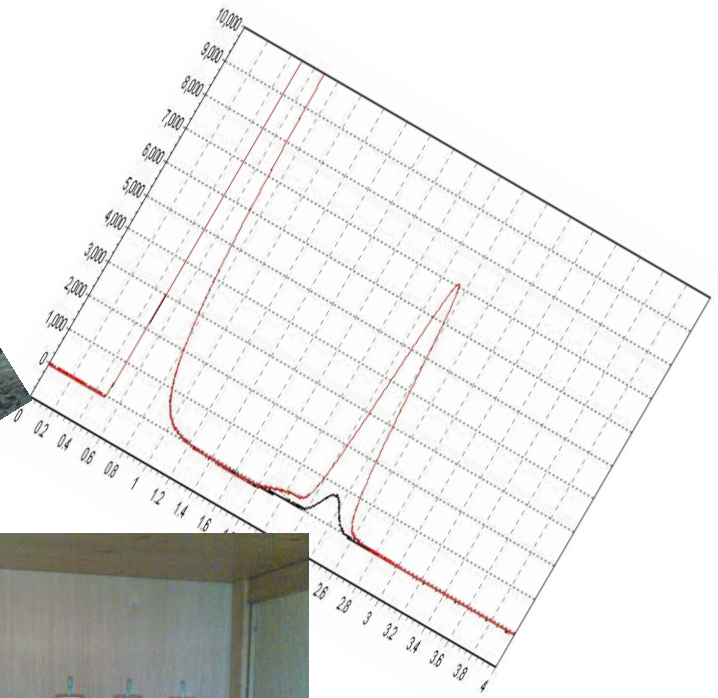


UNESCO-IHE INSTITUTE FOR WATER EDUCATION



LABORATORY STUDY ON THE EMISSIONS OF N_2O , CH_4 AND CO_2 FROM ACTIVATED SLUDGE

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A LABORATORY STUDY ON THE EMISSIONS OF N₂O, CH₄ AND CO₂ FROM ACTIVATED SLUDGE

Master of Science Thesis

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Abstract

The last 200 years, has witnessed human activities in agriculture, industry, waste disposal, deforestation and most significantly fossil fuel producing unprecedented impacts on levels of GHGs, thus changing the composition of these gases in the atmosphere. One of the main sources of these GHGs is wastewater treatment, where it expected grow.

The quest to meet the needs of the ever increasing human population is exerting a significant pressure on the global environment. The Millennium Development Goal 7 aims to ensure that by the year 2015, the proportion of people without access to portable drinking water and adequate basic sanitation is halved. This calls for accelerated expansion in portable water facilities. However, the use of water is associated with wastewater generation since about 75-85% of water used is returned as waste. As long as populations grow significantly, GHGs from wastewater sources will continue to have a major influence on the upward trends in CO₂, CH₄ and N₂O emissions.

Whereas it is obvious that wastewater treatment systems have a great potential in controlling water pollution from both point and non point sources, it is not certain if the secondary environmental burden and effects in terms of GHGs emissions makes them environmentally sustainable. This research is thus an attempt to identify and quantify the GHGs particularly N₂O, CO₂ and CH₄ from an activated sludge wastewater treatment system under different temperatures and nutrient conditions.

Active sludge samples from the Aerobic tank (AE), Anaerobic Digester (AN) and Denitrifying (Anoxic) tank (DE) of an activated sludge wastewater treatment system were incubated under four different temperatures namely 4°C, 20°C, 30°C and 37°C. Gas samples were collected and analysed in GC with specific settings to identify N₂O, CO₂ and CH₄. Experiments were also conducted under altered nutrient (15mg/l & 30mg/l nitrate and 30mg/l ammonia) concentrations and shaking (100rpm) at 30°C.

The results showed that elevated temperatures promote higher releases of N₂O, CO₂ and CH₄. Higher nitrate concentrations positively influence the release of N₂O and CH₄ but have no influence on CO₂ release. The relatively lower emissions measured for N₂O at 4°C and 20°C were due to a slowdown of the denitrification and nitrification processes at lower temperatures. Furthermore, the lower emissions of CO₂ and CH₄ at 4°C and 20°C were also attributable to a slowdown of biological activities at lower temperatures. Shaking at 100rpm led to less N₂O, CO₂ and CH₄ emissions. Additionally, it was found that activated sludge treatment system is an important source of GHGs with the anaerobic sludge having the highest potential for the release of the most important GHGs (N₂O, CO₂ and CH₄)

Key Words: Aerobic sludge, activated sludge, ammonification, anaerobic sludge, denitrifying sludge, denitrification, electron capturing detector, gas chromatography, global warming potential, greenhouse effect, greenhouse gases, methanogenesis, nitrification, thermal capturing detector.

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Dedication

To Nubania

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List of Acronyms

AE	Aerobic Sludge
AMIS	Anaerobic Methane Incubation System
AN	Anaerobic Sludge
AOB	Ammonia Oxidising Bacteria
AOM	Anaerobic Oxidation O f Methane
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DE	Denitrifying Sludge
DO	Dissolve Oxygen
ECD	Electron Capturing Detector
EF	Emission Factors
GC	Gas Chromatography
GHGs	Greenhouse Gases
GWP	Global Warming Potential
IPCC	Intergovernmental Panel on Climate Change
MDG	Millennium Development Goal
MOA	Methane Oxidising Acheae
ODS	Ozone Depleting Substances
TCD	Thermal Capturing Detector
TSS	Total Suspended Solids
UASB	Upflow Anaerobic Sludge Blanket
UN	United Nations
US EPA	United States Environmental Protection Agency
UNEP	United Nations Environment Programme
VFAs	Volatile Fatty Acids

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Wastewater may be defined as any water that has been adversely affected in quality by anthropogenic influence. According to UNEP (2000), wastewater is the main land based point source of pollutant responsible for contamination of water resources. Current wastewater disposal practices have many impacts on the environment. El-Fadel & Massoud (2002) emphasized that greenhouse gases are released when wastewater is handled under anaerobic condition. Added to this is the offensive odour emitted as a result of hydrogen sulphide generation. Wastewater also contains contaminants which are often harmful to water bodies. Water quality in many rivers is deteriorating and the marine environment is also being affected by pollution from wastewater (UNEP, 2004). Scott et al (2004) reported that 75-85% of water used may return as wastewater, the rest escape or is retained through some other means, while Zimmo et al. (2003) approximated that 95% of all wastewater generated globally is released to the environment without treatment. This has resulted in rivers downstream from large cities especially in developing countries being barely cleaner than open sewers. Thus wastewater management should be considered in the context of sustainable development¹. It also means that the technologies employed in managing wastewater should have environmental sustainability² as the key word and should therefore not put further burden on the environment.

There are however many technologies suitable for wastewater treatment including conventional and the so called eco-technological treatment systems. While conventional systems allow for process manipulation, eco-technological systems are known for their simplicity based on natural treatment principles. Nevertheless, both conventional and the natural treatment systems may generate environmental effects that impact on climate change due to different transformation and biochemical processes, environmental and operational factors (temperature, pH, aerobic or anaerobic) and conditions at different stages of operation of wastewater treatment plant. The performance of conventional and eco-technological treatment systems is affected by undesirable effects including emission of greenhouse gases³ (GHGs) and odour (Shilton and Walmsey, 2005; van der Steen et al., 2003; Reed et al., 2000; Crites et al., 1995)

Whereas it is certain that conventional systems have lower foot prints in terms of land requirements, it is however, not certain if the secondary environmental burden and effects in terms of GHGs emissions are lower for these systems. This is what has necessitated the demand for reliable emission data for precise assessment of the environmental sustainability of these systems.

¹ The Brundtland Commission defined sustainable development as development that "meets the needs of the present without compromising the ability of future generations to meet their own needs"

² Environmental sustainability: the process of making sure current processes of interaction with the environment is pursued with the idea of keeping the environment as pristine as naturally possible.

³ Greenhouse gases are those gaseous constituents—both natural and anthropogenic—of the Earth's atmosphere that absorb infrared radiation emitted from the Earth's surface, the atmosphere, and clouds. Water vapour (H₂O), carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are the primary greenhouse gases in the Earth's atmosphere.

1.2 Problem Definition

The last century has seen a drastic change in the earth's climate. There are a variety of causes for the global climate change, including natural and human-induced mechanisms. The IPCC concluded that human activities are changing the atmospheric concentrations and distributions of greenhouse gases.

These changes can produce a radiative forcing⁴ by changing either the reflection or absorption of solar radiation, or the emission and absorption of terrestrial radiation (IPCC 1996). Building on that conclusion, IPCC further asserted that “concentrations of atmospheric greenhouse gases and their radiative forcing have continued to increase as a result of human activities” (IPCC 2001). The increasing concentration in these GHGs from anthropogenic sources facilitates the formation of a thermal blanket around the earth and thus traps energy radiated from the earth's surface thereby causing the earth's temperature to rise: a phenomenon known as the Greenhouse Effect⁵ and its implications on Global warming⁶. The contribution of the major GHGs to the greenhouse effect are CO₂ 50%, CH₄ 18%, CFCs 14%, O₃ 8%, N₂O 6% and other gases 4%. In De's (1999) estimation, the annual increases in concentration of these GHGs in the atmosphere are 0.4%, 1.0%, 0.3% and 5.0% for CO₂, CH₄, CFCs and N₂O respectively and this plays a significant role in climate change.

The quest to meet the needs of the ever increasing human population is exerting a significant pressure on the global environment. The Millennium Development Goal 7 target 10, aims to ensure that by the year 2015, the proportion of people without adequate basic sanitation is halved (UN, 2008). This calls for accelerated expansion in portable water facilities. However, the use of water is often associated with wastewater generation since about 75-85% of water used is returned as waste (Scott et al 2004). Thus any extension of water supply and sanitation services without a comparable expansion of wastewater treatment facilities would exacerbate the existing problems by intensify emissions from wastewater and sanitation sources. Wastewater emissions are growing most rapidly especially in developing countries. Methane from wastewater in Africa and the Middle East is expected to approximately double between 1995 and 2020 (U.S. EPA, 2002). The main driver for increasing municipal wastewater emissions is population growth associated with countries that rely on less advanced, anaerobic treatment and collection systems such as latrines, septic tanks, open sewers, and lagoons. It is estimated that over 80% of domestic wastewater goes uncollected and untreated in China, S&E Asia, and Africa. Much of this untreated wastewater is found in open sewers, pits, latrines, or lagoons where there is potential for CH₄ and N₂O production. Nearly 75% of China's wastewater emissions come from latrines, with the majority of wastewater generated in rural China being untreated (USEPA, 2006). 62% of India's emissions also come from latrines while open sewers contribute some 34% as well (USEPA, 2006). As long as populations grow significantly without large scale advances in wastewater treatment, these areas will continue to have a major influence on the upward trend in wastewater CH₄ and N₂O emissions. Concerns over these impacts and their consequences on climate change means that it is essential to determine the types and

⁴ IPCC defines radiative forcing as a measure of how the energy balance of the Earth-atmosphere system is influenced when factors that affect climate are altered.

⁵ The change in the steady state temperature of the earth by the presence of an atmosphere containing gas that absorbs and emits infrared radiation (IPCC, 2007 Fourth Assessment Report)

⁶ According to IPCC (2007) Global warming is the increase in the average temperature of the Earth's near-surface, air and oceans since the mid-twentieth century and its projected continuation

amounts of GHGs emitted by current and possible future treatment technologies, so that appropriate measures can be adopted to reduce source emissions. It is therefore important to study the processes that lead to GHGs emissions from wastewater treatment systems and the important factors (both environmental and natural) that influence them. In this respect, the understanding of such processes as biochemical reactions, microbial activity, reaction pathways and factors like pH, temperature, Oxygen etc is essential to fully understanding design measures to mitigate GHGs emissions.

1.3 Objective of the research

The overall objective of this research is to assess and quantify GHG emissions (i. e. CO₂, CH₄ and N₂O) from an activated sludge wastewater treatment system.

1.3.1 Specific Objectives

The following specific objectives are clearly defined in order to achieve the overall objective:

- i) To develop a way to measure simultaneously, CO₂, CH₄ and N₂O in gas and liquid phases in activated sludge wastewater treatment systems.
- ii) To quantify GHG emissions (i. e. CO₂, CH₄, N₂O) from activated sludge wastewater treatment system.
- iii) To assess the influence of temperature on CO₂, CH₄ and N₂O emissions.
- iv) To assess the role of nutrients (i. e. NO₃⁻ and NH₄⁺) on CO₂, CH₄ and N₂O emission from activated sludge wastewater treatment systems.
- v) To assess the effects of shaking on CO₂, CH₄ and N₂O emissions from activated sludge wastewater treatment system

1.3.2 Hypotheses

Based on key and specific objectives of the research, the following hypotheses are proposed:

- i) Environmental factors (i. e. temperature) and operational factors (i. e. nutrients and shaking) influence CO₂, CH₄ and N₂O generation in activated sludge wastewater treatment system.
- ii) Activated sludge wastewater treatment systems emit less GHGs compared with other wastewater treatment systems.

1.3.3 Key Research Questions

In accordance with the main and specific objectives, the following research questions have been formulated.

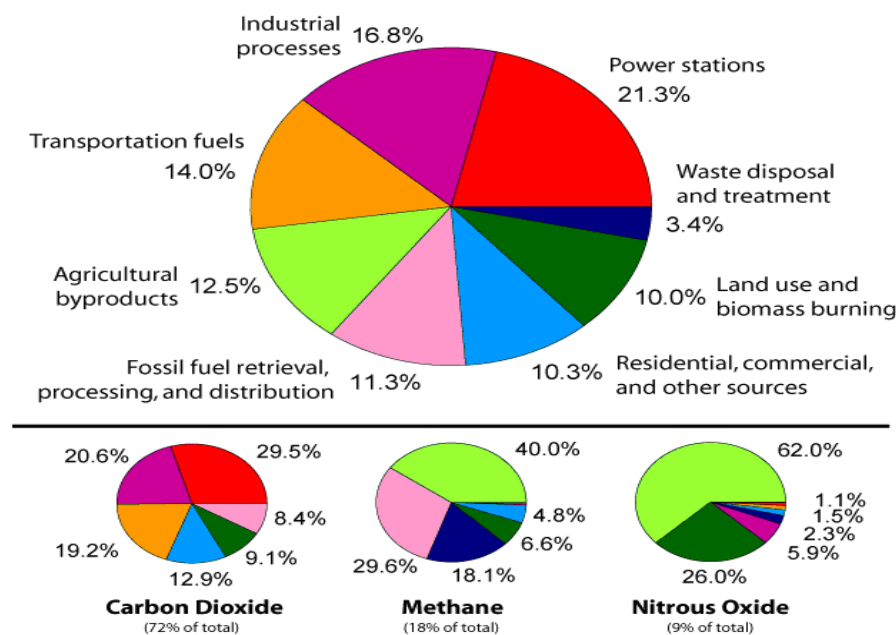
- i) Which greenhouse gases and what quantities are emitted from activated sludge wastewater treatment systems?
- ii) What is the influence of temperature on GHGs emissions from activated sludge wastewater treatment systems?
- iii) What is the role of nutrients (i. e. NO₃⁻ and NH₄⁺) on GHG emission from activated sludge wastewater treatment systems?
- iv) What is the role of shaking on GHG emission from activated sludge wastewater treatment systems?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GHGs from wastewater treatment sources

The sources of GHGs are multi-sectoral. The major anthropogenic sources of GHGs emissions include power stations, industrial processes, transportation fuels, agricultural by-products and land use related activities. Industrial and municipal wastewaters are also a source of GHGs emissions. Collection and treatment of wastewater are a source of CO₂, CH₄, and N₂O. Similarly, the sludge generated as by-product of treatment can further undergo microbial decomposition to CH₄ and N₂O. Figure 1 below depicts the annual GHGs emission by sector.



Source: http://www.globalwarmingart.com/images/e/e0/Greenhouse_Gas_by_Sector.png

Figure 2.1 Annual Greenhouse Gas Emissions by Sector

It can be inferred that wastewater represents less than 2% of the total GHGs emissions. Consequently, the footprint of wastewater in GHGs emissions can be considered negligible. If this is indeed the case then why should that be a key issue of environmental concern? The answer to this question is in three facets and discussed below:

First, the UN Millennium Development Goal 7 (MDG 7) is to ensure environmental sustainability with one of its targets directly related to wastewater i. e. "Halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation" (UN, 2008). To meet this target requires that East Asia and Pacific countries will increase their coverage of sanitation to 60% while Latin America will expand their coverage up to 80%. Regions whose emissions are expected to increase the most by 2020 (with regional increases of 40 to 95%) are Africa, the Middle East, the developing countries of South and East Asia, the Caribbean, and Central and South

America (US EPA, 2006). Conversely, any extension of water supply and sanitation services without a corresponding expansion of wastewater treatment capacities would aggravate the existing problems, create new ones and still miss the MDG target. Bogner et al. (2007) argued that to meet the MDG target will necessitate increase of GHGs emissions from wastewater treatment and this increase is likely to continue into 2020 particularly in South Asia (especially India and China). While it is expected that N₂O emission from wastewater will hold steady from 1990 to 2020 at 100 Mt CO₂-eq. yr⁻¹, CH₄ emission will increase by more than 45% over the same period (Silva, 2007). Figure 2 depicts the projected trends of CH₄ and N₂O emissions from 1990 to 2020.

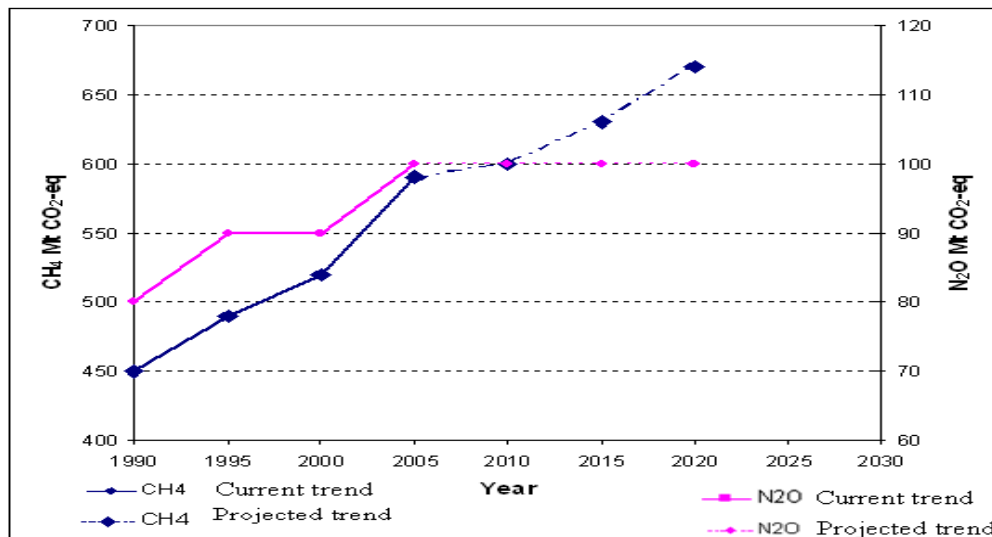


Figure 2.2 Trends of GHGs emission from wastewater treatment plants (adapted from Bogner et al. 2007, IPCC, 2007)

Secondly, there are uncertainties associated with the approximation of GHGs emissions from wastewater. Accordingly, there are considerable reservations with respect to direct and indirect emissions and reliability of wastewater discharge data particularly in developing countries.

2.2 Greenhouse gases and climate change- The global perspective

The earth's atmosphere consists of a mix layer of gases that surround its surface. It contains approximately (by molar content/volume) 78% Nitrogen, 21% Oxygen and less than 1% of trace amounts of other gases (Ruddiman, 2001) such as CO₂, N₂O, NO, CFCs, O₃ and a variable amount of water vapour. It is these trace gases which are called the greenhouse gases due to their contribution to the Greenhouse Effect.

The greenhouse effect is a naturally occurring process that helps in warming the Earth's surface and atmosphere and is therefore essential in maintaining the temperature of the earth. Without this heat trapping by the GHGs in the atmosphere, the surface of the earth would be about 30°C colder than it is (IPCC 2007). The gases act like a thermal shield around the earth and thus trap energy radiated from the earth's surface thereby causing changes in the energy distribution and contribute to the rise in temperature of the earth- the so called Global Warming. Conversely, an excess of GHGs concentrations from anthropogenic sources modifies the climate and can raise the temperature of the earth to lethal levels (IPCC 2007). During the last 200years, human activities in agriculture,

industry, waste disposal, deforestation and most significantly fossil fuel have been producing increasing levels of GHGs thus changing the composition of these gases in the atmosphere. Table 1 indicates the trends of GHGs since the pre-industrial period⁷; concentrations that have not been exceeded during the last thousand years.

