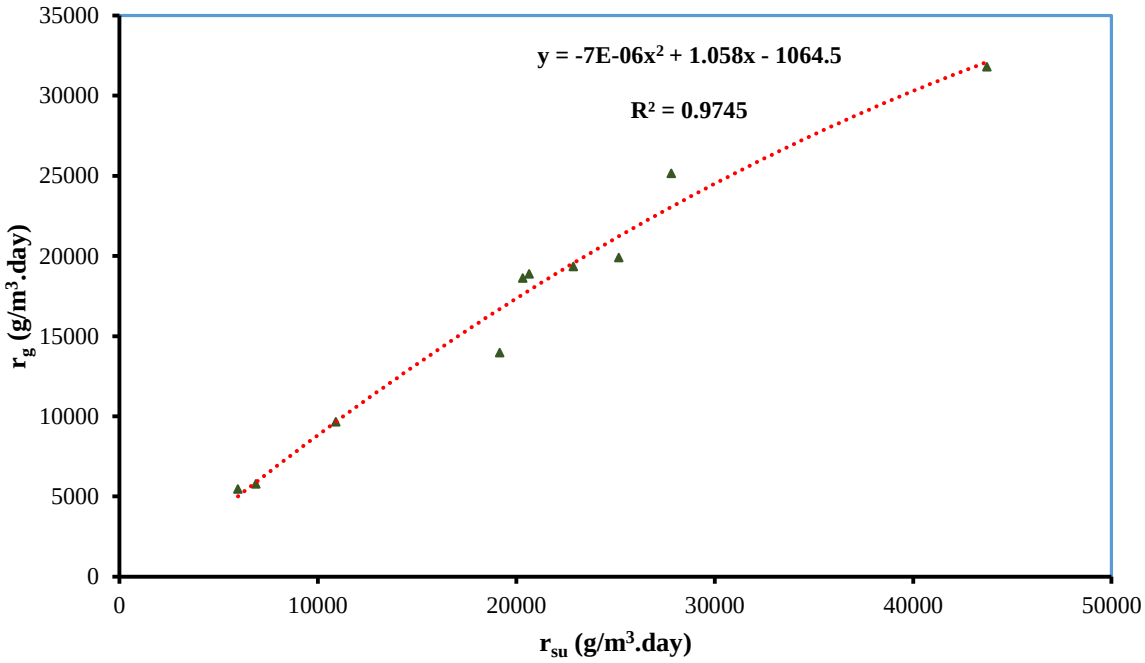


Figure 4.23: Microbial growth-rate (SBR-2) results.

4.8 Ammonia-Oxidising Rate and Ammonia Oxidising Bacteria (AOB) Growth Rate Kinetics Models.

In studying the ammonia-oxidising rate and AOB growth kinetics, the Monod kinetics models presented in equations [2.10] and [2.12] respectively, were implemented. All symbols are defined as per sub-section 2.5 and reference should be made to **Appendix D** for equation constant values.

$$r_{NH} = \mu_{max,AOB}\left(\frac{S_{NH}}{S_{NH}+K_{NH}}\right)\left(\frac{S_o}{S_o+K_{o,AOB}}\right)\left(\frac{QN_{ox}SRT}{V[1+b_{AOB}SRT]}\right) \quad [2.10]$$

$$\mu_{AOB} = \mu_{max,AOB}\left(\frac{S_{NH}}{S_{NH}+K_{NH}}\right)\left(\frac{S_o}{S_o+K_{o,AOB}}\right) - b_{AOB} \quad [2.12]$$

4.8.1 Results on Ammonia Oxidising Bacteria (AOB) Specific Growth Rate

According to Metcalf and Eddy (2014) and Monod (1942) the biodegradation of ammonia in wastewater is facilitated by the presence of AOB in activated sludge. **Figure 4.24** presents the findings of this study on the AOB specific growth rate kinetics. When studying **Figure 4.24**

below it can be seen that the AOB specific growth rate increased with increasing NH_3-N concentration being consumed in the reactor, giving an AOB specific growth rate ranging from 0.52 to 0.88 g VSS/g VSS.day with an average growth rate of 0.77 g VSS/g VSS.day. Moreover, a correlation between the NH_3-N concentration and AOB specific growth rate was attained, giving a polynomial fit correlation constant of R^2 = 0.5837. The low value of R^2 indicate that the ammonia oxidising bacteria specific growth rate was low. This is due to the fact that, the brewery wastewater which was used in this study had low concentrations of ammonia as presented in **Table 4.1** in the summary of results for the characteristics of brewery wastewater composition.

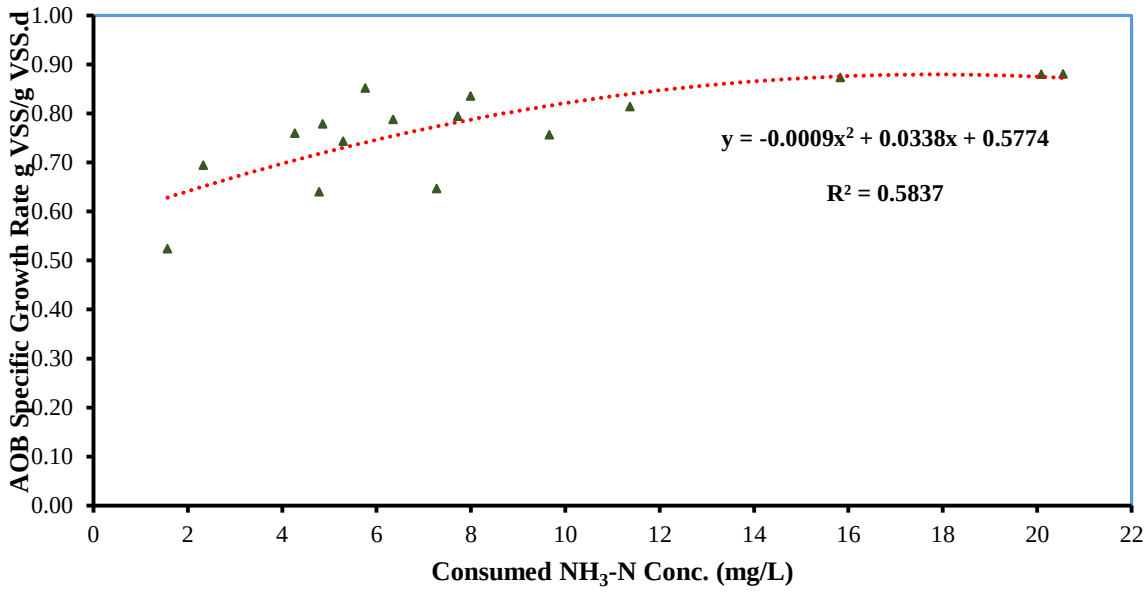


Figure 4.24: Findings of the study on AOB specific growth rate kinetics.

4.8.2 Overall Results on Microbial Growth Rate and Substrate Utilization Rate Kinetics

For the findings of the study discussed in this sub-section, reference should be made to the microbiology descriptive statistics results presented in **APPENDIX C**. **Table 4.4** present findings of the study on both microbial growth rate and substrate utilization rate kinetics. The findings are presented in terms of mean with standard deviation and range, and were statistically analysed at a 95 % confidence interval.

Table 4.4: Results on Microbial Growth Rate and Substrate Utilization Rate Kinetics.

	SBR-1		SBR-2	
Parameter	**Mean (±SD)**	**Range**	**Mean (±SD)**	**Range**
r_{su} (g COD/m^3.d)	15141±5499	4607 – 20010	20343±11107	5970 – 43713
r_g (g VSS/m^3.d)	12518±4794	4075 – 18121	16860±8346	5465 – 31790
μ_{AOB} (g VSS/g VSS.d)	0.75±0.09	0.52 – 0.88	---	---
r_{NH} (g NH_3-N/m^3.d)	937±33.9	2.27 – 134	---	---
F/M (g COD/g VSS.d)	2.86±0.78	1.89 – 4.37	2.86±0.78	1.89 – 4.37