Table 2.1 Historical trends of greenhouse gas concentration that are affected by human activities

Period	CO ₂	CH ₄	N ₂ O	SF ₆	CF ₄
<i>Pre-industrial</i>	About 280 ppmv	About 700 ppbv	About 270 ppbv	0	40
<i>Concentration 1998</i>	365 ppmv	1745 ppbv	314 ppbv	4.2 ppmv	80 pptv
<i>Rate of change</i>	1.5 ppmv/yr	7.0 ppbv/yr	0.8 ppbv/yr	0.24 ppbv/yr	-1.0 pptv/yr
<i>Atmospheric life time</i>	5-200 yr	12 yr	114 yr	3,200 yr	>50,000 yr

Source: IPCC, 2001

The rapid change in climate affects both the environment and humans. The predicted effects of climate change in terms of environment include rising sea levels, and coastal flooding, increase in precipitation in tropical areas, decreased precipitation in the sub-tropics and changes in precipitation patterns that could lead to drought or floods, changes in aquatic ecosystem, disruption of some agricultural practices and more frequent droughts/floods among others (US EPA 2001, IPCC 2001). It is thus known that climate change will exert a severe pressure on human society and induce many changes in the global climate system during the 21st century that would very likely be larger than those observed during the 20th century (Meehl et al., 2007, IPCC, 2007). This thus calls for an intervention to reduce GHGs emission. In order to achieve any meaningful outcome however, there is the need to first quantify GHGs emissions from both natural and anthropogenic sources. This is what this research seeks to do.

2.3 Overview of Greenhouse Gases and Climate Change

2.3.1 Greenhouse gases

Greenhouse gases are those gaseous constituents of the atmosphere, both natural and anthropogenic, that absorb and emit radiation at specific wavelengths within the spectrum of thermal infrared radiation emitted by the Earth's surface, the atmosphere itself, and by clouds (IPCC, 2007). They include, but are not limited to, water vapour, carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), chlorofluorocarbons (CFCs), hydro chlorofluorocarbons (HCFCs), ozone (O₃), hydro fluorocarbons (HFCs), per fluorocarbons (PFCs), and sulphur hexafluoride (SF₆) (USEPA, 2006). However, water vapour (H₂O), carbon dioxide (CO₂), nitrous oxide (N₂O), methane (CH₄) and ozone (O₃) are the primary greenhouse gases in the Earth's atmosphere (IPCC, 2007). The atmospheric concentrations of CO₂, CH₄, N₂O and CFCs increased from 337 to 360, 1.50 to 1.72, 0.302 to 0.320, and 4.68×10^{-4} to 7.69×10^{-4} g cm⁻³, respectively, during last decade (Rasmussen and Khalil, 1986; Battle et al., 1996).

Human actions intensify the blanketing effect through the release of greenhouse gases. For instance, the amount of CO₂ in the atmosphere has increased by about 35% in the industrial era, and this increase is known to be due to human activities, primarily the combustion of fossil fuels and removal of forests. Thus, humankind has dramatically altered the chemical composition of the global atmosphere with considerable implications

⁷ The period before the industrial revolution in the mid 18th century

for climate (Le Treut et al 2007, IPCC 2007). Some greenhouse gases such as CO₂ and N₂O occur naturally and are emitted to the atmosphere through natural processes. They are also emitted through human activities. Other greenhouse gases (e.g. fluorinated gases) are synthetic and emitted solely through human activities.

2.3.2 Greenhouse gases, sources and impacts on climate change

There is a general agreement that the observed increases in the atmospheric concentrations of greenhouse gases like N₂O, CH₄, and CO₂ due to use of fossil fuel, land-use changes, and intensive agriculture has led to a warming of the earth's climate (Søvik et al, 2006). The ratification of the Kyoto Accord therefore compels many countries to detail total GHG flux over their different sectors and understanding their influence on ecosystem processes (Mosier et al., 1998c). Greenhouse gases of particular importance to the human induced greenhouse effect are CO₂, CH₄ and N₂O, which according to IPCC, (2001) currently contributes with 60%, 20% and 6%, respectively, of the anthropogenic radiative forcing from long-lived and globally mixed greenhouse gases. Greenhouse gases of concern for the wastewater sector are CO₂, N₂O and CH₄ (Kong et al., 2002). Similarly, Jain and Hayhoe, (2003) estimated that CO₂, CH₄, N₂O and various halocarbons are responsible for 64, 20, 6 and 10%, respectively, of the increase in the greenhouse effect. Not all GHGs are often listed; nitrogen trifluoride (NF₃) with a higher global warming potential (GWP) is often not listed because it is only present in very small quantities (Prather and Hsu, 2008). NF₃ is a GHG, with a global warming potential GWP 17,200 times greater than that of CO₂ when compared over a 100 year period (IPCC, 2007). The GWP of a GHG is based on the specific wavelengths a molecule absorbs plus the biogeochemical residence time of the GHG (Braun, 2005). The contribution of a gas to the greenhouse effect depends on both the characteristics of the gas and its abundance. As an example, on a molecule-for-molecule basis CH₄ is a much stronger GHG than CO₂, but its presence in much smaller concentrations makes its total contribution smaller. Coe and Webb, (2003) highlighted that greenhouse gas molecules in the earth's atmosphere absorb long-wave radiation emitted by the earth's surface which according to Paltridge and Platt, (1976) is responsible for warming the earth's atmosphere. The greenhouse effect is a naturally occurring process that helps in warming the Earth's surface and atmosphere and is therefore essential in maintaining the temperature of the earth. Without this heat trapping by the GHGs in the atmosphere, the surface of the earth would be about 30°C colder than it is (IPCC 2007). The greenhouse effect therefore maintains the planet at a mean annual temperature of 14°C without which the average temperature would be -18°C (Wuebbles and Edmonds, 1991).

Concentrations of GHGs, and therefore the magnitude of the greenhouse effect have hanged over time. According to Wayne (2003), the estimated CO₂ and N₂O concentrations when the earth's atmosphere was first formed were 98% and 2%, respectively. Overall, the earth's atmosphere contains approximately 78% Nitrogen, 21% Oxygen and less than 1% of trace amounts of other gases (Ruddiman, 2001) such as CO₂, N₂O, NO, CFCs, O₃ and a variable amount of water vapour. It is known that variation in GHG concentrations and global temperatures are closely correlated in geological history (Petit et al., 1999).

The industrial revolution marked the beginning of major anthropogenic changes in the concentrations of GHGs and the magnitude of the greenhouse effect. Trenberth et al., (1996) attributed the amplification of the atmospheric concentrations of GHGs to burning

of fossil fuels, deforestation and land use changes while Jain and Hayhoe, (2003) stated that increases in GHGs have moved global temperatures out of their normal range of variation with average temperatures now exceeding maximum temperatures from the past half-million years

2.3.3 Carbon dioxide (CO₂)

CO₂ is the most important greenhouse gas from human sources. Human activity is not only producing more CO₂ but is also severely damaging the capacity of the earth to absorb its emissions by means of its natural sinks: the forests and oceanic plankton. While CO₂ occurs naturally, its concentration in the atmosphere is rapidly increasing due to burning of fossil fuel like coal, oil and natural gas.

Data from climate change studies points to the direct effect of human activities on the chemical composition of the global atmosphere. Observations of parallel trends in the atmospheric abundances of the ¹³CO₂ isotope (Francey and Farquhar, 1982) and molecular oxygen (O₂) (Keeling and Shertz, 1992; Bender et al., 1996) uniquely identified the rise in CO₂ with anthropogenic source particularly, fossil fuel burning, land use changes and cement manufacturing (Jain and Hayhoe, 2003). During the industrial era, CO₂ abundance rose roughly exponentially to 367 ppm in 1999 (Neftel et al., 1985; Prentice, 2001, IPCC, 2001a) and to 379 ppm in 2005 (IPCC 2007). The lifespan of CO₂ is approximately 120 years (Shallcross et al., 2003) and is assigned a global warming potential (GWP) of 1 and used as the reference value for determining the GWP of all other GHGs.

Abiotic sinks for anthropogenically produced CO₂ include absorption by ocean water and the atmosphere (Shallcross et al., 2003). Massive destruction of forests worldwide results in much fewer trees to take up CO₂. In addition the stored CO₂ in the trees is release into the atmosphere compounding the problem. Thus damage to the planet's carbon sinks through deforestation and ozone layer depletion makes a direct contribution to the enhanced greenhouse effect. Atmospheric CO₂ is part of this global carbon cycle and therefore its fate is a complex function of many biogeochemical processes.

Trees take up CO₂ from the air through photosynthesis. Destroying forests release CO₂ thus increases its levels in the atmosphere. Deforestation and fossil fuel combustion accounts for about 25% of CO₂ increase from human activities since the industrial revolution (Schneider, 1998). Until some 50 years ago, most of the CO₂ from deforestation was released from temperate zones: now tropical deforestation is the largest source (Boreal Europe). Tropical forests are being burned and cut for farming, mining and raising cattle. These activities increase the amount of CO₂ in the atmosphere and also contribute to the loss of biodiversity every year.

It is estimated that CO₂ concentrations in the atmosphere had increased by about 33% since the pre-industrial times (IPCC 2001, Blasing and Jones, 2003) and according to Stephen, (2004) it could double by the year 2065. The IPCC (1996) stated that a doubling of CO₂ would lead to a global temperature rise of 1.5 to 4.5°C.

2.3.4 Methane (CH₄)

CH₄ is another major GHG. Its primary source is the anaerobic decomposition of organic matter in biological systems such as wetland rice cultivation, decomposition of animals,

solid waste and wastewater treatment systems and the distribution of petroleum and natural gas. It is also a by-product of coal mining and incomplete combustion of fossil fuels. Anaerobic bacteria (methanogens) produce CH_4 in the waterlogged, anoxic part of wetland soils. The rate of CH_4 production relative to consumption, by aerobic bacteria (methanotrophs) in unsaturated soil horizons above the water table, determines the net flux to the atmosphere (Bubier and Moore, 1994). Several environmental variables have been identified as controls of methane production and ultimately of net CH_4 emission. These include temperature, depth of water table (Torn and Chapin 1993) and substrate type and quality. In addition presence and species composition of vascular plants can affect CH_4 exchange between wetland ecosystems and the atmosphere, because plants affect important aspects of CH_4 dynamics, e.g., production, consumption and transport (Joabsson et al. 1999).

About 80% of methane is produced biologically and the major source sites are rice paddies, wetlands, sediments, enteric fermentation, animal wastes treatment and landfills under low redox potential conditions by obligate anaerobes (Yang and Chang, 1999).

The atmospheric concentration of CH_4 has increased by about 50% since the pre-industrial times. However the rate of increase has declined over the last two decades (Dlugokencky et al., 1998). The IPCC (2001) stated that over half of the current CH_4 flux in the atmosphere has anthropogenic origins with agriculture, fossil fuels, and waste disposal being the biggest contributors while Barlett and Harriss (1993) opined that 60% of the total CH_4 emissions are contributed by tropical wetlands. CH_4 production is naturally carried out by the anaerobic breakdown of organic matter in wetlands, the gut of termites and in some other animals (Bréas et al, 2001). It also escapes from natural gas deposits. CH_4 is abiotically removed from the atmosphere by reacting with hydroxyl radicals (OH) to produce methyl radicals and water vapour and in the stratosphere with chlorine to produce methyl radicals and hydrochloric acid (Tyler, 1991).

The IPCC, (2001) estimates that the atmospheric concentration of CH_4 , whose GWP is 23 times greater than CO_2 , increased by 150% to 1,745 ppb since pre-industrial times. While the life span of CH_4 is estimated to be 9-15 years (Schimel et al., 1996), its major anthropogenic sources include enteric fermentation, fossil fuel related activities and rice paddies (IPCC, 2001). The atmospheric concentrations of CH_4 have more than doubled since the pre-industrial times and according to Stephen (2004) it could double again by 2050 if appropriate measures are not instituted to curtail the current trends.

2.3.5 Nitrous oxide (N_2O)

N_2O is produced through the microbial processes nitrification and denitrification (Smith 1997). Nitrification is an aerobic process and probably not of any major importance in wetlands. A draw down of the water table, however, in particular in nutrient-rich marsh, may cause significant N_2O emissions arising from these processes (Martikainen et al. 1993). In waterlogged soils with high nitrate concentrations denitrification can occur when nitrate is used by soil microorganism as an alternative electron acceptor in respiration. During this process nitrate is reduced to NO_2^- , NO, N_2O or N_2 . The availability of molecular oxygen is an important regulatory factor for both nitrification and denitrification and the water level is therefore decisive in regulating N_2O fluxes, although other aspects such as availability of organic matter also needs to be considered (Johansson, 2002). Little is known about the effect of vascular plants on N_2O emissions,

however since they affect both the availability of molecular oxygen and the availability of organic matter in the rhizosphere they may potentially also affect the fluxes of N_2O (Inamori et al., 2008).

Anthropogenic sources of N_2O include agricultural soils, fossil fuel combustion, nitric acid production, municipal solid waste and wastewater treatment and combustion and burning of biomass. N_2O with an average life span of 120 years (Prather et al., 1995) is estimated to have increased by about 17% from its pre-industrial concentrations of 270 ppb to 314 ppb in 1998 (IPCC, 2001) whereas according to Shallcross et al., (2003.) it has risen from a pre-industrial concentration of 275 ppb to the current concentration of 310 ppb. N_2O has a GWP 296 times that of CO_2 (IPCC, 2001). An abiotic sink of N_2O is its photolytic reactions with atmospheric constituents like ozone (Wang et al., 1976), the resultant destruction of which increases the amount of UV-B and UV-C rays that reach the earth's surface. Nitrous oxide is an intermediate product of both nitrification and denitrification; however it is more associated with denitrification (USEPA, 2006)

2.3.6 Ozone (O_3)

Ozone is formed by the reaction of sunlight on air containing hydrocarbons and nitrogen oxides that react directly at the source of the pollution or many kilometers down wind. The atmospheric lifetime of tropospheric ozone is about 22 days; its main removal mechanisms is deposition on the ground, producing OH, and by reactions with OH and the peroxy radical HO_2 (Stevenson et al, 2006)

Ozone has been present at ground level before the industrial revolution; however, current peak concentrations are now far higher than the pre-industrial levels. Background concentrations well away from sources of pollution are substantially higher. This increase in ozone raises further concern because in the upper troposphere it acts as a GHG, absorbing some of the infrared energy emitted by the earth. The GWP of ozone is difficult to quantify because of its non-uniform concentrations across the globe. However, the Third Assessment Report (IPCC, 2001) suggests that the radiative forcing of tropospheric ozone is about 25% that of CO_2

2.3.7 Halocarbons

Hydro fluorocarbons, per fluorocarbons, and sulphur hexafluoride are synthetic, powerful greenhouse gases that are emitted from a variety of industrial processes. Fluorinated gases are sometimes used as substitutes for ozone depleting substances (ODS) (i.e. CFCs, HCFCs, and halons). These gases are typically emitted in smaller quantities, but because they are potent greenhouse gases, they are sometimes referred to as High Global Warming Potential gases (US EPA, 2006).

Halocarbons are primarily anthropogenic in origin and have found uses as cooling agents in refrigeration, propellants and cleaning solvents in many applications. The most familiar of these halocarbons are CFCs. Though the levels of CFCs are declining due to a phase out of ozone depletion substances under the Montreal Protocol, their long term atmospheric lifetimes guarantee that they will continue to contribute to the greenhouse effect for some time. Another group of halocarbons are the hydrofluorocarbons (HFCs). They are less stable in the atmosphere and therefore have shorter lifetimes. At lower altitudes, halocarbons function as a warming gas while at higher altitudes, they exert a

cooling effect through their interaction with O₃ (Devkota, 2007). The ultimate impact of halocarbons is therefore uncertain.

2.4 Impacts of Greenhouse gases

The role GHGs play in shaping the global climate can not be over emphasised. The direct impact of GHG emissions is the rise in average global temperatures leading to global warming. A rise in average surface temperature of 1°C may adversely affect world food production (IPCC, 2007). Cunningham et al. (2007) reported that as temperatures rise, more water evaporates from rivers, lakes, oceans etc. Cunningham et al. (2007) further asserted that the relative humidity of the atmosphere increases with increase in temperature thus leading to more water vapour being absorbed in the atmosphere arguing that higher concentrations of water vapour in turn make the atmosphere to absorb more thermal infrared radiation from the earth resulting in further warming of the atmosphere. The warmer atmosphere can then hold more water vapour and the cycle continues. Global warming will probably result in large swings in global climate patterns.

The predicted effects of climate change in terms of environment include rising sea levels, and coastal flooding, increase in precipitation in tropical areas, decreased precipitation in the sub-tropics and changes in precipitation patterns that could lead to drought or floods, changes in aquatic ecosystem, disruption of some agricultural practices and more frequent droughts/floods among others (USEPA 2001, IPCC 2001). It is thus known that climate change will exert a severe pressure on human society and induce many changes in the global climate system during the 21st century that would very likely be larger than those observed during the 20th century (Meehl et al 2007, IPCC 2007)

2.5 Overview of wastewater and wastewater treatment systems

2.5.1 Wastewater

Wastewater is defined as any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic residences, commercial properties, industry, storm water and/or agriculture and may contain a wide range of potential contaminants and concentrations. According to Metcalf and Eddy (2003) wastewater is characterised in terms of its physical, chemical and biological composition. The key physical characteristics are associated with temperature, odour, colour and solid concentrations. While the chemical parameters are related to dissolve metals, organic pollutants, inorganics, oils and grease, the important biological characteristics in wastewater are related to pathogens such as bacteria, viruses, and parasitic worms and non-pathogenic bacteria, archae, algae, fungi, protozoa and helminths (Silva, 2007).

2.5.2 Wastewater treatment processes

There are many treatment regimes for wastewater. These may be generally classified into biological, chemical and physical forms of treatment.

2.5.3 Biological wastewater treatment

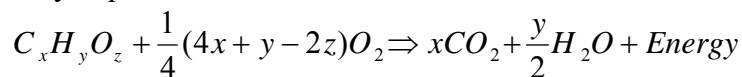
The idea behind biological methods of wastewater treatment is to introduce contact with bacteria (cells), which feed on the carbonaceous organic materials in the wastewater, thereby degrading it. The carbonaceous (organic) matter in the wastewater is either

biodegradable or inert. While the inert material leaves the treatment system without any change in its chemical composition, the biodegradable matter is soluble or particulate.

Generally speaking, in biological treatment, simple bacteria (cells) feed on the organic material present in the wastewater. Through their metabolism, the organic material is transformed into cellular mass, which is no longer in solution but can be precipitated at the bottom of a settling tank or retained as slime on solid surfaces or vegetation in the system. According to Van Haandel and Lettinga, (1994) the biological conversion of carbonaceous biodegradable matter can occur under aerobic or anaerobic environment.

2.5.3.1 Aerobic conversion of organic matter

In aerobic oxidation, organic molecules are metabolized to CO₂ and H₂O, and released energy. This conversion of biodegradable organic matter by heterotrophic bacteria may be generally expressed as:



C_xH_yO_z represents an organic molecule which serves as an electron donor, while oxygen serves as the acceptor. The principal characteristics of aerobic oxidation process are:

- Stabilisation of organic matter
- Release of energy
- Utilisation of oxygen
- Production of carbon dioxide which is a GHG

2.5.3.2 Anaerobic conversion of organic matter and methane emissions

Anaerobic conversion of organic matter is the biological breakdown of the organic matter without the use of air or elemental oxygen. Grady et al (1999) stated that anaerobic processes characterise a diverse array of biological wastewater treatment systems from which dissolved oxygen and nitrate-N are excluded. The ultimate product of anaerobic digestion in most cases is methane, though this process can take place when denitrifying and sulphate reducing bacteria can be utilized to convert nitrate and sulphate (SO₄²⁻) or sulphite (SO₃²⁻) to nitrogen and sulphide (S²⁻) respectively. Generally, the anaerobic process involves four key biological and chemical stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

Hydrolysis

This is the process of breaking down long chain polymeric and complex organic molecules such as carbohydrates, proteins, lipids and dissolving the smaller molecules into solution. Therefore hydrolysis of these high molecular weight polymeric components is the necessary first step in anaerobic digestion. Through hydrolysis the complex organic molecules are broken down into simple sugars, amino acids, and fatty acids (Silva, 2007). The resultant molecules are subsequently used in acidogenesis. However, acetate and hydrogen produced in this stage can be used directly by methanogens

Acidogenesis

The biological process of acidogenesis is where there is further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here a soluble mix of short chain volatile fatty acids (VFAs) like acetic, propionic, formic, lactic acids and acetates

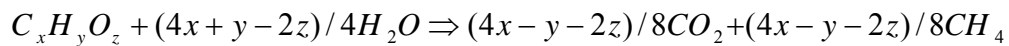
are created along with ammonia, carbon dioxide and hydrogen sulphide as well as other by-products.

Acetogenesis

The third stage in anaerobic digestion is acetogenesis. Here simple molecules created through the acidogenesis phase are further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen; the key substrates for methanogens in the final stage of anaerobic digestion. This stage regulates the cumulative concentrations of VFAs which affects the subsequent stage.

Methanogenesis

The terminal stage of anaerobic digestion is the biological process of methanogenesis. Here methanogens utilise the intermediate products of the preceding stages and convert them into the two most important GHGs (CH₄ and CO₂) and water. Methanogenesis is sensitive to both high and low pHs and occurs between pH 6.5 and pH 8. Pearson, (2005) described methanogenesis as the rate determining step of the entire anaerobic digestion process because it has a cell ‘doubling time’ of a few days compared with the few hours required for acetogenic bacteria. According to Van Haandel and Lettinga (1994), the following simplified generic equation may represent the overall anaerobic processes:



C_xH_yO_z represents organic matter. The simplified metabolic sequences of the four key processes discussed above are represented below:

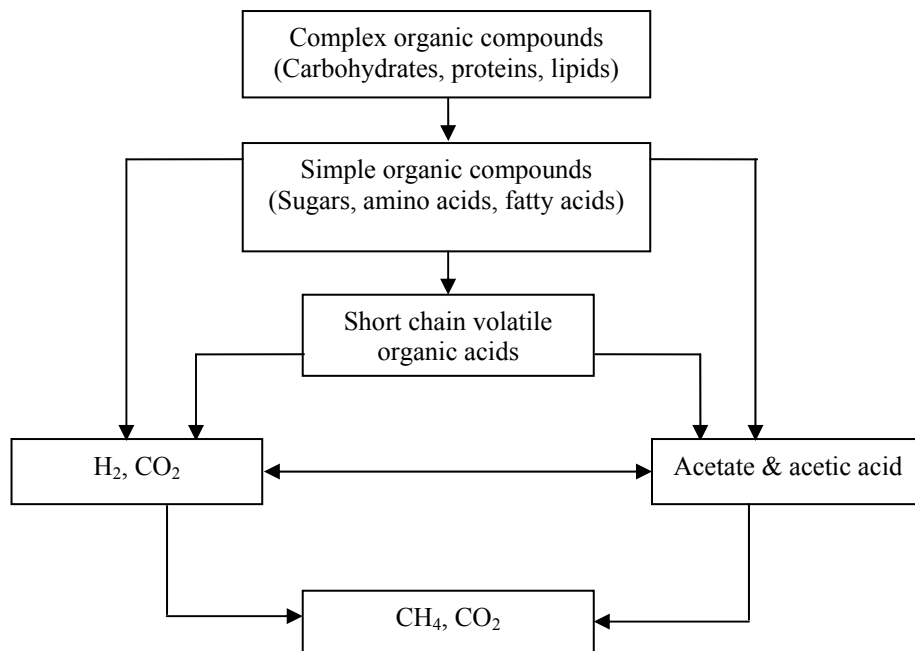
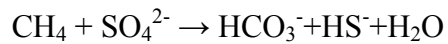


Figure 2.3 The four metabolic sequences of the key biological and chemical stages in anaerobic digestion

2.5.4 Anaerobic oxidation of methane (AOM)

Anaerobic oxidation of methane (AOM) is a microbial process occurring mainly in anoxic marine sediments. During AOM, CH₄ is oxidised using sulphate as the terminal electron acceptor. The relation can be summarised as follows:



The driving force for interest in AOM processes is that microbial communities intercept and consume methane from anoxic environments, which would otherwise enter the atmosphere. AOB is therefore biogeochemically important because CH₄ is a potent greenhouse gas in the atmosphere and is abundant in anoxic/anaerobic environments. Mingxing and Jing (2002) stated that oxidation of CH₄ reduces the emission from the soil of rice field to the atmosphere.

Methane can be oxidised by both aerobic and anaerobic bacteria. Anaerobic oxidation of methane is still a poorly understood process, but appears to be an important methane sink in certain types of anaerobic environments. Aerobic oxidation has been better characterised. Aerobic methane-oxidizing bacteria (methanotrophic) need oxygen for growth, but the required oxygen may be very low under certain conditions (Mingxing and Jing 2002). Bender and Conrad (1994) reported that the CH₄ oxidation activity was strictly coupled to the presence of oxygen and indicated that anaerobic CH₄ oxidation was not important. Conversely, about 1% of the carbon dioxide annually fixed by photosynthesis is converted back to carbon dioxide by microorganisms via CH₄, which amounts to 1 billion tonnes of CH₄ formed and consumed per year (Thauer and Shima 2006).

In theory, prokaryotes should be capable of using nitrate to oxidise CH₄ anaerobically, but such organisms have neither been observed in nature nor isolated in the laboratory, thus microbial oxidation of CH₄ is considered to proceed only with oxygen or sulphate (Raghoebarsing et al, 2006).

Though AOM is observed much more in marine environments, research also found organisms capable of AOM in fresh water environments and possibly in wastewater treatment (Wang et al., 2008). To date no methane oxidising archaea (MOA), have been isolated in pure culture. However, MOA have been grown in mixed communities during anaerobic incubations on a novel continuous-flow bioreactor called AMIS (Girguis et al., 2005). AOM has been detected at temperatures from 4°C to over 30°C and at different locations like lakes and seashores. Recent investigations however, indicate that some consortia of archaea and bacteria are able to oxidise CH₄ using nitrate instead of sulphate as the terminal electron acceptor (Raghoebarsing et al, 2006).

2.5.5 Nitrogen conversion processes and nitrous oxide emissions

Nitrogen is present in municipal wastewater in two forms: organic (urea, amines, amino acids and proteins) and inorganic (ammonia). These must be eliminated during treatment because together with phosphorus, it represents a major source of concern for eutrophication of surface water. In particular, wastewater from biological odour removal processes and effluent from anaerobic wastewater treatment processes are characterised by a high concentration of nitrogen (Waki et al, 2007). As nitrogen-rich liquid waste, the potential for NO₃⁻ leaching, NH₃ volatilisation and N₂O emission may pose immediate

problems (Zhou et al, 2008). Generally, the removal of nitrogen in wastewater is governed by three important sequential processes: ammonification, nitrification and denitrification and lately the anaerobic ammonia oxidation (anammox) process.

Ammonification

Ammonification is the first step in the transformation of organic nitrogen in wastewater. Ammonification refers to the processes by which organically bound forms of nitrogen occurring in dead biomass (such as amino acids and proteins) are oxidised into ammonia and ammonium. The process of ammonification is carried out in wastewater by a diversity of microbes and is one of the many types of chemical transformations that occur during the decomposition of dead organic matter. The ammonification of organic compounds in wastewater starts in sewer systems and continues in the primary and biological units of the wastewater treatment system. Once ammonia is formed a fraction of it is assimilated by the bacteria while the rest is nitrified and then denitrified into molecular nitrogen in the treatment system (Silva, 2007).

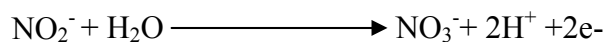
Nitrification

Nitrification is a microbial process by which reduced nitrogen compounds (primarily ammonia) are sequentially oxidised to nitrite and nitrate (US EPA, 2002). The nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonia or nitrite. The carbon source is usually from CO₂. In the first step of nitrification, ammonia-oxidizing bacteria oxidise ammonia to nitrite according to equation



Nitrosomonas is the most frequently identified genus associated with this step, although other genera, including *Nitrosococcus*, and *Nitrosospira* can also autotrophically oxidise ammonia (Watson et al. 1981).

In the second step of the process, nitrite-oxidizing bacteria oxidise nitrite to nitrate according to equation



Nitrobacter is the most frequently identified genus associated with this second step (Watson et al. 1981).

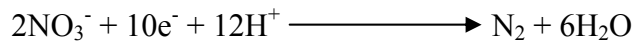
The principal characteristics of these processes are the consumption of oxygen (4.57gO₂/gN oxidised), the release of H⁺ lowering the alkalinity of the medium (7.14g of alkalinity as CaCO₃ is required) and possibly the reduction of pH if there is no sufficient buffering capacity. However, these reactions can be inhibited by chemicals such as organic solvents, proteins, amines as well as high NH₃ concentrations, resulting in decrease or suspended NH₃ oxidation due to the presence of un-ionised NH₄ (Metcalf and Eddy, 2003).

Denitrification

Denitrification is an anoxic microbially facilitated process in which denitrifying bacteria reduce nitrate to ultimately produce molecular nitrogen (N₂) through a series of intermediate gaseous nitrogen oxide products. This respiratory process reduces oxidised forms of nitrogen in response to the oxidation of an electron donor such as organic matter. The preferred nitrogen electron acceptors in order of most favourable include: nitrate (NO₃⁻), nitrite (NO₂⁻), nitric oxide (NO), and nitrous oxide (N₂O).



The bacteria that catalyse nitrification are heterotrophic. They use NO₃⁻ instead of O₂ as electron acceptor and organic matter as a carbon source. The carbon source may come from the influent BOD of the wastewater or an exogenous source such as methanol, ethanol or acetate if there is no sufficient BOD in the influent wastewater. The complete denitrification process can be expressed as a redox reaction:



The importance of denitrification is seen is highlighted by:

- The absence of O₂ represents an economy in the stabilisation of organic matter
- The consumption of H⁺ leading to the recovery of alkalinity in the process

Ammonification nitrification and denitrification are a key component in the biogeochemical cycling of nitrogen.

Anammox process

In the anammox process NH₃ is oxidised into N₂ by bacteria that use NO₂⁻ as electron acceptor and CO₂ as carbon source. Waki et al (2007) reported that the anammox process is dependent on NO₂⁻; therefore, in many cases a partial oxidation process is applied before the anammox process occurs or the NO₂⁻ compound is added. The anammox process is a viable option for biological wastewater treatment. The bacterial responsible for the process do not need either exogenous source of carbon or oxygen. Instead of producing CO₂, anammox bacteria consume it thus rendering N₂ removal through the anammox process environmentally friendly. According to Pilcher (2005), a major advantage of the anammox process for wastewater treatment is a 90% reduction in operational cost and a further 50% reduction in space requirement compared with other conventional methods.

2.5.6 Wastewater treatment systems

Earlier disposal methods for wastewater relied on the natural assimilative capacities of rivers and other water bodies. It was thought that surface water had an infinite capacity to absorb all the pollutants in wastewater and still re-establish the equilibrium conditions within the aquatic environment, through self purification, after the alterations induced by effluent discharged. The impact of population rapid growth however rendered this self purification capacity of water bodies insufficient and therefore new approaches had to be engineered. Earlier engineered treatment objectives were principally based on:

- Removal of floatable, suspended and colloidal materials
- Removal of biodegradable organics and
- Removal of pathogens

Though the earlier treatment objective remain valid today, latter developments in environmental regulations and legislations especially in the 1970s to 1980s raised the stake in wastewater treatment objectives to include but not limited to:

- BOD removal,
- Suspended solids (TSS) removal
- Treatment of biodegradable organics (sludge)
- Elimination of pathogens and lately
- Removal of nutrients (nitrogen and phosphorus)

Currently, wastewater treatment processes are categorised according to the following:

- Preliminary: this includes simple processes such as screening and grit removal to remove coarse solids and therefore gross solid pollution.
- Primary: usually plain sedimentation; simple settlement of the solid material in wastewater can reduce the pollution load by significant amounts.
- Secondary: for further treatment and removal of common pollutants (organic matter, proteins), usually by a biological process.
- Tertiary: usually for removal of specific pollutants e.g. nitrogen or phosphorous, or specific industrial pollutants.

It is however, worth knowing that according to the level of treatment desired, many treatment options could be combined and integrated into many unit operations that make up a single treatment system that takes into account treatment objectives, cost, minimise environmental impacts and high performance efficiencies.

There are many treatment systems for wastewater. However, they can broadly be classified into conventional and eco-technological systems.

A Conventional systems

These are mostly based on external mechanics and energy input. They may be generally classified into aerobic and anaerobic systems.

Aerobic

- Activated sludge
- Sequencing batch reactor
- Trickling filters
- Rotating Biological contactor
- Surface-aerated basins
- Filter beds (oxidizing beds)
- Biological aerated filters
- Membrane bioreactors

Anaerobic

- Anaerobic filter
- UASB
- Fluidized bed reactor

Aerobic systems

The aerobic systems are generally efficient, however, they are characterised by high investment, operational and maintenance cost and high energy requirements. They may require chemical input in addition to the high biomass (sludge) turnover which are not reused or recovered to attenuate their environmental impacts, thus fixing the objective of the treatment in obtaining a cleaner effluent (Gijzen, 2001).

Anaerobic systems

The anaerobic systems are equally efficient with high energy recovery efficiency. They however, require high investment, operational and maintenance cost. Furthermore, they are characterised by low sludge production.

Generally, the conventional systems have a good advantage due to the small land requirements. They however, they are disadvantaged by their lack of efficiency in phosphorus and pathogen removal in addition to their sensitivity to load variations and toxic compounds. Moreover, they are typified by high skill manpower demand.

Activated sludge treatment system

The activated-sludge process is a biological method of wastewater treatment that is performed by a variable and mixed community of microorganisms in an aerobic aquatic environment. These microorganisms derive energy from carbonaceous organic matter in aerated wastewater for the production of new cells in a process known as synthesis, while simultaneously releasing energy through the conversion of this organic matter into compounds that contain lower energy, such as carbon dioxide and water, in a process called respiration. A variable number of microorganisms in the system obtain energy by converting ammonia nitrogen to nitrate nitrogen in a process termed nitrification. This consortium of microorganisms, the biological component of the process, is collectively known as activated sludge. The activated sludge process is thus a suspended culture process with sludge return and may either be completely mixed or plug-flow process (Lin, 2001).

In the activated sludge process, the oxygen utilization rate usually exceed the rate of natural replenishment, thus some artificial means of adding oxygen must be used. With the exception of the pure oxygen system, oxygen is supplied by aerating the mixed liquor in the biological reactor (Lin, 2001). Mechanical aerators produce turbulence at the air liquid interface and this turbulence capture air into the liquid. Mechanical aerators may have high speed impellers that add large quantities of air to relatively small quantities of water. Brush type aerators are used to provide both aeration and momentum to wastewater in the oxidation ditch (Peavy et al., 1985)

The overall goal of the activated-sludge process is to remove substances that have a demand for oxygen from the system. This is accomplished by the metabolic reactions (synthesis-respiration and nitrification) of the microorganisms, the separation and settling of activated-sludge solids to create an acceptable quality of secondary wastewater effluent, and the collection and recycling of microorganisms back into the system or removal of excess microorganisms from the system.

B Eco-technological systems

These systems function on principle of natural processes. They combine ecological principles of natural systems with strong engineering principles to improve the removal of nutrients and pathogens from wastewater. The primary energy input is solar. The various technologies include: natural wetland, constructed wetland, stabilisation pond, facultative pond, anaerobic pond, duckweed pond, high rate algal and anaerobic ponds, advance pond and many others.

Several advantages of these technologies have been demonstrated: stabilisation pond (Mara, 2005; Pena et al., 2002; Arthur, 1983), constructed wetlands (Kadlec et al, 2005; Rousseau et al, 2004) and duck week pond (El-Shaffai et al, 2007; Nhapi and Gijzen, 2005; Caicedo, 2005; Nhapi et al. 2003; Alaerts et al., 1996).

They are however, not without disadvantages. Some of these disadvantages include large footprint in terms of land requirements, the influence of varied climatic conditions (especially in non-tropical areas) and other effects including GHGs and odour emissions (Shilton and Walmsey, 2005; Van der Steen et al., 2003; Reed et al., 2000; Crites et al., 1995). Furthermore, constructed wetlands have high N₂O and CH₄ emissions (Teiter and Mander, 2005; Liikanen et al., 2006).

2.6 Overview of methodologies for estimating greenhouse gases

There are a number of methodologies for estimating GHG emissions from wastewater treatment systems. These include techniques that make use of emission factors, mass balances and experimental setups that employ dynamic and static chambers.

Emission factors (EF)

An emission factor may be defined as the average emission value or quantity of a specific pollutant emitted to the atmosphere from a given source, relative to the intensity of a specific activity associated with the emission of that pollutant. These factors are usually expressed as the weight of pollutant divided by a unit weight, volume, distance, or duration of the activity emitting the pollutant. Such factors facilitate estimation of emissions from various sources of air pollution. Emissions from wastewater treatment are a function of the amount of organic waste present and an emission factor characterises the extent to which this waste generates GHGs. Thus, the estimation of GHGs from wastewater treatment based on emission factors can be calculated as follows:

$$\text{Emission of GHG} = \text{EF}_j * \text{activity, and } \text{EF}_j = \text{Bo} * \text{CF}_j$$

Where:

- EF_j = emission factor for each treatment/discharge pathway or system, kg CH₄/kg COD,
- j = each treatment/discharge pathway or system
- Bo = maximum producing capacity, kg CH₄/kg COD
- CF_j = correction factor (fraction)

According to IPCC (2006) for methane emitted from wastewater treatment, its emission factor is defined by a function of the maximum potential amount of CH₄ emitted from a given quantity of organics expressed in BOD or COD. In the same way the emission

factor for N_2O is expressed as a function of the ration of kgN_2O/kgN . However, in the case of emissions from centralised wastewater treatment facility that involves nitrification and denitrification steps the emission factor for N_2O is $gN_2O/cap/year$.

Though emission factors are generally derived from measurements on a number of sources presumed to be representative of a particular source sector, El-Fadel and Massoud (2002) stressed that there are many uncertainties related to insufficient data that do not account for many other factors including nutrient limitations, extent of decomposition, biological inhibition, physico-chemical interactions and requirements for bacterial synthesis. Moreover, emission factors are applied independently of the type of wastewater treatment and seasonal variations and their impact on wastewater treatment (Silva, 2007). These are important limitations in the use of EFs in estimating GHG emission.

Mass balance

The general concept of the law of conservation of mass takes into account inputs, outputs and transformations of matter based on the application of mass balance. Mass balance may be used to estimate GHGs from wastewater treatment within an appropriate system boundary i. e. the treatment plant. The mass balance applications make use of both theoretical (stoichiometric) and experimental (in situ measurements) data combined to create representative equations of a system. Though mass balance is a flexible, fairly accurate and reliable methodology in estimating GHGs (Cakir and Strenstrom, 2005), the major drawback is the inadequate boundary definition, use of non-representative stoichiometric coefficients and the fact that it does not take into account the type of wastewater treatment technology and data collection problems which may all lead to erroneous estimation of GHG emissions (Silva, 2007).

Experimental setup for measuring GHGs

There are a few methods for estimating GHGs emissions experimentally. Two typical setups are widely used; the dynamic and static chambers. These apparatus use special setups to entrap gas samples that are subsequently analysed by gas chromatography to determine gas concentrations.

Dynamic chamber

In the dynamic chamber apparatus, the emission rate of the test compound is determined by the increase in mass of the sample over time. In its design, a pump is used to introduce dry clean sweep air into a chamber at a fixed rate. The volumetric flow rate of the sweep air through the chamber is recorded and the concentration of specimen (gas) of interest is determined at the chamber exit. This method can be used to quantify a wide range of different gas fluxes including CH_4 , CO_2 , N_2O and H_2S in addition to NH_3 volatilisation (Zimmo et al, 2003). The key advantage of this apparatus is the in situ sample analysis as it can be injected directly into an analyser. However, inadequate flow rate and pressure deficit (or surplus) that leads to incorrect measurements is a major setback.

Static chamber

This device can be used to determine emission rates from wastewater treatment. The method employs a chamber on a fixed base at a point in the wastewater treatment system. It is the simplest setup in use because there is neither sweep gas to deal with nor any

valves to control flow rate. However, since the air inside the chamber is not circulating, a longer sampling period is required to achieve steady state conditions. Static chambers have been widely used in GHG measurements from wastewater in ponds (Stardmark and Leonardson, 2005), in constructed wetlands (Johansson et al., 2004; Teiter and Mander, 2005; Liikanen et al., 2006). However, according to Purvaja et al.,(2005) some adjustment may be done on the regular static chamber to adapt it to free floating static chamber with added advantage of avoiding sampling artifacts⁸ due to long hours of sampling at a single location and disturbance of sediment surface.