From **Table 4.4**, it can be seen that SBR-1 and SBR-2 recorded high substrate utilization rates and microbial growth rates of 15 141 and 20 343 g COD/m^3.d and 12 518 and 16 860 g VSS/g VSS.d on mean averages respectively. The high substrate utilization rates were attained due to the fact that the reactor influent stream recorded high organic volumetric loading rates in teams of kg COD/m^3.d, which gave higher substrate utilization rates which resulted in a hike in microbial growth rate. Moreover, it was observed that both the substrate utilization rate and microbial growth rate were higher for SBR-2 when compared to SBR-1. This means that the microbial population and reactor operating conditions favoured the PAOs over the AOB, since the brewery wastewater samples used in this study were rich in orthophosphates. Furthermore, an AOB growth rate of 0.75 g VSS/g VSS.d on mean average was attained for SBR-1. This means that for the microbial population produced on a daily basis by the system, 75% was AOB, giving an ammonia-oxidising rate of 937 g NH_3-N/m^3.d at a food to microorganism ratio (F/M) of 2.86 g COD/g VSS.d on mean average.

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The aim of this experimental research study was to investigate the performance of a cyclic aerobic-anaerobic SBR inoculated with activated sludge for the removal of orthophosphates and nitrogen group pollutants from industrial wastewater generated from the brewery. This section presents the conclusions and recommendations made on the basis of the findings presented in Chapter Four. In achieving the aim of the research study, the objectives of the study were defined as follows:

- Characterising wastewater generated from the brewery.
- Determine the sequencing batch reactor treatment efficiency in treating brewery wastewater for biological nutrient removal in terms of percentage removal.
- Investigate the effect of influent organic pollutant strength/organic volumetric loading rate in terms of chemical oxygen demand in treating brewery wastewater for biological nutrients.
- Use Monod and *Michaelis-Menten's* empirical models to study the microbiology kinetics in terms of substrate utilisation rate kinetics, and microbial growth rate kinetics.

5.1.1 Characterisation of brewery wastewater composition

The results which were attained in this study on characterisation of brewery wastewater composition explicitly showed that wastewater generated from the brewery contained high organic, inorganic, and biological nutrients pollutants. It was also observed that the composition of brewery wastewater samples used in this study fluctuated greatly which was congruent to previously done studies on brewery wastewater characterisation. Moreover, the brewery effluent did not meet the industrial wastewater discharge limits set by the South African Department of Water Affairs, therefore brewery wastewater required treatment prior to discharge into water receiving bodies.

5.1.2 Effect of SRT and HRT on biological nutrient removal

The results obtained from this study showed that higher biological nutrient removal was achieved with increasing SRT particularly on orthophosphates removal. The findings of the study on mass balance results explicitly showed that biodegradation of biological nutrients were mostly achieved through the solids retention time more than the HRT for both the aerobic and anaerobic phases. It was also observed that an increase in HRT resulted in an increase in biological nutrient removal. Moreover, the HRT of 18 hours and SRT of 5 and 7 days for nitrogen and orthophosphates respectively was found to be optimum for the biodegradation of biological nutrients from industrial wastewater generated from the brewery.

5.1.3 Effect of organic volumetric loading rate (OVLR) on biological nutrients removal

The findings of the study showed that brewery wastewater samples used in this research study fluctuated greatly in terms of OVLR for all experimental runs. It was observed that the fluctuations in OVLR had a minimal effect on biological nutrient removal. On average an OVLR of 3.17 kg COD/m^3.d was attained. This was due to the fact that the microbial population and brewery wastewater samples used in this study were harvested from the same brewery, thus the microbial population was adaptable to the fluctuations in OVLR. It may be said that an OVLR of 3.17 kg COD/m^3.d did not inhibit the biodegradation process of biological nutrients in brewery wastewater.

5.1.4 Orthophosphates removal

The findings of the study on the investigation of the SBR for orthophosphates removal efficacy demonstrated high removal efficacies ranging from 33 to 81% and recording 69% on average. From the findings of the study, it was found that higher orthophosphates removal efficacies were achieved at a SRT of 5 days and above. Furthermore, the system was found to be stable at a SRT of 3 days with minimal effects from OVLR fluctuations. Based on the findings of the study attained, it may be concluded that the SBR demonstrated high orthophosphate removal efficacies at a SRT of 5 days and above, moreover, the SBR was found to be stable at a SRT of 3 days.