Analytical method

Gas chromatography is the main analytical method for measurement of GHGs concentrations. The flame ion detector can be used for CH₄ chromatography (Toda et al., 1994, Kreye et al., 2007, Picek et al., 2007) while electron capture detector chromatography (Toda et al., 1994, Kreye et al, 2007, Picek et al., 2007, Zhou et al., 2008) is employed for N₂O. Thermal capturing detector (TCD) can be used to quantify CH₄ and CO₂ concentrations (Picek et al., 2007)

⁸ Sampling artifact: an error or misrepresentation introduced by a sampling technique and/or technology

CHAPTER THREE

3.0 RESEARCH APPROACH AND METHODOLOGY

3.1 Wastewater Process Flow and Sample Collection Points

Figure 4.1 indicates the wastewater treatment processes at the activated sludge wastewater treatment plant at Hoek van Holland. Sludge samples were collected from the aeration, anoxic (denitrifying) and anaerobic digestion units as indicated in the figure below. The samples were used immediately or stored under 4°C.

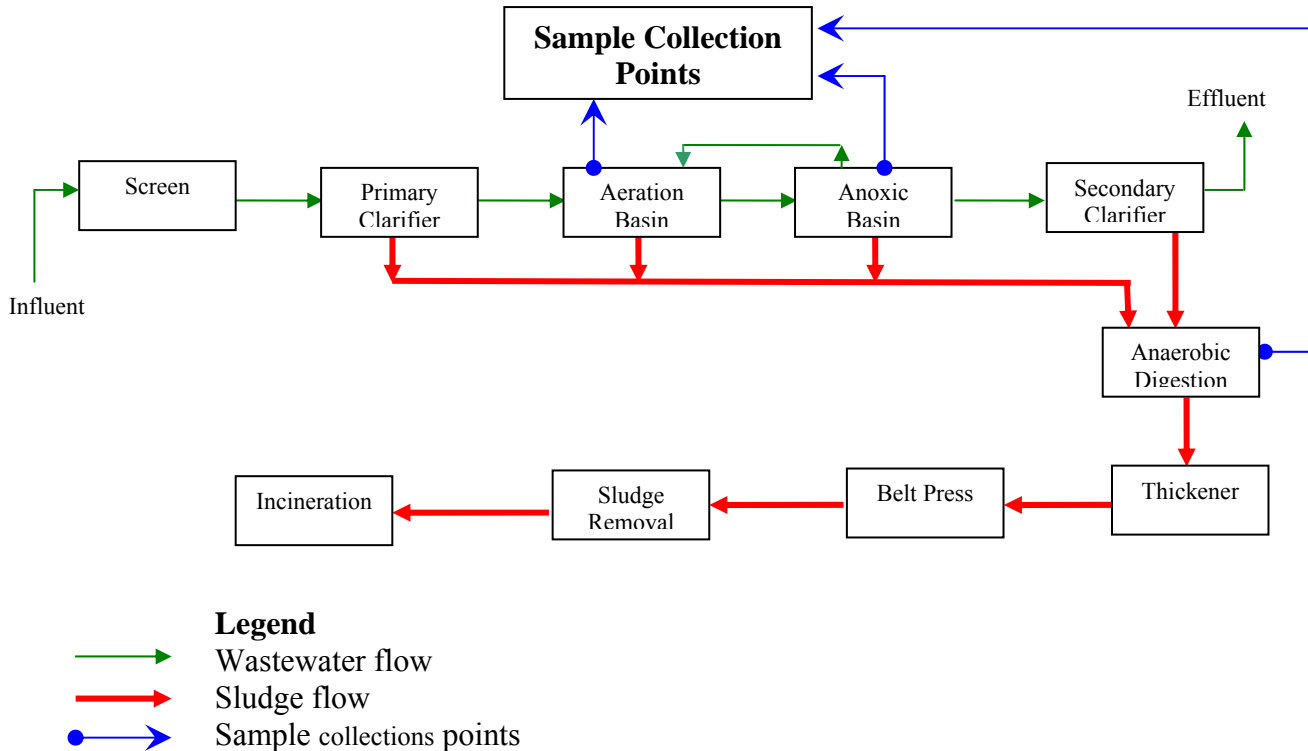


Figure 4.1 Process flow chart showing sludge sample collection points

3.2 Research Concept and Experimental Approach

The research was conducted in UNESCO-IHE Delft, The Netherlands. The research strategy was limited to laboratory studies. The influence of temperature and nutrient (NO_3^- , NH_4^+) variation and shaking on GHGs emission from wastewater treatment systems can only be investigated in such places where those parameters can be manipulated. In view of the fact that these parameters can be accurately manipulate in the laboratory, the research was done on laboratory scale under regulated conditions. Presented below in Figure 5 is the general description of the methodology and approaches that were applied to achieve the set objectives.

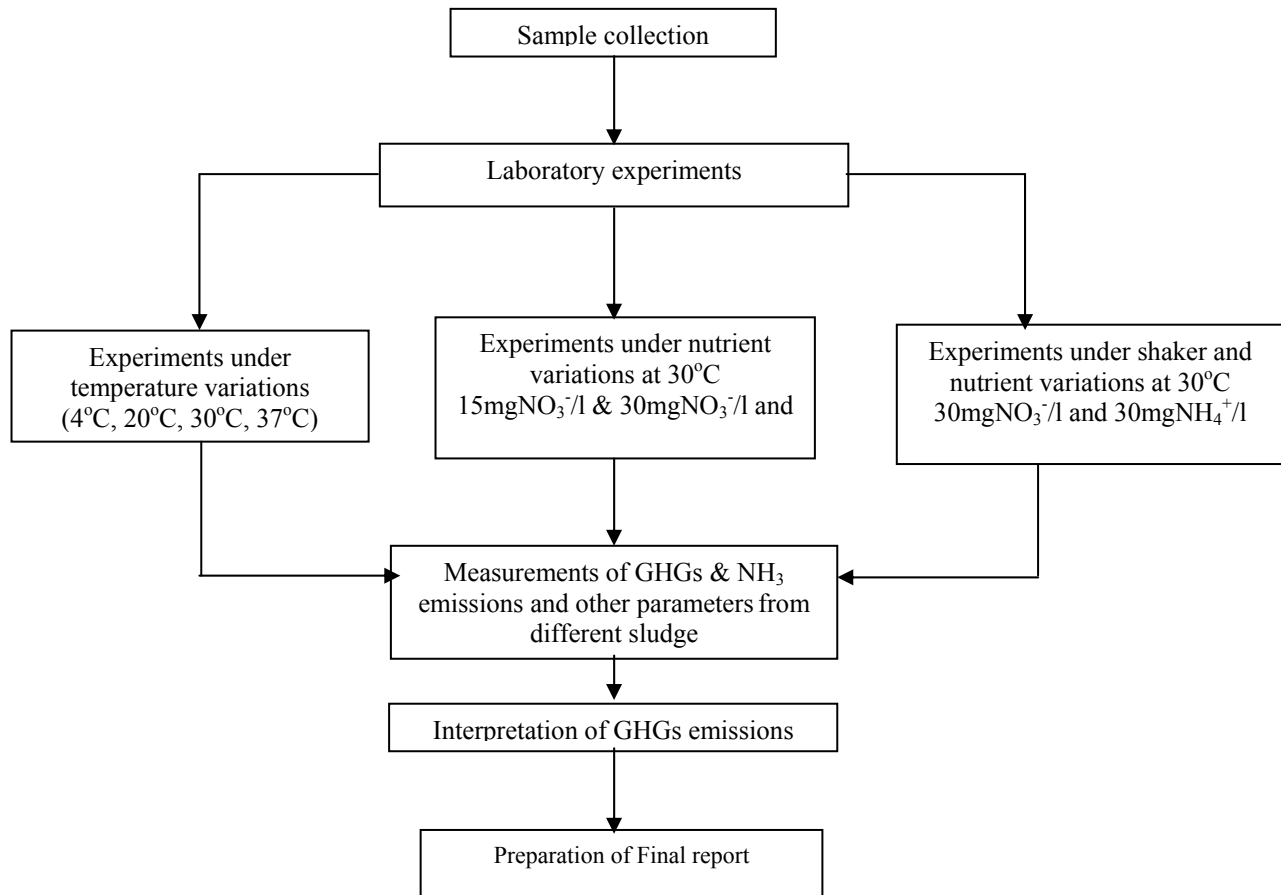


Figure 4.2 General methodological approaches.

The first stage of the research was to adapt, modify and standardised experimental setups on laboratory scale inline with the objectives. In the same way, a methodology to identify and quantify greenhouse gases emitted from the activated sludge system was standardised.

Thus, in order to achieve the overall and specific objectives, laboratory scale prototypes of the wastewater treatment systems was setup using active sludge from existing wastewater treatment plant (activated sludge system) from Hoek van Holland.

3.3 Materials and Methods

3.3.1 Instrument and chemicals required

In order to attain the set objectives a number of equipment, instruments and chemicals were required to carry out the experiments and their associated analyses. The complete list is attached as *Appendix 3.1A*. In addition laboratory chemicals were used in the experiments and the analyses. The list is attached as *Appendix 3.1B*.

3.3.2 Preparation of bacterial substrate (sodium acetate) and nutrients

The bacterial feed was prepared in accordance with the recipe provided by UNESCO-IHE Laboratory. The sodium acetate (CH_3COONa) solution was prepared by dissolving 6.5g of it in 1 liter demineralised water. The nutrients: Nutri I, Nutri II and Nutri IV were

readily in stock in the laboratory. The compositions of the nutrient solutions are as follows:

Table 4.1 Composition of feed nutrients

No	Name	Composition
1	Nutri I	52.7g urea, 25.5g (NH ₄) ₃ PO ₃ 6.7g Na ₂ SO ₄ , 1ml H ₂ SO ₄ dissolved in 1000ml distilled water.
2	Nutri II	19.5g MgSO ₄ , 0.55g CaCl ₂ in 200ml distilled water
3	Nutri IV	5g yeast extract in 100ml distilled water

3.4 Experimental Approach

The experiments were setup in accordance with the specific objectives outlined in Chapter 1 and described below. In all, methodologies were developed for calibrations, identifying and quantifying the influence of temperature, the role of nutrients (NO₃⁻ and NH₄⁺) and the influence of shaking on GHGs emissions. Gas samples were analysed for CO₂, CH₄ and N₂O using gas chromatography (GC) (Farrell et al., 2002). The gas chromatographic data was processed with *Galaxy Chromatography Workstation* software (version.1.9).

3.4.1 Sample analysis

Gas samples were analysed with Varian 3800 gas chromatography (Varian Netherlands, Middelburg). pH was measured using pH meter (WTW pH 340), while Chemical Oxygen Demand (COD) and ammonium were determined in accordance with Standard Methods (APHA, 2005). The initial and final acetate concentrations were also estimated for each reactor using the Chrompack 9001 (Chrompack, Bergen op Zoom, Netherlands) gas chromatograph. The nitrate concentrations in each reactor were determined using the Ion Chromatograph; Dionex Ion Chromatography System, ICS-1000 (Dionex, Breda, Netherlands) with automated sample injector Dionex ASI 100.

3.4.2 Methodological approach to calibration

In order to quantify the GHGs emitted from the sludge, standardised methodologies were developed for calibration of CO₂, CH₄ and N₂O.

Standardised methodology for calibration for CO₂ estimation

Bottles each of known volume (580ml) was filled with air over a 5 minute period and tightly closed to prevent the escape of the air. Standard concentrations of pure CO₂ (UNESCO-IHE Laboratory) in the air were prepared in each bottle. 29ml of pure CO₂ represents 50g/l CO₂ in the bottle. The CO₂ air mix was stirred for 5 minutes to ensure uniform mixing and distribution of CO₂ in the bottle. 5ml of the standard was withdrawn using a syringe (B-D Plastipak) and needle. It was analysed using the gas chromatograph equipped with a Thermal Conductivity Detector (TCD) for CO₂. The peak area signal was recorded. The experiment was repeated for different concentrations of CO₂ (i. e. 100, 150, 200, 300, 400 and 500 g/l), each time recording the peak area signal. A plot of peak area signal (y-axis) against the standard concentrations (x-axis) was done and linear, least squares regression to the standard CO₂ concentrations (g/l) was obtained. CO₂ concentrations in the headspace samples were then computed automatically from the regression equation.

Standardised methodology for calibration for CH₄ estimation

The same procedural approach as described for CO₂ was followed for CH₄ calibration, except that pure CH₄ (Praxair NV, Oevel, Belgium) was used in place of CO₂.

Standardised methodology for calibration for N₂O estimation

Standard concentrations of N₂O were prepared by mixing standard volumes (2, 4, 8, 10, 15, 20, 30, 50 and 100uL) of pure N₂O (Praxair NV, Oevel, Belgium) with air in a 2320ml bottle. The standard volumes represent 0.86, 1.72, 3.44, 4.30, 6.45, 8.60, 12.90, 21.50 and 43.00mg/l N₂O concentrations in the 2320ml respectively. The N₂O air mix was stirred for 5 minutes to ensure uniform mixing and distribution of N₂O in the bottle. 5ml of each standard concentration was analysed using the gas chromatograph equipped with an Electron Capturing Detector (ECD). The peak area signals were recorded for each standard concentration. A plot of peak area signal (y-axis) against the standard concentrations (x-axis) was done and linear, least squares regression to the standard N₂O concentration (mg/l) was obtained. N₂O concentrations in the headspace samples were then calculated automatically from the regression equation

3.4.3 Methodological approach to Objective 1

The first specific objective was:

- *To develop a way to identify and measure simultaneously, CO₂, CH₄, N₂O and NH₃ in gas and liquid phases in eco-technological wastewater treatment systems.*

To achieve this objective, methodological approaches were considered for identification and quantification of GHGs in liquid and gas phases.

A1 Standardised methodology to identify GHGs in the gas phase of activated sludge system.

Sludge samples from the Aerobic tank (AE), Anaerobic Digester (AN) and Denitrifying (Anoxic) tank (DE), were incubated in a reactor at 30°C and gas samples collected and analysed in GC with specific settings to identify CO₂, CH₄ and N₂O. The sample reactor is shown in Figure 6



Figure 4.3 Reactors for quantifying GHGs

It consisted of a reactor made from 500ml plain glass bottle filled with 250ml sludge. 25ml of CH₃COO⁻, 1.25ml of Nutri I, 0.25ml of Nutri II and 0.25ml of Nutri IV were

added and incubated at constant temperatures. The reactor has a capillary vent from the cork of the bottle to relieve any pressure build-up within it. Gas samples were extracted from the headspace of the reactor with plastic syringe (B-D Plastipak). The GHGs were identified and their peak areas determined using the Thermal Conductivity Detector (TCD) for CO₂ and CH₄ (Picek et al., 2007) and Electron Capture Detector (ECD) for N₂O (Picek et al., 2007, Zhou et al., 2008).

B 1 Standardised methodology to quantify GHGs in the gas phase of the activated sludge system

Carbon dioxide (CO₂) and Methane (CH₄) and Nitrous oxide (N₂O) analyses were carried out sequentially using a Varian CP 3800 GC (Varian Netherlands, Middelburg) equipped with a Thermal Conductivity Detector (TCD) for CO₂ and CH₄ analyses and Electron Capture Detector (ECD) for N₂O analyses.

For CO₂ and CH₄ analyses, the oven temperature was 22 °C and the detector temperature was 80°C. The analysis time was 12 minutes. Separations were carried out using a Hayesep T column with Helium (He) as the carrier gas of pressure 4 psi, detection range of 0.5 (mid sensitivity). 5 ml gas samples were manually injected using 5ml plastic (Perpex) syringes. The system was calibrated using standard gases (CO₂ from UNESCO-IHE Laboratory and CH₄ in air from Praxair NV, Oevel, Belgium) as described above.

The N₂O system was analysed with oven temperature at 40°C and detector temperature at 250°C. Separations were carried out using Hayesep T (and in some cases Hayesep S) columns with nitrogen as the carrier gas and P5 (95:5 Ar: CH₄ mix) as the make-up gas. The analysis time was 3 minutes for the Hayesep T and six minutes for the Hayesep S columns. 5 ml of gas samples were manually injected into the column using a plastic syringe. The system was calibrated using standard gas (pure N₂O) obtained from Praxair NV, Oevel, Belgium.

Data was processed with *Galaxy Chromatography Workstation* software (version 1.9). Peak areas for all three gases were quantified. Internal calibration curves as described above were obtained by applying linear, least squares regression to the standard CO₂, CH₄ and N₂O concentration (mg/l) versus peak area data. CO₂, CH₄ and N₂O concentrations in the headspace samples were then calculated automatically from the regression equation.

C 1 Standardised methodology to quantify GHGs in the liquid phase of eco-technological wastewater treatment systems.

Two setups were used. The same setup as described in A1 was utilised. In addition to the 25ml sodium acetate, 1.25ml Nutri I, 0.25ml Nutri II and 0.25ml Nutri IV, 10ml of 15mgNO₃⁻/l and 30mgNO₃⁻/l respective concentrations were introduced to each set of reactors and incubated at 30°C and thereafter added every two weeks. However, one setup was placed on a shaker and shaken at 100 revolutions per minute (rpm) during the experimental period. The GHG concentrations in the headspace were measured twice weekly and quantified. The concentrations of GHGs in the headspace were expected to be higher in the shaker setup. The difference in concentrations between the shaker and the non-shaker were recorded and compared. The difference represented the dissolved GHGs concentrations in the liquid phase.

3.4.4 Methodological approach to Objective 2

- *To assess the influence of temperature on GHGs emissions.*

A 2 Standardised methodology to assess the influence of temperature

Four (4) sets of three (3) reactors each as described above respectively containing Aerated sludge (AE), Anaerobic sludge (AN) and Denitrifying sludge (DE) were placed under four different temperatures namely 4°C, 20°C, 30°C and 37°C. Each reactor was filled with 250ml sludge and 25ml sodium acetate (substrate) and 1.25ml Nutri I, 0.25ml Nutri II and 0.25ml Nutri IV under constant temperature. Each set comprised three reactors respectively containing the Aerated sludge (AE), Anaerobic sludge (AN) and Denitrifying sludge (DE). The table below gives the details of the reactor sets and their incubation temperatures.

Table 4.2 Reactor set and incubation temperature

Set	Reactor	Incubation temperature (°C)
1	AE-1, AN-1, DE-1	4
2	AE-2, AN-2, DE-2	20
3	AE-3, AN-3, DE-3	30
4	AE-4, AN-4, DE-4	37
	<i>NO₃⁻ treatment</i>	
5	AE-15, AN-15, DE-15	30
6	AE-30, AN-30, DE-30	30
7	AE-30, AN-30, DE-30	30
	<i>NO₃⁻, NH₃ Treatment & Shaking</i>	
8	AE-0, AN-0, DE-0	30
9	AE-NO ₃ ⁻ , AN-NO ₃ ⁻ , DE-NO ₃ ⁻	30
10	AE-NO ₃ ⁻ -S, AN-NO ₃ ⁻ -S, DE-NO ₃ ⁻ -S	30
11	AE-S, AN-S, DE-S	30
12	AE-NH ₃ , AN-NH ₃ , DE-NH ₃	30
13	AE-NH ₃ -S, AN-NH ₃ -S, DE-NH ₃ -S	30

Measurements for GHGs (i. e. CO₂, CH₄, N₂O) were done using the Gas Chromatography equipment as described above. The GHGs were quantified after calibration curves were obtained by applying linear, least squares regression to the standard CO₂, CH₄ and N₂O concentration (mg/l) versus peak area data. CO₂, CH₄ and N₂O concentrations in the headspace samples were then calculated automatically from the regression equation. The GHGs concentrations under the different temperatures were then compared.

3.4.5 Methodological approach to Objective 3

- *To assess the role of nutrients (i. e. NO₃⁻ and NH₄⁺) on GHG emission from activated sludge wastewater treatment systems*

A 3 Standardised methodology to assess the influence of NO₃⁻ on greenhouse gas emission

Here two set of reactors were used. The same setup as described in A1 was utilised except the use of Hayesep S column as a substitute for Hayesep T. However, in addition to the 25ml sodium acetate, 1.25ml Nutri I, 0.25ml Nutri II and 0.25ml Nutri IV, 10ml of 15mgNO₃⁻/l and 30mgNO₃⁻/l respective concentrations were introduced to each set of

reactors and incubated at 30°C and thereafter added every two weeks. The GHG concentrations in the headspace were measured twice weekly and quantified after calibration as discussed above. The GHGs concentrations from the reactors were compared. The nitrate concentrations in each reactor at the beginning and end of each experiment were determined using the Ion Chromatograph Dionex Ion Chromatography System, ICS-1000 with automated sample injector Dionex ASI 100 and compared

B 3 Standardised methodology to assess the influence of NH_4^+ on greenhouse gas emission

Two set of reactors were used. The same setup and procedures as described in A3 above was used. However, in addition to the 25ml sodium acetate, 1.25ml Nutri I, 0.25ml Nutri II and 0.25ml Nutri IV, 10ml of 15mg NH_4^+ /l and 30mg NH_4^+ /l respective concentrations were introduced to each set of reactors and incubated at 30°C and thereafter added every two weeks. The GHG concentrations in the headspace were measured twice weekly and quantified. The GHGs concentrations from the reactors were compared. The NH_4^+ concentrations in each reactor at the beginning and end of each experiment were determined.

3.4.6 Methodological approach to Objective 4

- *To assess the effects of shaking on GHGs emissions from sludge*

A 4 Standardised methodology to assess the influence of shaking on GHG emissions from sludge

Two set of reactors were used. The same setup and procedures as explained in A3 were utilised. However, one set of reactors were placed on a shaker at 100 revolutions per minute (rpm) and both sets put under 30°C during the experimental period. The concentrations of CO_2 , CH_4 and N_2O were measured from the headspace, quantified and their concentrations over time compared. .

CHAPTER FOUR

4.0 RESULTS

This chapter presents the results of the experiment. In all three (3) types of sludge were used. These were **AE**: Aerobic sludge, **AN**: Anaerobic sludge and **DE**: Denitrifying sludge. The details are provided in Materials and Methods in Chapter Three. The general data are attached as appendices (**Appendix 4.1**-N₂O, **Appendix 4.2**-CO₂, **Appendix 4.3**-CH₄, **Appendix 4.4**-COD, **Appendix 4.5**-Ammonia, **Appendix 4.6**-Nitrate, **Appendix 4.7**-Acetate and **Appendix 4.8**-pH). The chapter highlights the following results.

- 4.1 Effects of temperature (37°C, 30°C, 20°C & 4°C) on N₂O, CO₂ and CH₄ emissions
- 4.2 Effects of nitrate on N₂O, CO₂ and CH₄ emissions
- 4.3 Effects of ammonium on N₂O, CO₂ and CH₄ emissions and
- 4.4 Effects of shaking on N₂O, CO₂ and CH₄ emissions

4.1 Effects of Temperature on N₂O, CO₂ and CH₄ Emissions

4.1.1 N₂O, CO₂ and CH₄ Emissions at 37°C

N₂O

Figure 4.1a illustrates N₂O releases at 37°C below. Prior to incubation, smaller concentrations of 0.3mg/l, 0.4mg/l & 0.9mg/l were respectively measured for reactors AE-4, AN-4 & DE-4. This however, fell to 0mg/l on day 2 until day 28 when minimal releases resumed

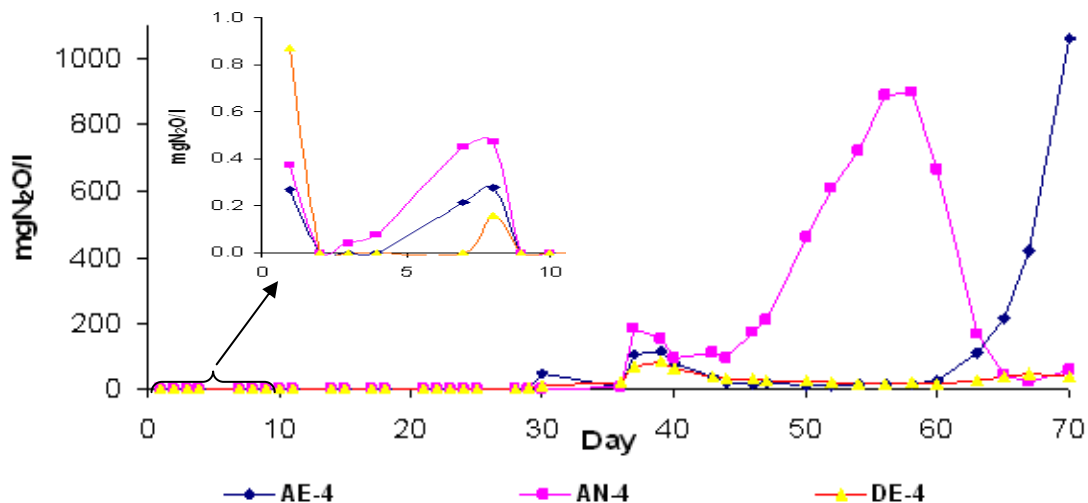


Figure 4.1a N₂O emissions in sludge at 37°C

From day 36 noticeable N₂O productions were detected especially for AN-4. An exponential increase in N₂O production was observed for AN-4 and AE-4 after 44 and 60 days respectively until day 58 when production reached its peak at 899.27mg/l for AN-4. Unexpectedly however, an exponential decline in concentration was subsequently observed for AN-4 as indicated in Figure 4.1a. This may suggest consumption of N₂O

within the reactor. Conversely the sharp increase recorded for AE-4 continued exponentially to a maximum of 1065.14mg/l during the experimental period. On the other hand DE-4 maintained the least N_2O production with the maximum concentration of only 85.18mg/l occurring on day 29.

CO₂

CO₂ emission trends as shown in Figure 4.1b indicate similar emission patterns for all 3 sludge with production starting from day 1.

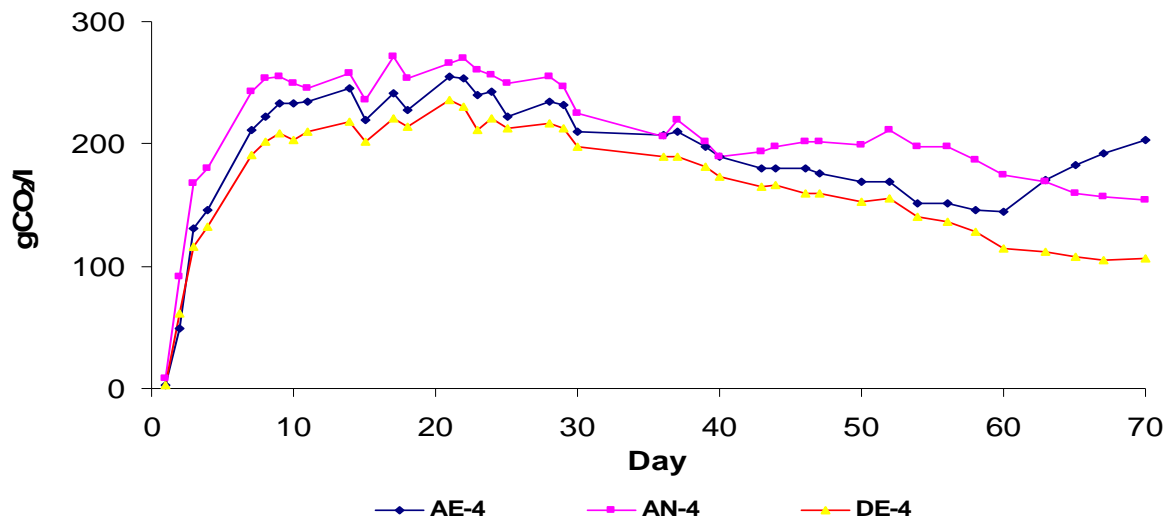


Figure 4.1b CO₂ emission in sludge at 37°C

Peak emissions concentrations for all 3 sludge were reached in 17 days with AN-4 recording the highest of 271.17g/l, AE-4 241.97g/l and DE-4 the lowest of 220.99g/l. Thereafter emission concentrations remained more or less constant, possibly a result of a simultaneous production and consumption of CO₂.

CH₄

CH₄ production started on day 3 for AE-4 and AN-4 and day 4 for DE-3 with all reactors exhibiting identical trends in emissions as indicated in Figure 4.1c.

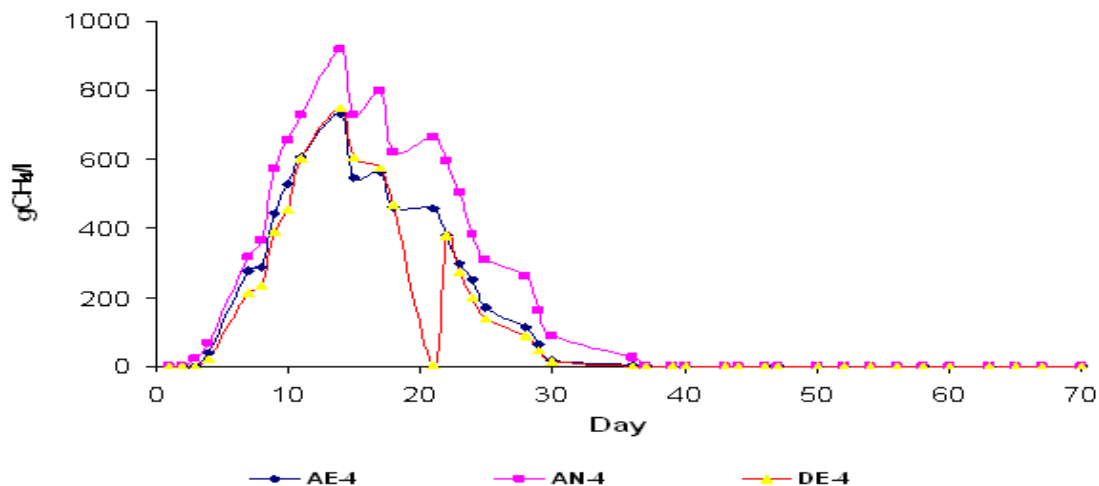


Figure 4.1c CH₄ emission in sludge at 37°C

Peak emission concentrations were reached in 14 days for all reactors with AE-4, AN-4 and DE-4 recording respective concentrations of 730.6g/l, 914.1 g/l and 747.0g/l. Conversely however, concentrations continued to decline and by day 36 reached almost 0 in all reactors. This may suggest consumption of CH₄ within the reactors.

Acetate, NH₄⁺, COD, NO₃-N and pH trends

A comparison of the trends in influent and effluent acetate (Figure 4.1d), NH₄⁺ (Figure 4.1e), COD (Figure 4.1f), NO₃-N (Table 4.1) concentrations and pH (Figure 4.1g) for all 3 reactors.

The results showed a sharp reduction (over 99%) in acetate, a build up (about 86% increase) of NH₄⁺ (37.38-273.63, 39.23-273.41 & 48.31-336.29mg/l respectively for AE-4, AN-4 & DE-4.), a marked reduction (over 70%) in COD, an unexpected build up of NO₃-N in AE-4 and DE-4 (0.12 to 4.26 & <0.10 to 0.45 respectively) and a stable pH (7.1<pH<7.7) in all 3 reactors over the experimental period.

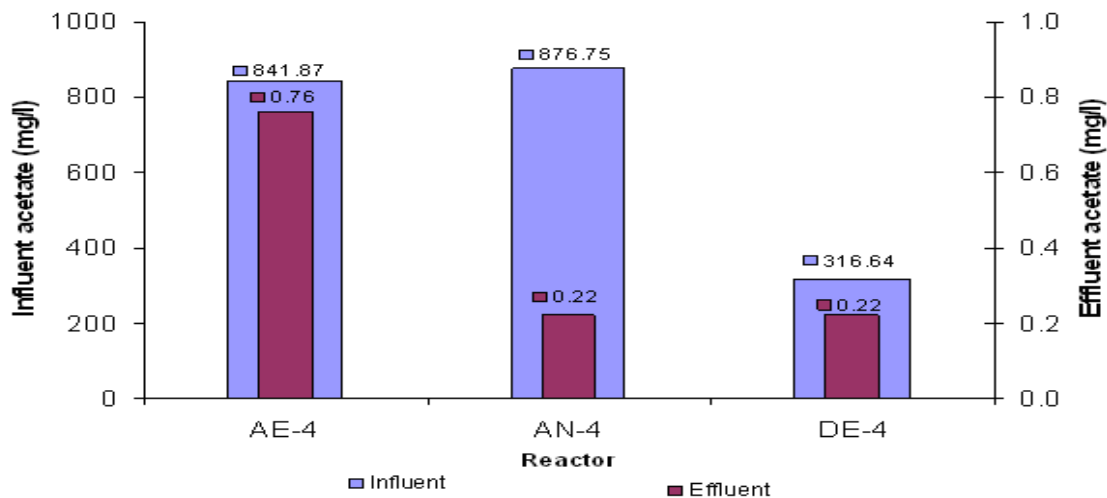


Figure 4.1d Acetate concentrations at 37°C

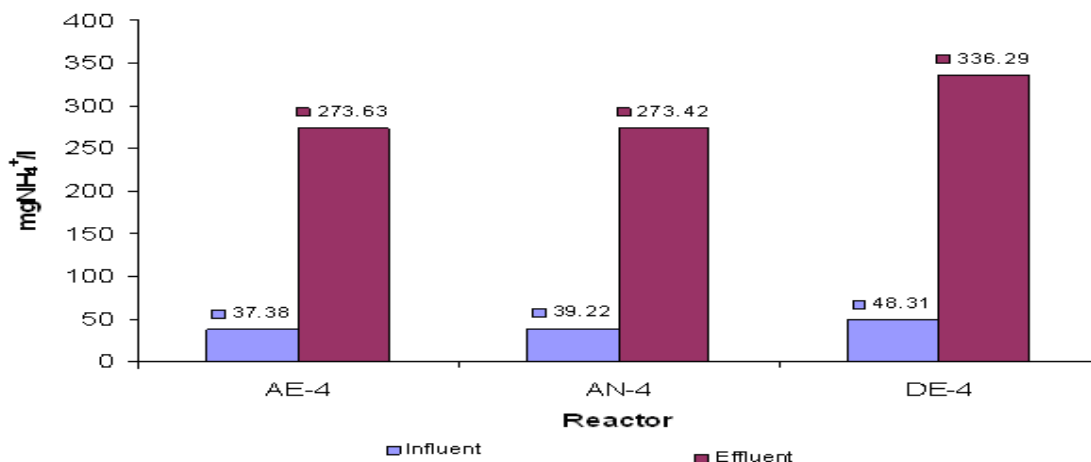


Figure 4.1e Ammonia concentrations at 37°C

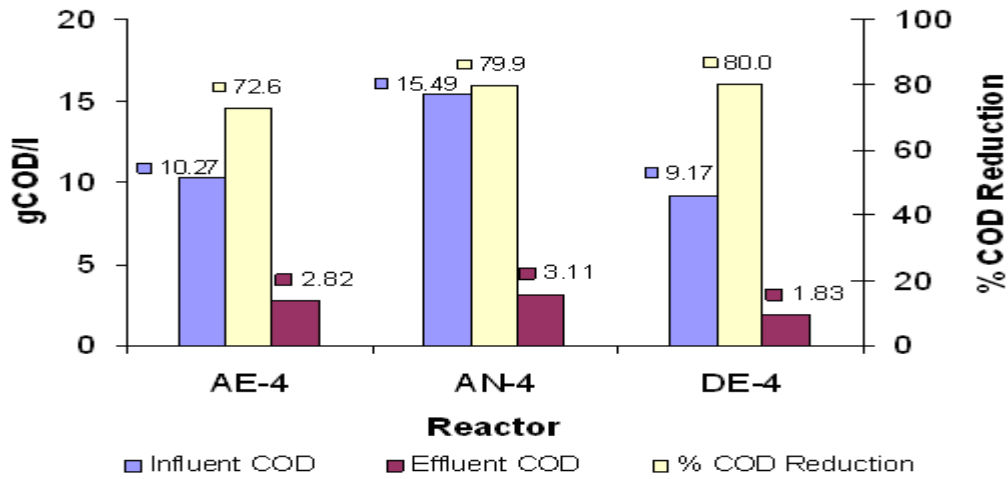


Figure 4.1f COD concentrations at 37°C

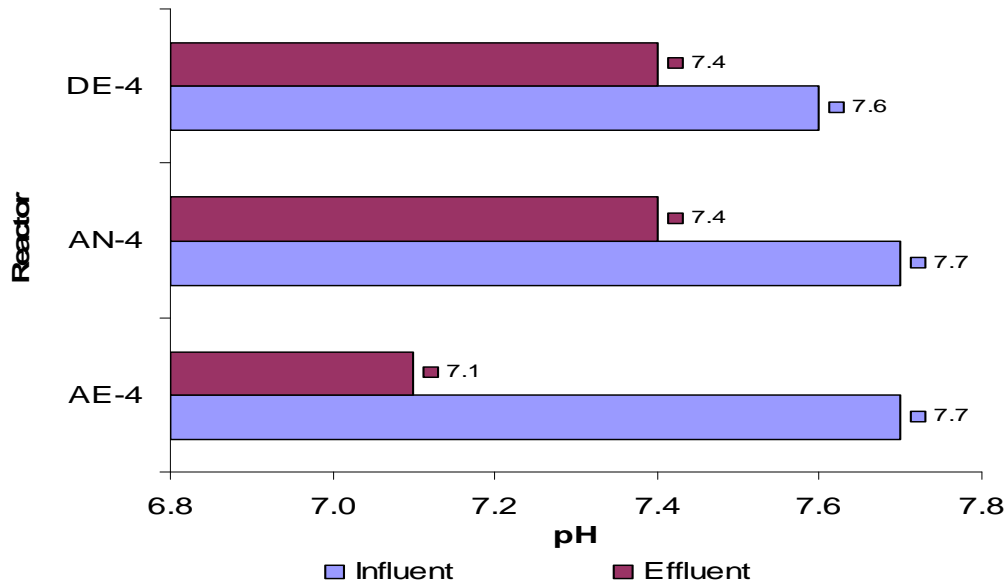


Figure 4.1g pH at 37°C

Table 4.1 Nitrate concentrations 37°C

Influent and Effluent NO ₃ -N Concentration (mg/l)		
Reactor	Influent	Effluent
AE-4	0.12	4.26
AN-4	0.20	0.03
DE-4	<0.10	0.45

4.1.2 N₂O, CO₂ and CH₄ Emissions at 30°C

N₂O

Figure 4.2a shows the trends in N₂O emission patterns over the experimental period. Prior to incubation, 0.28, 0.40 and 0.67mg/l respectively were recorded for AE-3, AN-3 & DE-3 rising to 8.17, 7.87 and 2.76mg/l for AE-3, AN-3 and DE-3 in day 3.

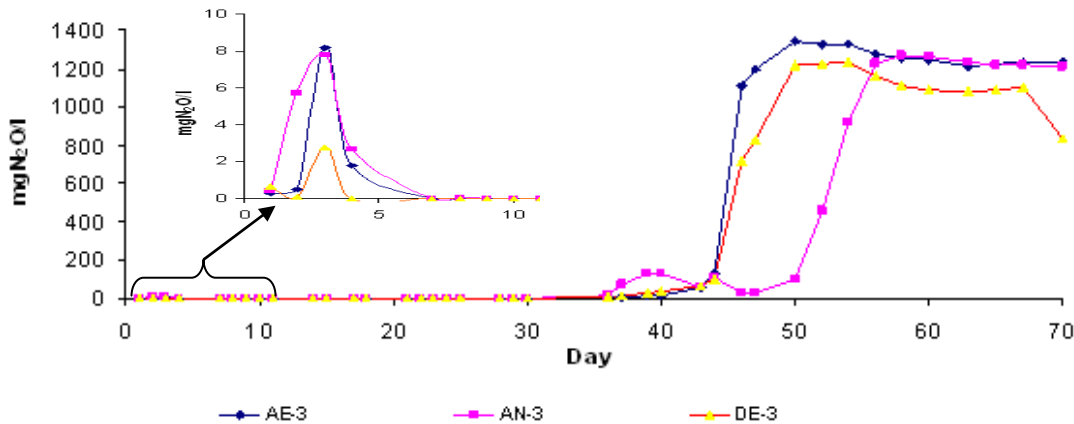


Figure 4.2a N₂O emission in sludge at 30°C

Thereafter a decline in N₂O was observed till day 36 when considerable production resumed rising exponentially (day 40 for AE-3 & DE-3 and day 47 for AN-3) to a peak of 1342.6, 1272.1 and 1230.6mg/l for AE-3, AN-3 & DE-3 on day 50, 58 and 54 respectively. It can also be inferred from the Figure 4.2a that, though the reactors showed similar response patterns at 30°C, the exponential phase for AE-3 and DE-3 (day 40) preceded that for AN-3 (day 47). The N₂O concentration more or less remained constant after the peaks for all reactors thus suggesting a possible consecutive production and consumption of N₂O in all reactors.

CO₂

The CO₂ emission trends for AE-3, AN-3 and DE-3 at 30°C are depicted in Figure 4.2b. All reactors showed identical trends in CO₂ emission patterns.