5.1.6 Nitrogen Removal

The SBR system under cyclic aerobic-anaerobic configuration was also investigated for nitrogen pollutant removal by measuring the percentage removal of NH_3-N, TKN, NO_3-N + NO_2-N, TN, TON, and TIN. The findings of the study on SBR cyclic anaerobic-aerobic configuration indicated good nitrogen pollutant removal efficacies of 69% NH3-N, 67% TIN, 64% TON, 60% TN, 59% TKN, and 56% NO_3-N+NO_2-N on average. Based on the experimental results, the SBR under cyclic anaerobic-aerobic configuration demonstrated good biological nutrient removal efficacies from industrial wastewater generated from the brewery characterised with high organic load. Therefore, the SBR system demonstrated to be a sound technology to be implemented as a treatment process for industrial wastewater with high organic load prior to discharge into water receiving bodies, thus eliminating nitrogen pollutants from waste streams, which will enable the remediation of nitrogen pollution.

5.1.7 Substrate utilization rate kinetics and microbial growth rate kinetics

As discussed in detail in the literature presented in Chapter Two, it is imperative to study microbiology kinetics for the understanding of the dynamic manifestations of microbial life. Higher substrate concentration in the form of readily biodegradable COD were found to impact positively on the substrate utilization rate. Higher COD concentrations resulted in higher substrate utilisation rates. The findings of the study showed strong congruence with the *Michaelis-Menten*'s empirical model for substrate utilisation rates. According to the *Michaelis-Menten's* empirical model, the substrate utilization rate increases with an increase in the substrate concentration. Furthermore, based on the findings of the study, it was found that higher substrate utilisation rates gave an increase in microbial growth rate. Microbial growth rates of 12 518 and 16 860 g VSS/g VSS.d on average were recorded for SBR-1 and SBR-2 respectively, with an AOB growth rate of 0.75g VSS/g VSS.d. Based on the findings of the study on microbial growth and substrate utilisation rate, the microbial population used in this study demonstrated higher substrate utilisation rates and good microbial population growth rates. Thus, facilitating the biodegradation process of biological nutrients from industrial wastewater with high organic load.

5.2 Recommendations

From the knowledge attained in this research work, the following recommendations are proposed for further studies to be conducted on biological nutrient removal from industrial wastewater characterised with high organic load.

5.2.1 Wastewater composition C:N:P ratio balance

This study indicated that brewery wastewater pollutants fluctuate greatly which led to fluctuations in nutrient removal efficacies, particularly nitrogen pollutants. It is recommended that an investigation on biological nutrient removal using brewery wastewater with a well-balanced C:N:P ratio should be done to improve biological nutrient removal efficacies.

5.2.2 Temperature variation within the reactor

For this research work, the temperature within the reactor was left un-adjusted. It is recommended that further studies has to be done on SBR systems for biological nutrient removal with temperature variation to investigate the effect of temperature on the SBR performance for biological nutrient removal.

5.2.3 Use of acclimated sludge

The SBR was inoculated with un-acclimated sludge, since both the sludge and brewery wastewater were taken from the same plant. A recommendation is made that further studies could be conducted using well acclimated sludge under cyclic aerobic-anaerobic configuration. This could improve the biodegradation of biological nutrients during the cyclic operating configuration of the SBR system.