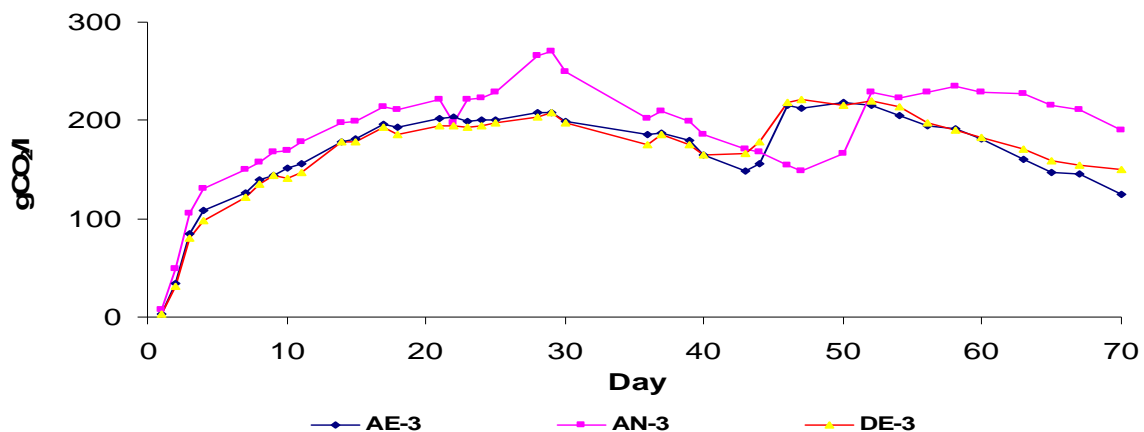


Figure 4.2b CO₂ emission in sludge at 30°C

Emission peaks were reached on day 29 for all reactors with AN-3 showing the highest peak concentration of 269.9g/l and about 207g/l for both AE-3 and DE-3. Thereafter emission concentrations remained more or less flat; a probable indication of a simultaneous production and consumption of CO₂.

CH₄

As indicated in Figure 4.2c, CH₄ production started on day 7 for all 3 reactors with AN-3 showing the highest emission. Though similar patterns were observed in all reactors, peak emission concentrations of 205.8, 466.5 and 159.1g/l were reached in 17, 29 and 15 days respectively for AE-3, AN-3 and DE-3.

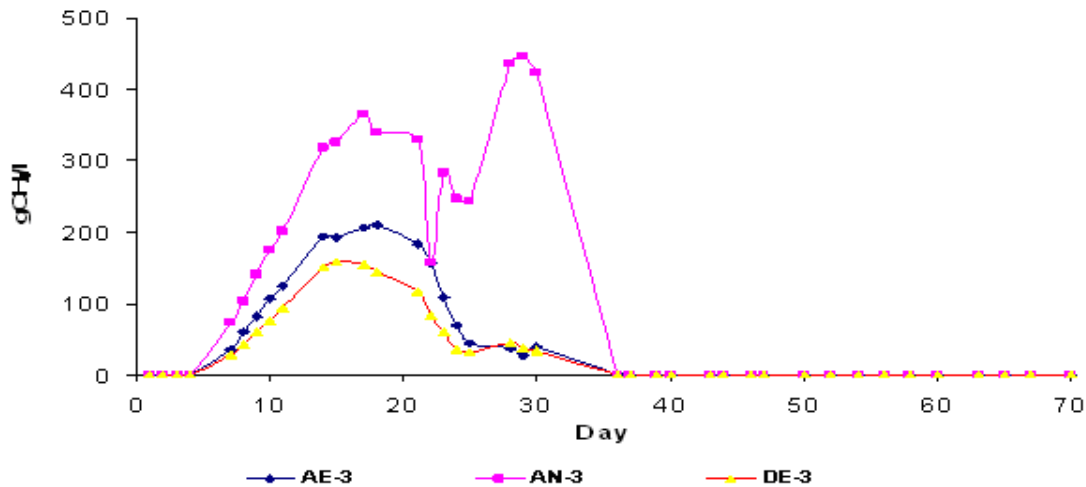


Figure 4.2c CH₄ emission in sludge at 30°C

Gradual declines in concentration for AE-3 & DE-3 were recorded thereafter, while a sharp fall was observed for AN-3 and by day 36 reached almost 0 for all reactors suggesting a likely consumption of CH₄ within the reactors.

Acetate, NH₄⁺, COD, NO₃-N and pH trends

The trends in influent and effluent acetate, NH₄⁺, COD, NO₃-N concentrations and pH are depicted in Figure 4.2d, Figure 4.2e, Figure 4.2f, Table 4.2 and Figure 4.2g respectively for all 3 reactors AE-3, AN-3 and DE-3.

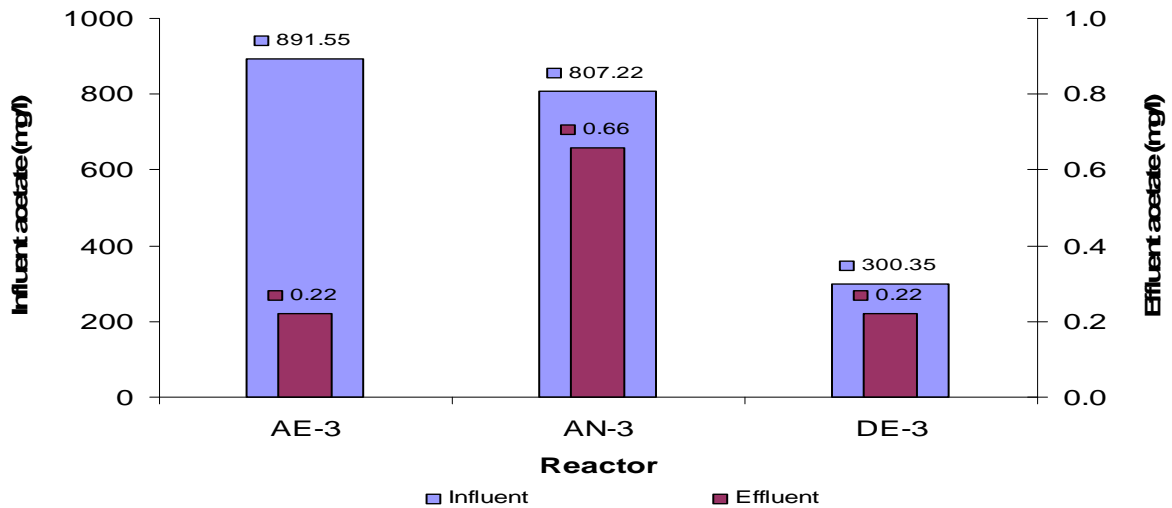


Figure 4.2d Acetate concentrations at 30°C

While the data showed a sharp reduction (over 99%) in acetate (Figure 4.2d), it recorded a build up (about 86% increase) of NH_4^+ corresponding to 46.4%, 76.1% & 58.9% respectively for AE-3, AN-3 & DE-3 (Figure 4.2e).

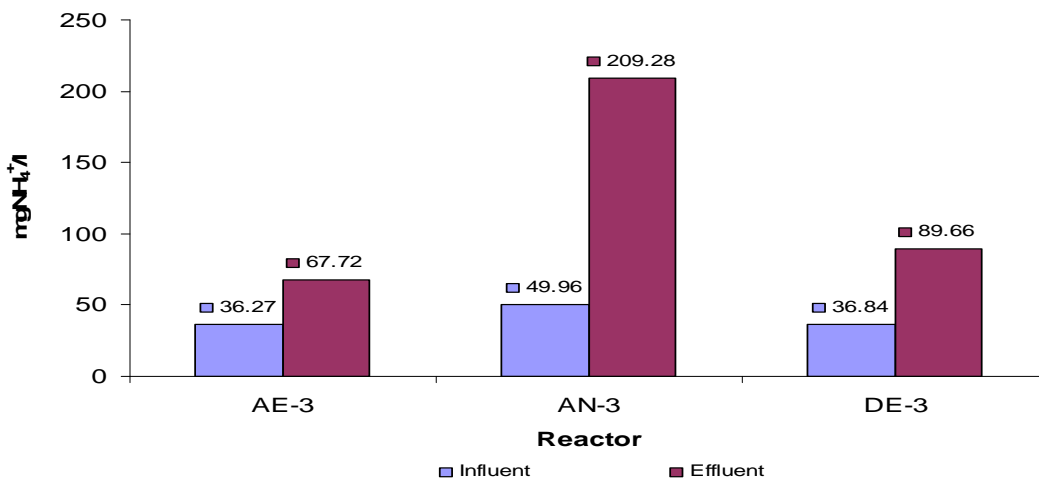


Figure 4.2e Ammonia concentrations at 30°C

As indicated in Figure 4.2f a marked reduction (over 60%) in COD was recorded in all reactors while an unexpected build-up of $\text{NO}_3\text{-N}$ (<0.10 -3.39mg/l, <0.10-0.24mg/l & <0.10-38.81mg/l respectively) was observed (Table 4.2) in all reactors. A relatively stable pH (7.1<pH<7.7) in all 3 reactors was also noted over the experimental period (Figure 4.2g).

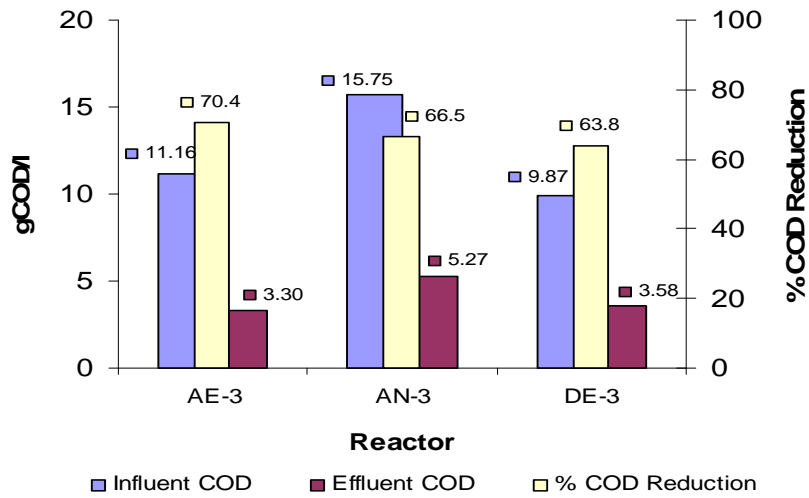


Figure 4.2f COD concentrations at 30°C

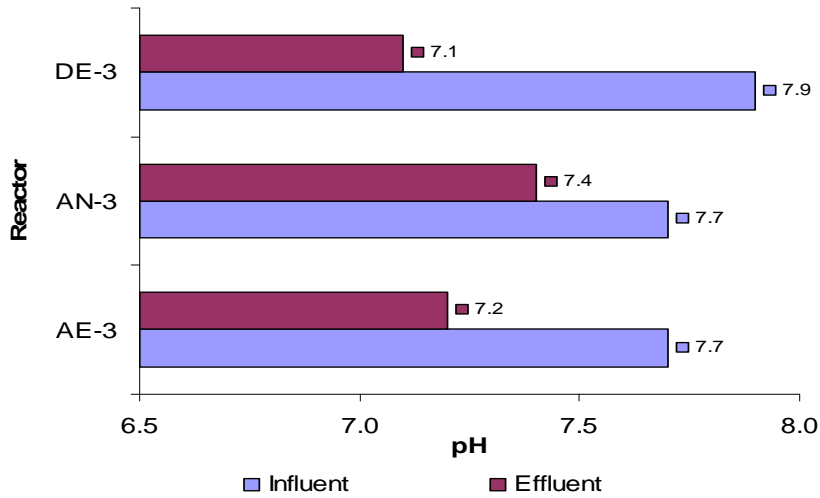


Figure 4.2g pH at 30°C

Table 4.2 Nitrate concentrations at 30°C

Influent and Effluent NO ₃ -N Concentration (mg/l)		
Reactor	Influent	Effluent
AE-3	<0.1	3.39
AN-3	<0.1	0.24
DE-3	<0.1	38.81

4.1.3 N₂O, CO₂ and CH₄ Emissions at 20°C

N₂O

The emission of N₂O during the experimental period was most important in the first week.

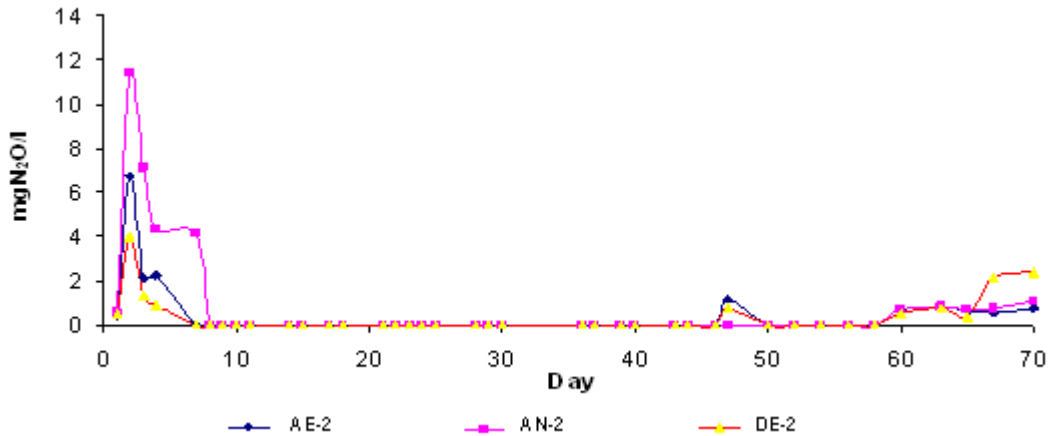


Figure 4.3a N₂O emission in sludge at 20°C

As Figure 4.3a indicated N₂O production reached peak concentrations in all reactors on the second day (6.7, 11.4, 3.9mg/l respectively for AE-2, AN-2 & DE-2) after which daily emission concentrations were markedly maintained at 0 till day 60 when smaller concentrations were detected, reaching a respective 0.78, 1.1 & 2.4mg/l for AE-2, AN-2 & DE-2 at the end of the experimental period.

CO₂

Figure 4.3b illustrates CO₂ emission trends at 20°C. Similar production and release patterns were observed in all reactors with AN-2 exhibiting the highest concentrations over the experimental period.

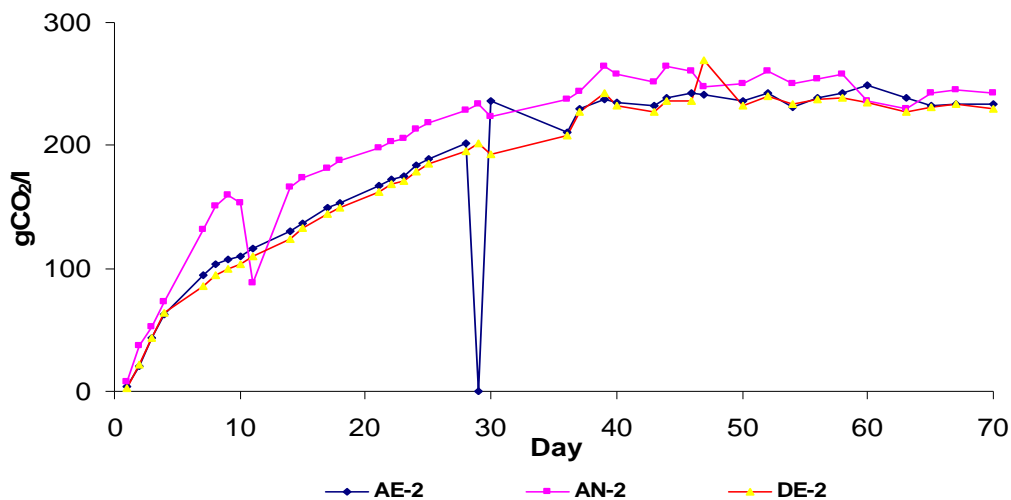


Figure 4.3b CO₂ emission in sludge at 20°C

Peak emissions concentrations for all 3 reactors were reached in 39 days with corresponding 237.1g/l, 264.7g/l and 242.4g/l for AE-2, AN-2 and DE-2. Subsequently, emission concentrations decreased gradually for the rest of the experimental period, perhaps as a consequence of the consumption of CO₂ within the reactors.

CH₄

CH₄ production was observed on day 8 of the experiment as shown in Figure 4.3c. Concentrations rose gradually, reaching the maximum for all 3 reactors on day 36.

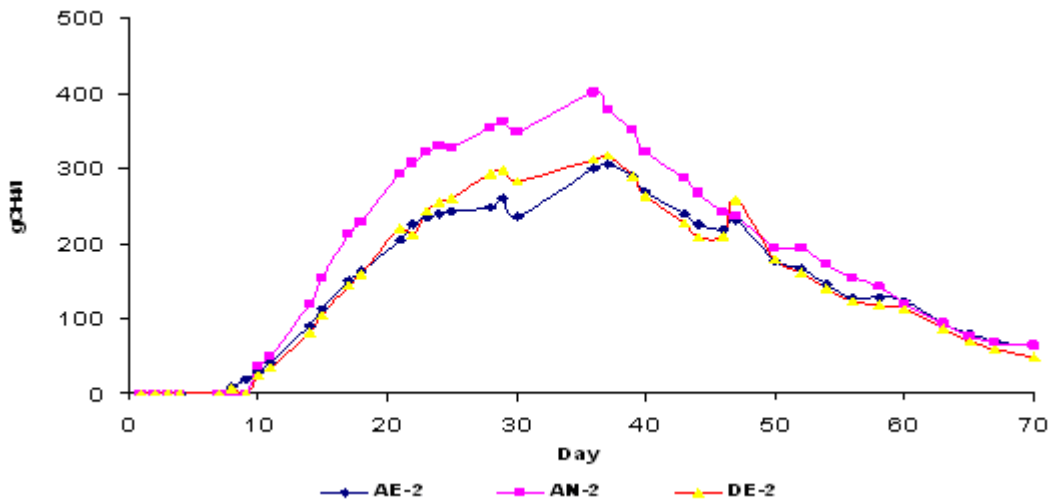


Figure 4.3c CH₄ emission in sludge at 20°C

Reactor AN-2 showed the highest emission concentrations over the period with peak concentration values of 300.1, 401.6 & 316.9g/l corresponding to reactors AE-2, AN-2 and DE-2. Gradual drops in concentration in all reactors were observed after day 36, reaching respective lows of 65.1g/l, 63.4g/l & 48.1g/l on the last day of the experiment.

Acetate, NH₄⁺, COD, NO₃-N and pH trends

The Figures below (4.3d-g & Table 4.4) followed the acetate, NH₄⁺, COD, NO₃-N concentrations and pH changes at the beginning and end of the experiment.

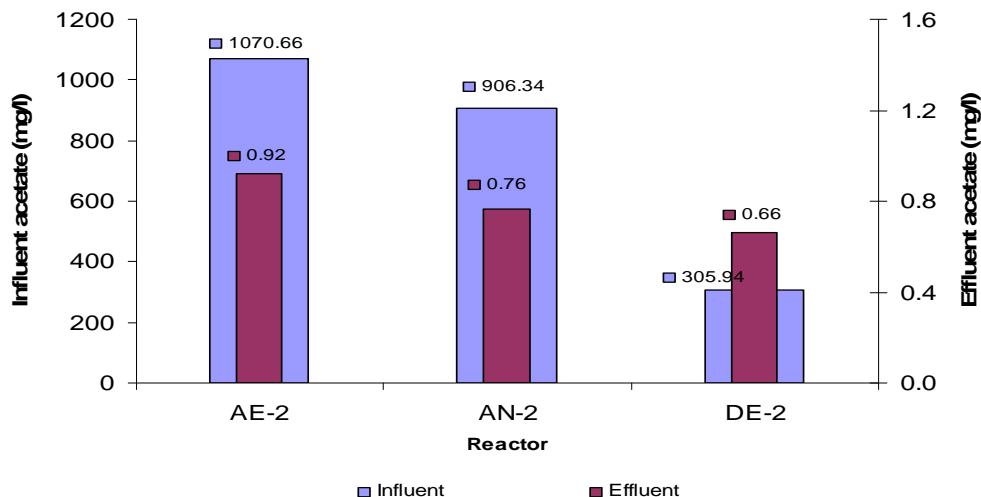


Figure 4.3d Acetate concentrations at 20°C

Acetate (Figure 4.3d), NH_4^+ (Figure 4.3e), COD (Figure 4.3f), $\text{NO}_3\text{-N}$ (Table 4.3) concentrations and pH (Figure 4.3g) shows more than 99% utilisation of acetate, a high buildup of NH_4^+ representing about 89.3%, 78.2% & 88.5% upsurge respectively for AE-2, AN-2 & DE-2.

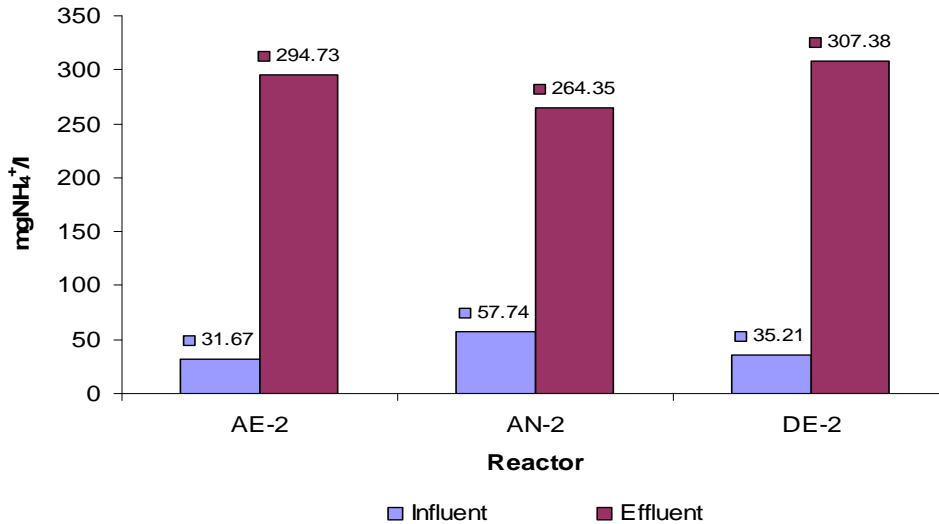


Figure 4.3e Ammonia concentrations at 20°C

A considerable reduction in COD representing 50%, 66% & 44% was attained while a slight buildup of $\text{NO}_3\text{-N}$ was observed in the effluent of the respective reactors (Table 4.3). The pH difference between influent and effluent ranged from a high of 7.99 in the influent to a low of 6.99 in the effluent (Figure 4.3f).

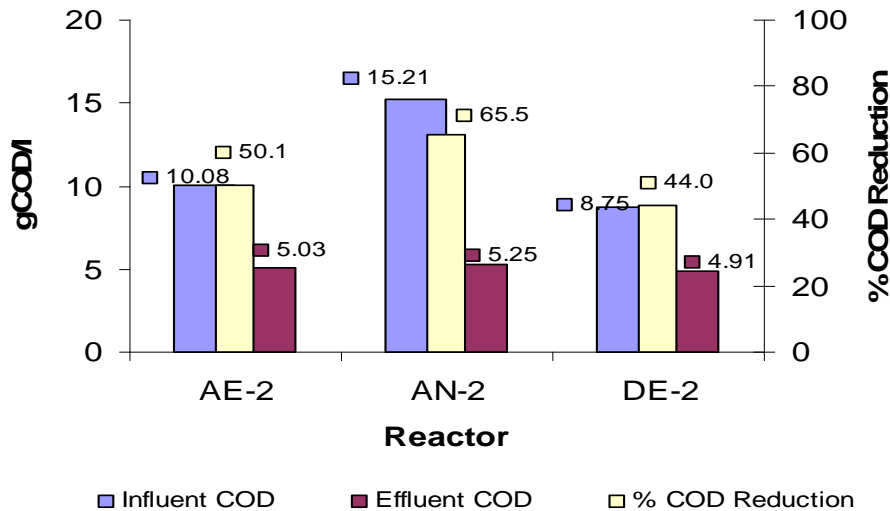


Figure 4.3f COD concentrations at 20°C

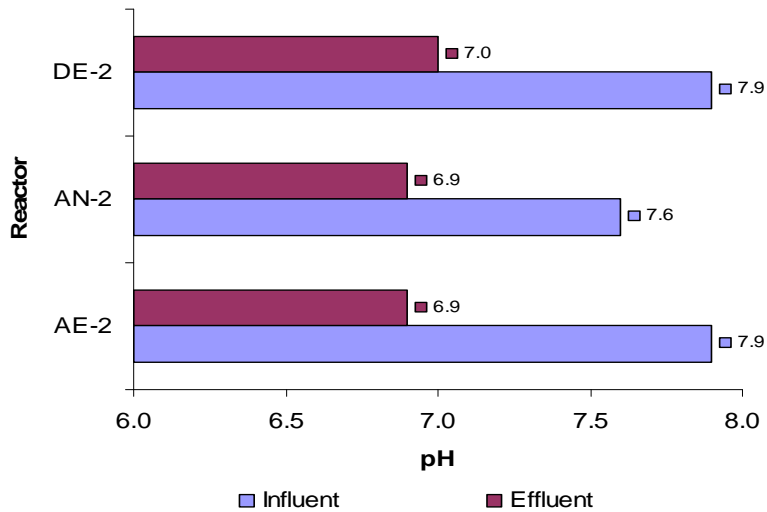


Figure 4.3f pH at 20°C

Table 4.3 Nitrate concentrations at 20°C

Influent and Effluent NO ₃ -N Concentration (mg/l)		
Reactor	Influent	Effluent
AE-2	0.16	0.27
AN-2	<0.10	0.48
DE-2	<0.10	0.22

4.1.4 N₂O, CO₂ and CH₄ Emissions at 4°C

N₂O

As Figure 4.4a shows, the emission of N₂O at 4°C, during the experimental period was most crucial in the first week with AE-1, AN-1 & DE-1 reaching daily peak concentrations of 2.7mg/l, 8.4mg/l and 16mg/l on day 4, day 2 and day 3 respectively

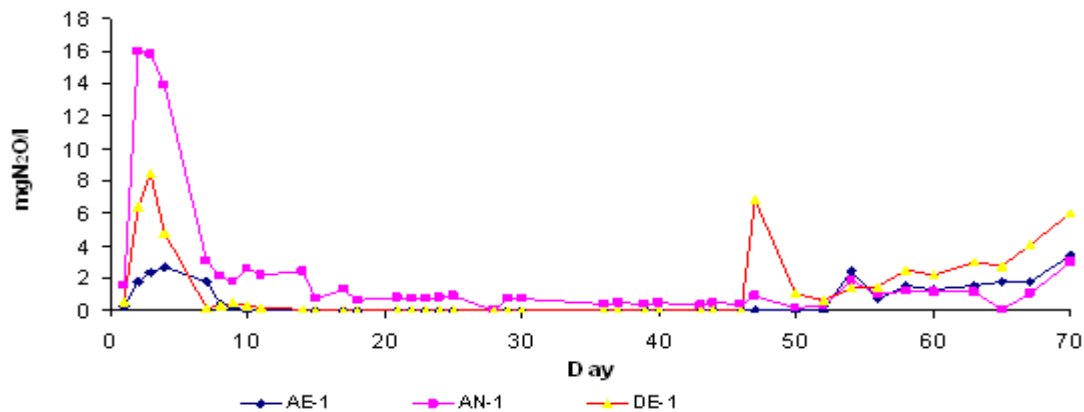


Figure 4.4a N₂O emission in sludge at 4°C

However, with the clear exception of AN-1 which maintained a daily average emission concentration of 1.2mg/l during the experimental period, production in AE-1 and DE-2 fell to 0 till considerable concentrations were detected on day 54 and day 47 respectively reaching 3.4mg/l and 5.9mg/l.

CO₂

CO₂ emission trends at 4°C are depicted in Figure 4.4b.

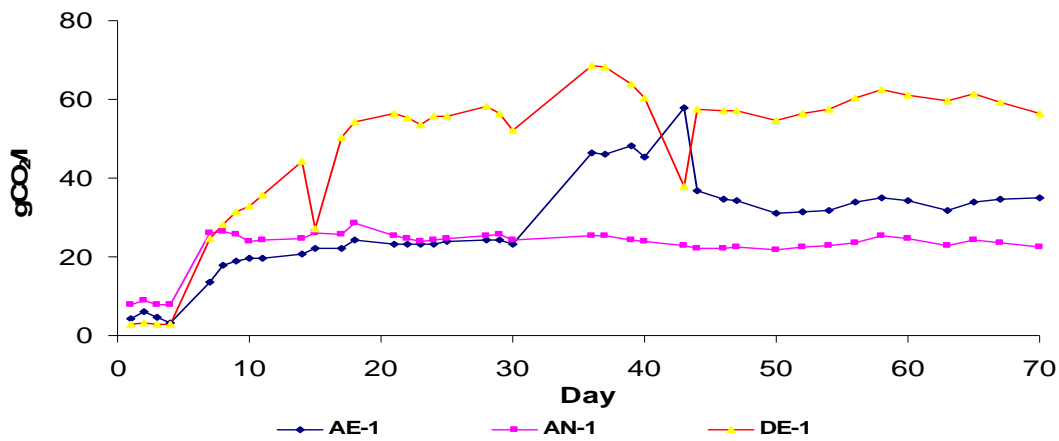


Figure 4.4b CO₂ emission in sludge at 4°C

The figure indicates that CO₂ emissions for all reactors was below 10g/l in the first 4 day, rising sharply to 17.9g/l, 26.3g/l and 28.3g/l respectively with reactor AN-1 remaining relatively constant for the rest of the experimental period.. However, reactors AE-1 and DE-1 reached higher daily emission concentrations of 68.5g/l & 57.7g/l in 43 and 36 days and subsequently remaining constant for the rest of the experimental period.

CH₄

The Figure below (Figure 4.4c) indicates CH₄ emissions at 4°C. As expected, no CH₄ productions were detected at 4°C in any of the reactors during the experimental period.

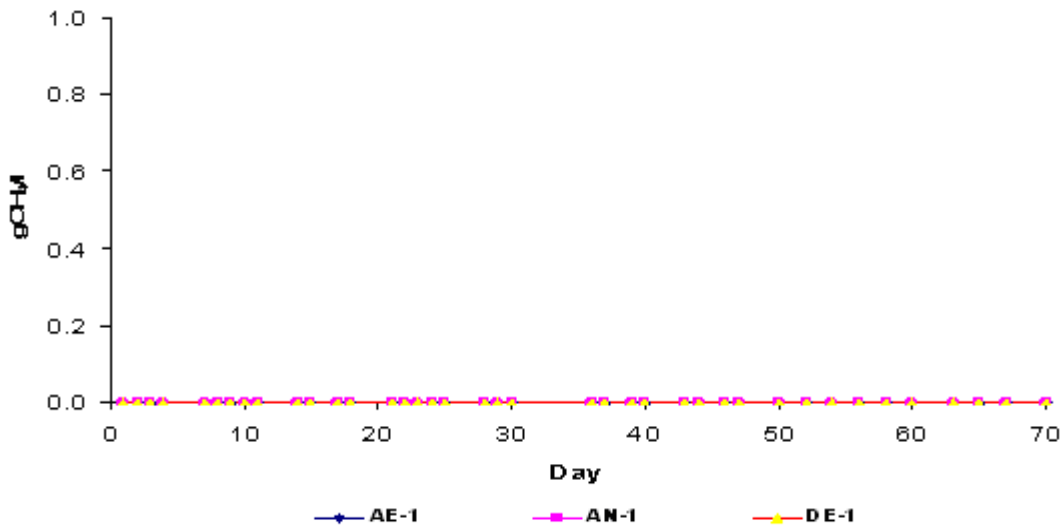


Figure 4.4c CH₄ emission in sludge at 4°C

Acetate, NH₄⁺, COD, NO₃-N and pH trends

A comparison of the trends in influent and effluent acetate (Figure 4.4d), NH₄⁺ (Figure 4.4e), COD (Figure 4.4f), NO₃-N (Table 4.4) concentrations and pH (Figure 4.4g) for all

3 reactors shows a marked reduction (over 99%) in acetate, an increase of NH_4^+ representing about 60%, 43% & 90% respectively for AE-1, AN-1 & DE-1 (Figure 4.4e)

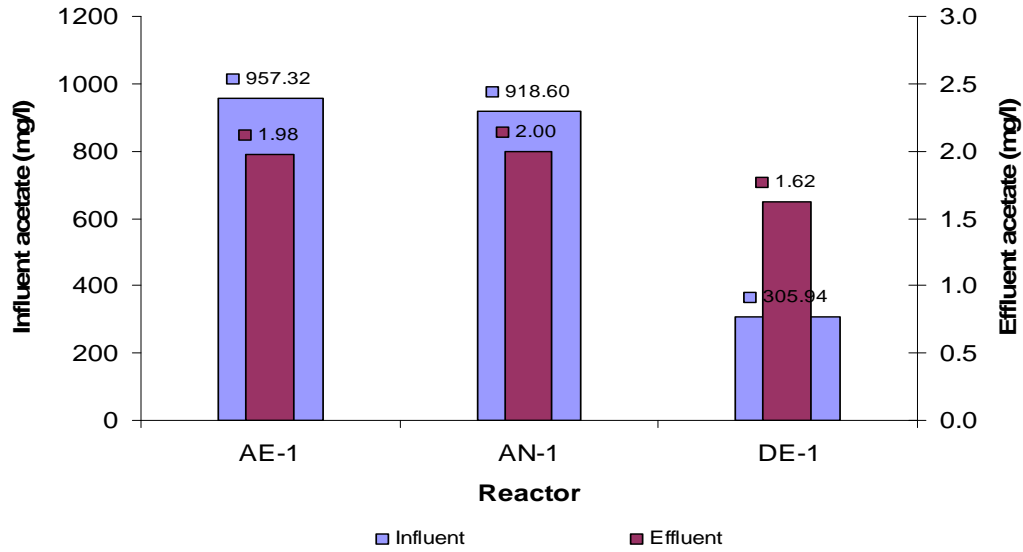


Figure 4.4d Acetate concentrations at 4°C

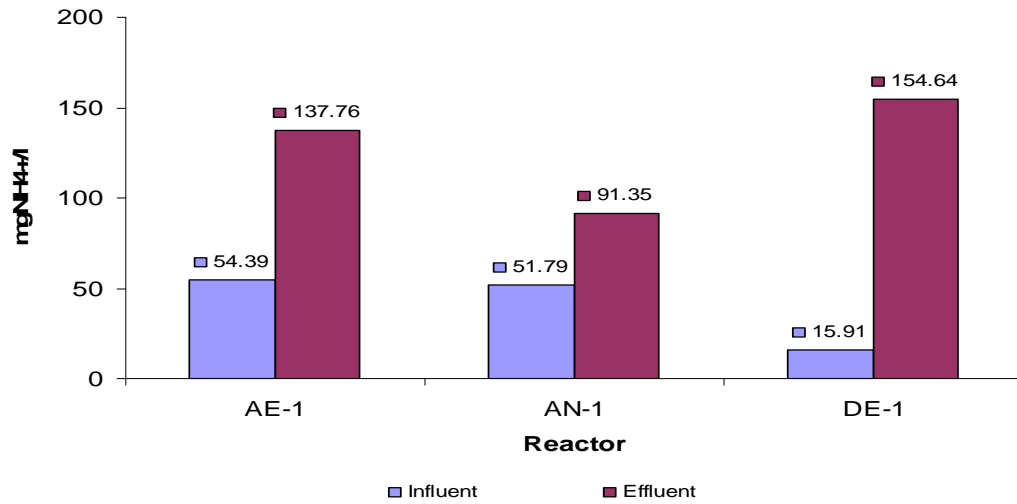


Figure 4.4e Ammonia concentrations at 4°C

A smaller reduction in COD, representing 29.3%, 19.4% & 58.6% was attained whereas an expected reduction of $\text{NO}_3\text{-N}$ in AE-1 and AN-1 was observed with a small increase recorded for DE-4 (0.18 to 0.39) (Table 4.4). A pH difference between influent and effluent varied from a high of 7.9 in the influent to a low of 7.2 in the effluent (Figure 4.2g) over the experimental period.

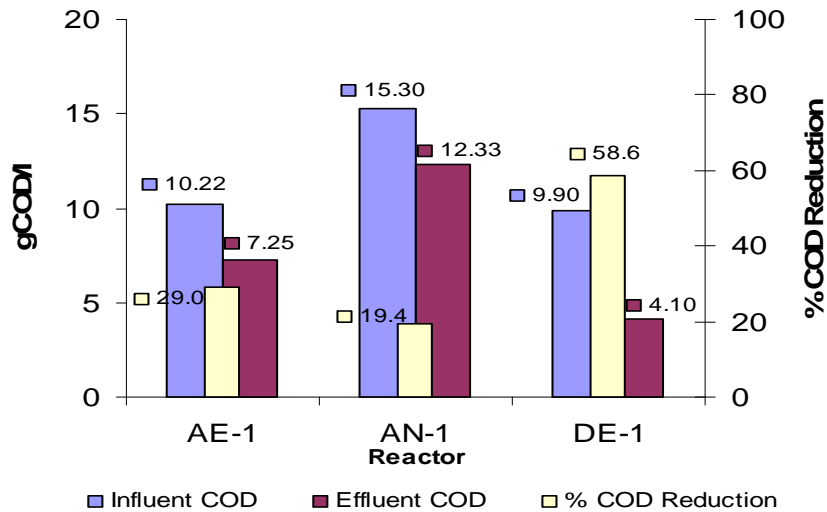


Figure 4.4f COD concentrations at 4°C

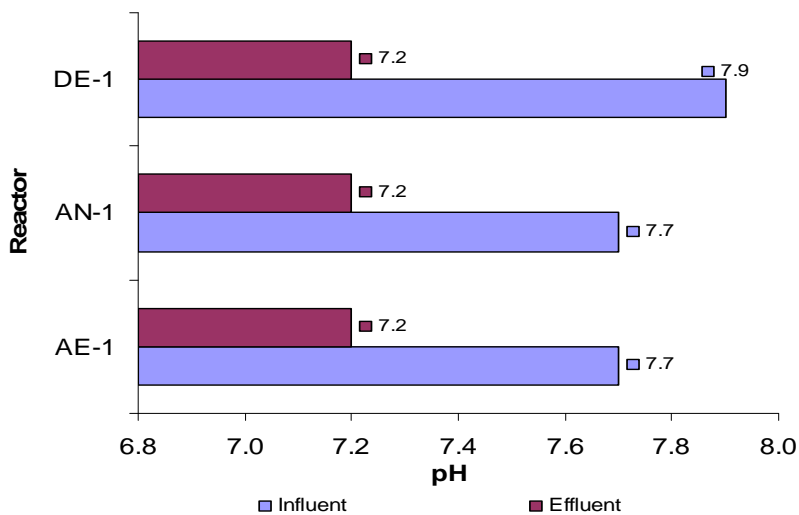


Figure 4.2g pH at 4°C

Table 4.4 Nitrate concentrations at 4°C

Influent and Effluent NO ₃ -N Concentration (mg/l)		
Reactor	Influent	Effluent
AE-1	0.47	<0.10
AN-1	<0.10	<0.10
DE-1	0.18	0.39

4.1.5 Comparing the Effects of Temperature on N₂O, CO₂ and CH₄ emissions

Comparing the effects of temperature on N₂O emissions

Aerobic sludge

The release of N₂O by Aerobic sludge under varying temperatures is illustrated in Figure 4.5a. The emission patterns indicated that under 4°C, the highest concentrations of N₂O were recorded on day 4 (2.67mg/l), day 54 (2.46mg/l) and day 70 (3.39mg/l).

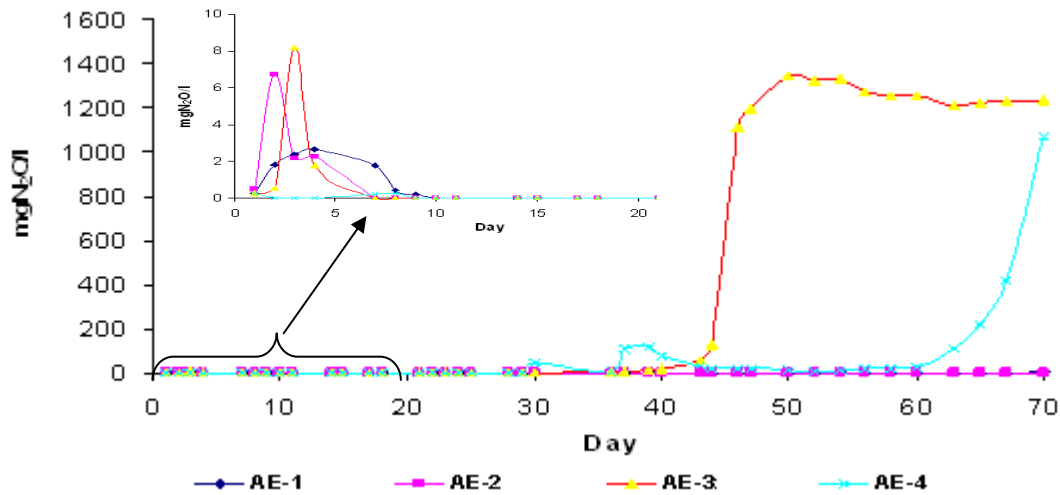


Figure 4.5a N_2O emissions from aerobic sludge at different temperatures

Similarly, under $20^{\circ}C$, slight emission concentrations were recorded on day 2 (6.71mg/l), day 60 (0.72mg/l) and only 0.78mg/l on the final day of the experiment. Conversely, considerable emissions were recorded for $30^{\circ}C$ and $37^{\circ}C$. These ranged from a low of 0.28mg/l on day 1 to a peak concentration of 1342.58mg/l on day 50 for $30^{\circ}C$ (AE-3) after which a more or less constant emissions were observed; a probable indication of production and consumption of the N_2O . At $37^{\circ}C$, significant emissions started from day 29 and rose steadily until day 60 when an exponential increase was recorded for the rest of the experimental period. Generally, lower emissions were initially recorded till day 54 ($4^{\circ}C$), day 47 ($20^{\circ}C$), day 37 ($30^{\circ}C$) and day 30 ($37^{\circ}C$) when important concentrations were measured.