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APPENDIX A

Minitab 15 Descriptive Statistics Results on Raw Brewery Wastewater Characteristics

Table A.1: Descriptive Statistics Results on BWW Characteristics

Variable	Mean	StDev	CoefVar	Minimum	Median	Maximum	Range	Skewness	Kurtosis
Temperature	30,680	3,885	12,66	24,400	30,300	37,000	12,600	0,01	-1,01
pH	6,444	2,371	36,78	4,400	5,440	12,200	7,800	1,50	1,13
ORP	17,4	135,9	779,75	-305,0	77,4	135,0	440,0	-1,52	-1,52
Conductivity	2677	1009	37,70	1893	2418	6017	4124	2,35	6,09
Turbidity	562,7	163,3	29,01	303,0	559,5	1039,0	736,0	1,12	2,62
TCOD	7753	1997	25,76	3447	8095	11813	8366	-0,23	0,07
SCOD	6297	1529	24,28	2287	6317	8627	6340	-0,85	1,13
PCOD	1453	892	61,42	127	1451	3693	3566	0,86	0,67
PO_4^{3-}	340,0	63,8	18,75	229,0	356,5	424,0	195,0	-0,35	-1,24
NH_3-N	12,11	7,25	59,91	2,21	10,77	27,80	25,59	0,86	0,25
TON	8,66	10,74	124,11	0,00	4,66	39,10	39,10	1,83	3,39
TIN	33,78	21,64	64,06	7,78	26,60	93,00	85,22	1,52	2,78
TKN	29,12	24,75	84,97	6,24	21,75	94,70	88,46	1,53	2,03
TN	37,94	28,10	74,05	13,70	26,55	106,00	92,30	1,53	1,48
NO_2N+NO_3N	9,67	11,58	119,69	2,87	5,00	49,40	46,53	3,00	10,19
TS	5951	3387	56,92	2942	4751	14981	12039	1,87	3,30
TDS	4164	1433	34,43	2198	4260	7400	5202	0,80	1,81
FVS	2360	1066	45,19	825	2313	4975	4150	1,27	3,52
VSS	1809	543	30,00	1043	1907	2572	1529	-0,22	-1,44

APPENDIX B

Biological Nutrients and TCOD Removal Student's t-Test Results

Table B.1: Orthophosphates removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	343,4210526	101,9368421
Variance	4044,701754	2973,190234
Observations	20	20
Hypothesized Mean Difference	0	
df	35	
t Stat	12,56499153	
P(T<=t) one-tail	7,85945E-15	
t Critical one-tail	1,689572458	
P(T<=t) two-tail	1,57189E-14	
t Critical two-tail	2,030107928	

Table B.2: TCOD removal in SBR-2 statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	7752,5	3579,7
Variance	3989553,316	2429240,642
Observations	20	20
Hypothesized Mean Difference	0	
df	36	
t Stat	7,365730929	
P(T<=t) one-tail	5,47036E-09	
t Critical one-tail	1,688297714	
P(T<=t) two-tail	1,09407E-08	
t Critical two-tail	2,028094001	

Table B.3: Material balance on SBR-2 statistical analyses.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	0,34	0,1061345
Variance	0,004065895	0,003154008
Observations	20	20
Hypothesized Mean Difference	0	
df	37	
t Stat	12,30879305	
P(T<=t) one-tail	5,98194E-15	
t Critical one-tail	1,68709362	
P(T<=t) two-tail	1,19639E-14	
t Critical two-tail	2,026192463	

Table B.4: Presents NH3-N removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	12,10625	3,62544
Variance	52,599718	4,31914
Observations	16	16
Hypothesized Mean Difference	0	
df	17	
t Stat	4,4964457	
P(T<=t) one-tail	0,0001591	
t Critical one-tail	1,7396067	
P(T<=t) two-tail	0,0003182	
t Critical two-tail	2,1098156	

Table B.5: NO_3-N+NO_2-N removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	9,67375	2,86
Variance	134,06169	2,9372
Observations	16	16
Hypothesized Mean Difference	0	
df	16	
t Stat	2,3285612	
P(T<=t) one-tail	0,0166588	
t Critical one-tail	1,7458837	
P(T<=t) two-tail	0,0333175	
t Critical two-tail	2,1199053	

Table B.6: TIN removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	33,783125	10,20625
Variance	468,31922	22,284412
Observations	16	16
Hypothesized Mean Difference	0	
df	16	
t Stat	4,2577568	
P(T<=t) one-tail	0,0003007	
t Critical one-tail	1,7458837	
P(T<=t) two-tail	0,0006014	
t Critical two-tail	2,1199053	

Table B.7: TON removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	Variable 2
Mean	8,656875	3,6351875
Variance	115,43234	43,112313
Observations	16	16
Hypothesized Mean Difference	0	
df	25	
t Stat	1,5952688	
P(T<=t) one-tail	0,0616088	
t Critical one-tail	1,7081408	
P(T<=t) two-tail	0,1232175	
t Critical two-tail	2,0595386	

Table B.8: TKN removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	29,123125	10,443125
Variance	612,3532	68,944903
Observations	16	16
Hypothesized Mean Difference	0	
df	18	
t Stat	2,8626501	
P(T<=t) one-tail	0,0051731	
t Critical one-tail	1,7340636	
P(T<=t) two-tail	0,0103461	
t Critical two-tail	2,100922	

Table B.9: TN removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	37,94375	13,39625
Variance	789,35729	66,798145
Observations	16	16
Hypothesized Mean Difference	0	
df	18	
t Stat	3,3557604	
P(T<=t) one-tail	0,0017599	
t Critical one-tail	1,7340636	
P(T<=t) two-tail	0,0035198	
t Critical two-tail	2,100922	

Table B.10: SBR-2 TCOD removal statistical analyses results.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	7635,5	3505,25
Variance	4749257,5	2855975,3
Observations	16	16
Hypothesized Mean Difference	0	
df	28	
t Stat	5,9907346	
P(T<=t) one-tail	9,381E-07	
t Critical one-tail	1,7011309	
P(T<=t) two-tail	1,876E-06	
t Critical two-tail	2,0484071	

APPENDIX C

Microbiology Kinetics and OVLR Descriptive Statistics Results

Table C.1: SBR-1 substrate utilisation rate descriptive analyses results.

Descriptive Analyses: SBR-1 r_{su} Rate	
Mean	15141,2995
Standard Error	1739,10498
Median	17630,8313
Mode	#N/A
Standard Deviation	5499,53284
Sample Variance	30244861,5
Kurtosis	-0,5808327
Skewness	-0,8289732
Range	15402,8946
Minimum	4607,63355
Maximum	20010,5281
Sum	151412,995
Count	10
Confidence Level (95,0%)	3934,1288

Table C.2: SBR-2 substrate utilisation rate descriptive analyses results.

Descriptive Analyses: SBR-2 r_{su} Rate	
Mean	20342,99191
Standard Error	3512,461357
Median	20481,35546
Mode	#N/A
Standard Deviation	11107,37808
Sample Variance	123373847,9
Kurtosis	1,189262281
Skewness	0,714740679
Range	37743,41246
Minimum	5969,874372
Maximum	43713,28683
Sum	203429,9191
Count	10
Confidence Level (95,0%)	7945,739618

Table C.3: SBR-1 microbial growth rate kinetics descriptive analyses results.

Descriptive Analyses: SBR-1 r_g	
Mean	12517,76235
Standard Error	1516,067765
Median	13475,95682
Mode	#N/A
Standard Deviation	4794,227226
Sample Variance	22984614,69
Kurtosis	-1,013051928
Skewness	-0,523092276
Range	14046,10942
Minimum	4075,118738
Maximum	18121,22816
Sum	125177,6235
Count	10
Confidence Level (95,0%)	3429,583555

Table C.4: SBR-1 microbial growth rate kinetics descriptive analyses results.

Descriptive analyses: SBR-2 r_g	
Mean	16860,04164
Standard Error	2639,211423
Median	18757,66167
Mode	#N/A
Standard Deviation	8345,919324
Sample Variance	69654369,37
Kurtosis	-0,32588259
Skewness	0,172900148
Range	26325,06288
Minimum	5465,421915
Maximum	31790,4848
Sum	168600,4164
Count	10
Confidence Level (95,0%)	5970,311025

Table C.5: AOB growth rate descriptive analyses results.

Descriptive Analyses: AOB Growth Rate	
Mean	0,766732846
Standard Error	0,024835155
Median	0,783563276
Mode	#N/A
Standard Deviation	0,099340621
Sample Variance	0,009868559
Kurtosis	0,885661545
Skewness	-1,014664789
Range	0,356905379
Minimum	0,524296378
Maximum	0,881201758
Sum	12,26772554
Count	16
Confidence Level (95,0%)	0,05293488

Table C.6: Ammonia-oxidising rate statistics descriptive analyses results.

Descriptive Analyses: SBR-1 r_{NH3N}	
Mean	37,465584
Standard Error	8,4571182
Median	27,719892
Mode	#N/A
Standard Deviation	33,828473
Sample Variance	1144,3656
Kurtosis	3,6197055
Skewness	1,8517368
Range	131,6001
Minimum	2,2751009
Maximum	133,8752
Sum	599,44934
Count	16
Confidence Level(95,0%)	18,025921

Table C.7: OVLR descriptive analyses results.

Descriptive Analyses: OVLR	
Mean	3,1714773
Standard Error	0,182712
Median	3,3115909
Mode	#N/A
Standard Deviation	0,8171127
Sample Variance	0,6676732
Kurtosis	0,0655219
Skewness	-0,2332925
Range	3,4224545
Minimum	1,4101364
Maximum	4,8325909
Sum	63,429545
Count	20
Confidence Level (95,0%)	0,3824205

Table C.8: F/M ratio descriptive analyses results.

Descriptive Analyses: F/M Ratio	
Mean	2,861933565
Standard Error	0,248219321
Median	2,677664873
Mode	#N/A
Standard Deviation	0,784938412
Sample Variance	0,616128311
Kurtosis	0,717472055
Skewness	1,208009977
Range	2,481458396
Minimum	1,891927042
Maximum	4,373385438
Sum	28,61933565
Count	10
Confidence Level(95,0%)	0,561511114

APPENDIX D
MICROBIOLOGY KINETICS AND ORGANIC LOADING RATE

Substrate Utilisation Rate Kinetics Models Constants

Table D.1: Microbiology kinetics equations constants of k and Ks (Monod 1942; Bailey and Ollis 1986).

Coefficient	Range
k	8 – 12 g COD/g VSS
K_s	10 – 40 g COD/m^3

Food to Microorganism (F/M) Ratio Equation Model

$$\frac{F}{M} = \frac{total\ applied\ substrate\ rate}{total\ microbial\ biomass} = \frac{QS_o}{VX} \qquad [1D]$$

Where, $F/M =$ food to biomass ratio, g BOD or COD/g VSS.d

$Q =$ Influent wastewater flowrate, m^3/d

$S_o =$ Influent BOD or COD concentration, g/m^3

$V =$ Aeration tank volume, m^3

$X =$ Mixed liquor biomass concentration in the aeration tank, g/m^3

According to Metcalf and Eddy (2014) the F/M ratio is useful for the understanding of transient loads on a system. The higher the organic loading rate the faster is the substrate utilization rate and thus the reactor would have a higher substrate concentration.

Organic Volumetric Loading Rate

$$L_{org} = \frac{QS_o}{(V)(10^3 g/1\ kg)} \qquad [2D]$$

Where, $L_{org} =$ Volumetric organic loading rate, kg COD/m^3.d

Relative Nitrification rate at a given pH, the model was adopted from (EPA 1993)

$$NR_{pH} = (0.0004017)e^{1.0946pH} \qquad [3D]$$

Where, NR_{pH} = Relative nitrification rate at particular pH

Table D.2: Activated sludge design kinetic coefficients at 20˚C (EPA 1993; Metcalf and Eddy 2014).

Coefficient	Unit	COD Oxidation	NH_4 Oxidation	NO_2 Oxidation
μ_{max}	g VSS/g VSS.d	6.0	0.90	1.0
K_s, K_{NH_4}, K_{NO_2}	mg/L	8.0	0.50	0.20
Y	g VSS/g substrate	0.45	0.15	0.05
b	g VSS/g VSS	0.12	0.17	0.17
K_{O_2}	mg/L	0.20	0.50	0.90
θ value				
μ_{max}	unitless	1.07	1.072	1.063
b	unitless	1.04	1.029	1.029
K_s, K_{NH_4}, K_{NO_2}	unitless	1.0	1.0	1.0