Anaerobic sludge

Figure 4.5b depicts the emissions of N_2O from Anaerobic sludge at different temperatures namely $4^{\circ}C$, $20^{\circ}C$, $30^{\circ}C$ and $37^{\circ}C$. The data indicated that at $4^{\circ}C$ a constant average release concentration of 1.2mg/l was maintained throughout the experimental period except the first 4 days where the highest concentrations were recorded. At $20^{\circ}C$, the notable release days are the first 3 days and the last 10 days with the highest concentration recorded on day 2.

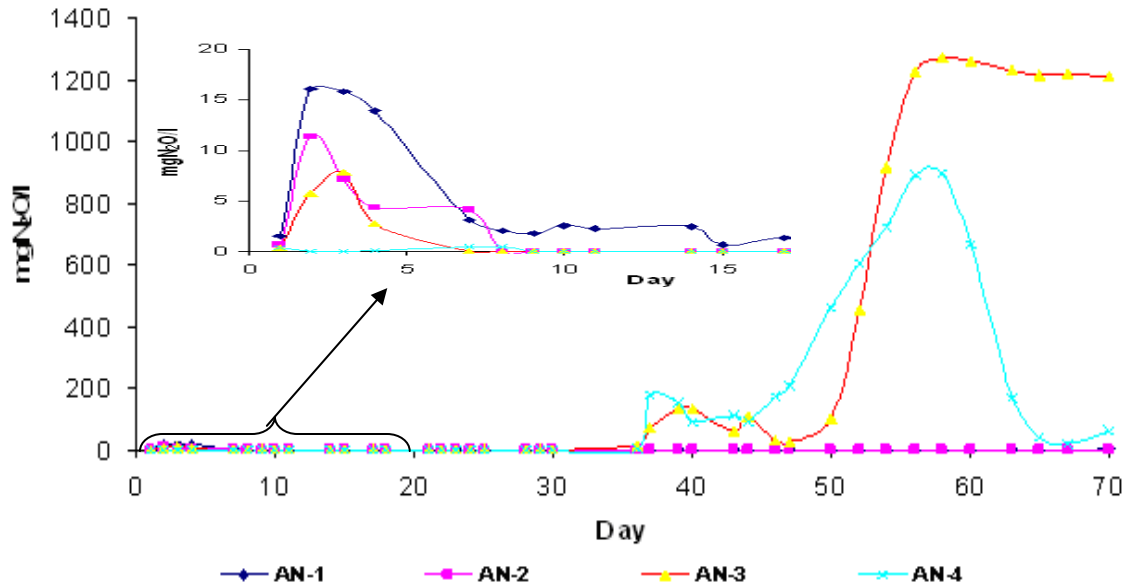


Figure 4.5b *N₂O emissions from anaerobic sludge at different temperatures*

Major N₂O production at 30°C began on day 36 and rose exponentially to attain a peak concentration of 1263.13mg/l on day 60 and thereafter maintained a constant level for the rest of the period. On the contrary, though important releases at 37°C started on day 37, a peak concentration (900mg/l) was reached on day 58 and thereafter fell sharply to 60mg/l at the end of the experiment.

Denitrifying sludge

The release of N₂O from Denitrifying sludge at different temperatures is indicated in Figure 4.5c. With the clear exception of 30°C which shows important emission concentrations, the other temperatures seemed to have less influence on N₂O emissions.

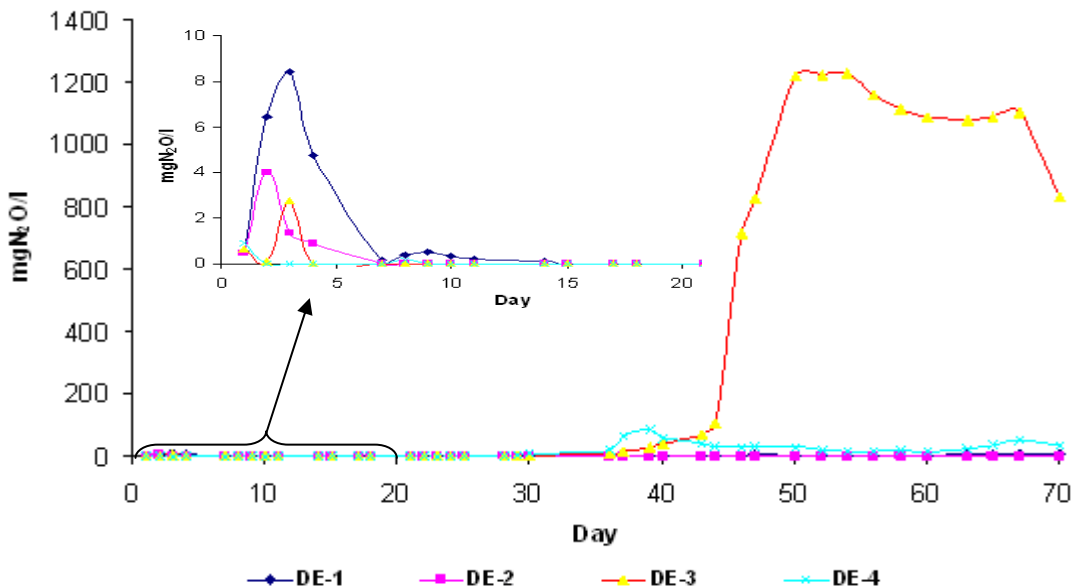


Figure 4.5c *N₂O emissions from denitrifying sludge at different temperatures*

Thus while emission concentrations varied from a low of 0.11 mg/l on day 2 to the highest of 1230.56 mg/l at 30°C, under 4°C and 20°C, N₂O emissions were less important. Emissions at 37°C are surprisingly much less only reaching a peak concentration of 85.18 mg/l on day 39.

Comparing the effects of temperature on CO₂ emissions

Aerobic sludge

The response of Aerobic sludge to the release of CO₂ under different temperatures is shown in Figure 4.5d. The trends under all temperatures is similar with 37°C showing the highest release concentrations which peaked on day 14 (245.93 g/l).

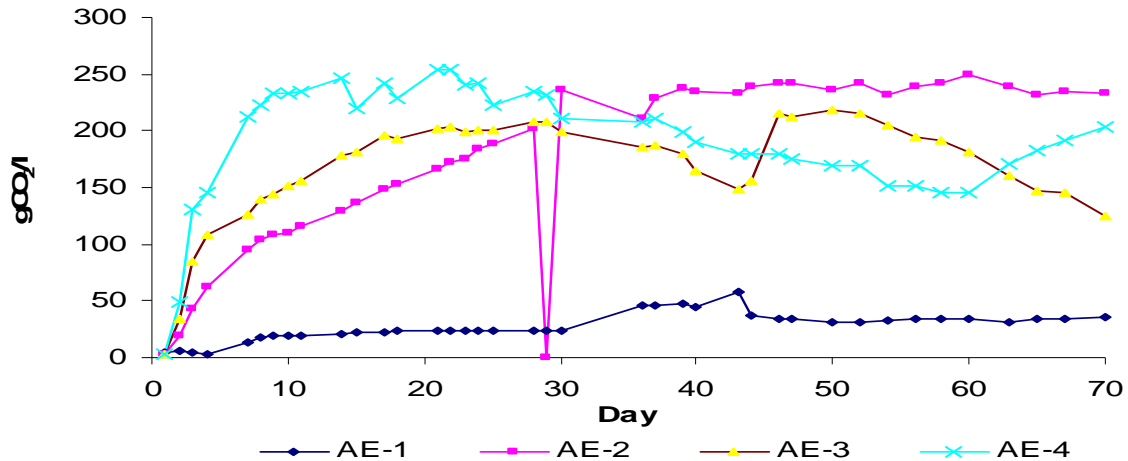


Figure 4.5d CO₂ emissions from aerobic sludge at different temperatures

As expected this is followed by release at 30°C, 20°C and finally the lowest emissions occurring under 4°C. Under 30°C, the maximum concentration of 202.99 g/l was reached on day 22 while in the case of 20°C and 4°C peak concentrations of 236.46 g/l and 57.72 g/l were reached on days 30 and 40 respectively.

Anaerobic sludge

The Anaerobic sludge response to CO₂ emissions under varying temperatures is depicted in Figure 4.5e. The emission patterns indicated quite similar patterns for all temperatures with 37°C showing the highest emissions concentrations reaching its maximum of 257.41 g/l in 14 days.

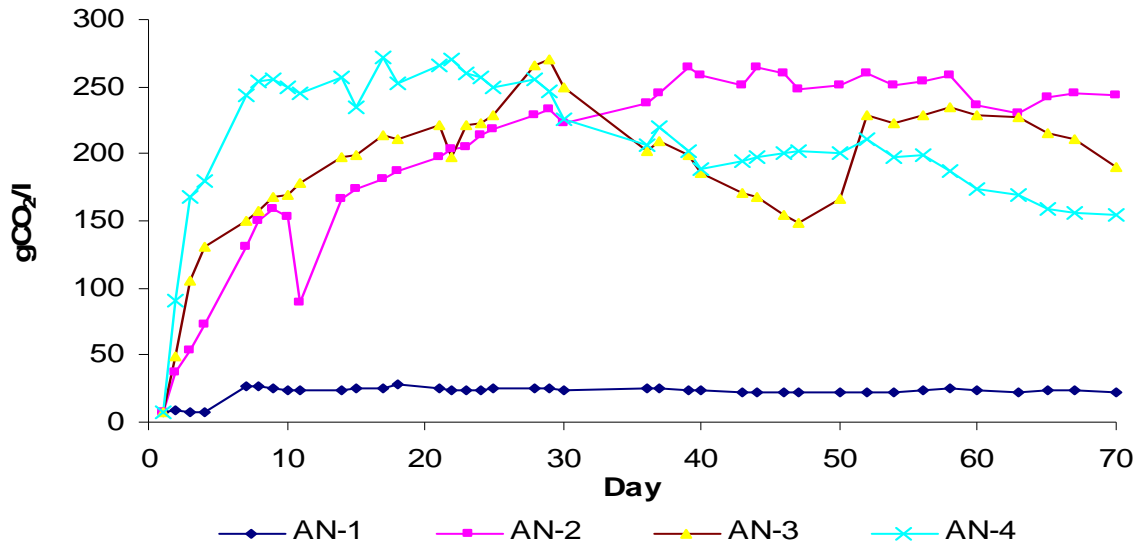


Figure 4.5e *CO₂ emissions from anaerobic sludge at different temperatures*

Under 30°C, the peak concentration of 269.90g/l was reached in 29 days while maximum concentrations of 260.13g/ and 28.40g/l were respectively reached for 20°C and 4°C in 44 and 18 days.

Denitrifying sludge

Figure 4.5f below indicates the effects of temperature on CO₂ releases from Denitrifying sludge. It is observed that the response trends for all temperatures are identical. As expected and observed in other sludge types, the higher temperatures recorded higher release concentrations than the lower temperatures. Thus, while the maximum concentrations of 235.38g/l and 207.44g/l were recorded at 37°C and 30°C in 21 and 29 days respectively, peak concentrations of 242.34g/l and 68.49g/l were correspondingly measured for 20°C and 4°C in 39 and 36 days respectively.

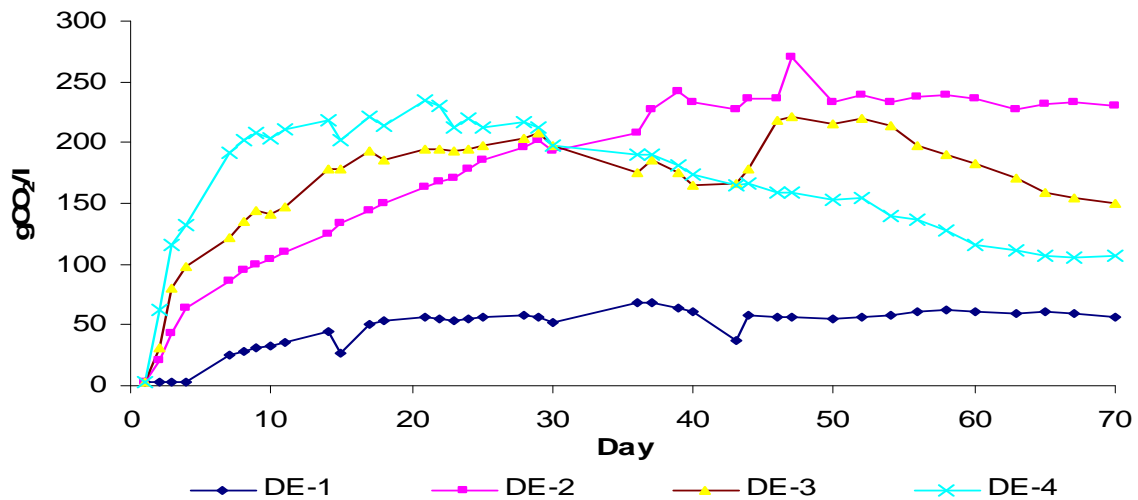


Figure 4.5f *CO₂ emissions from denitrifying sludge at different temperatures*

Comparing the Effects of Temperature on CH₄ emissions

Aerobic sludge

The response of CH₄ emissions from Aerobic sludge under different temperatures is presented in Figure 4.5g. The trends showed that 37°C recorded the most important CH₄ release reaching its highest emission concentrations of 730.6g/l in just 14 days.

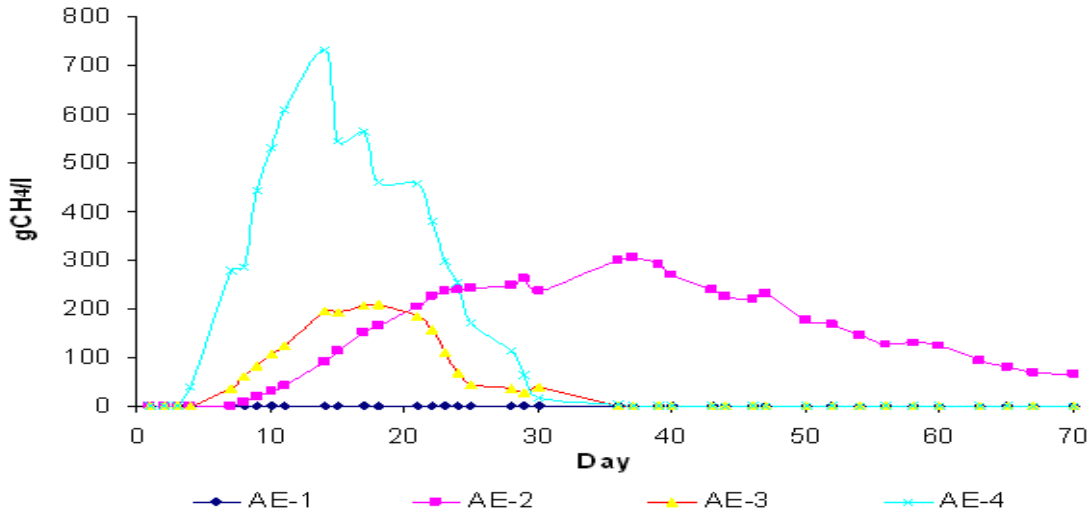


Figure 4.5g CH₄ emissions from aerobic sludge at different temperatures

Similarly, 30°C reached its peak concentration (205.8g/l) in 17 days with much less than peak concentration at 37°C. At 20°C, the process of CH₄ release was slower but nevertheless reaching its maximum concentration of 300.1g/l in 36 days. It is however worth mentioning that as expected, no CH₄ release was observed under 4°C.

Anaerobic sludge

Illustrated in Figure 4.5h is the CH₄ releases from Anaerobic sludge under different temperatures. The emission patterns under various temperatures were all similar to a varying degree. While the highest emissions occurred under 37°C, no observable releases were recorded under 4°C.

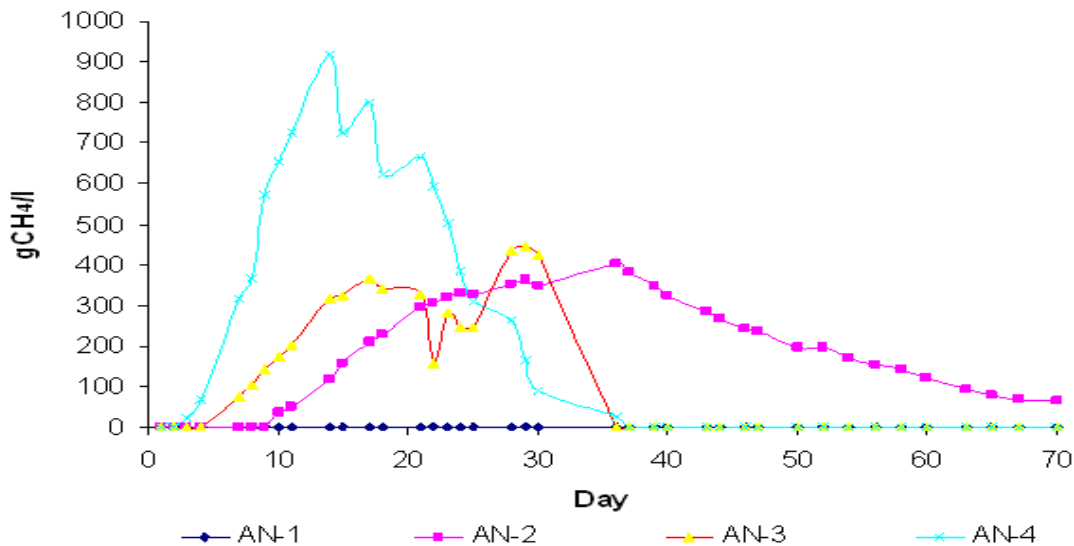


Figure 4.5h CH₄ emissions from anaerobic sludge at different temperatures

Under 37°C the highest concentration of 914.1g/l was attained in 14 days while the highest concentration of 466.5g/l for 30°C was reached in 29 days. It was observed that daily emission concentrations for 37°C were twice as high as daily emissions at 30°C. Conversely, at 20°C the maximum concentration of 401.6g/l was reached in 39 days with average daily concentration of about half those at 30°C

Denitrifying sludge

Figure 4.5i shows CH₄ emission patterns from Denitrifying sludge under different temperatures.

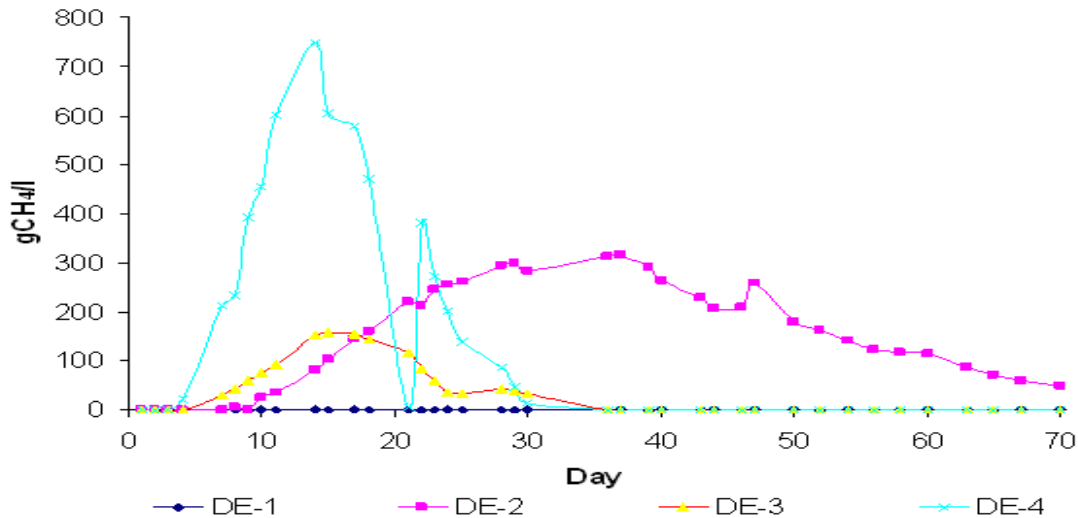


Figure 4.5i CH₄ emissions from denitrifying sludge at different temperatures

The highest emissions daily releases are associated with 37°C reaching its maximum concentration (747.0g/l) in 14 days. To a lesser extent the highest emission concentrations of 159.1g/l was reached for 30°C in 15 days. However the average daily concentrations at 30°C are less than half those recorded for 37°C. Initial lower daily emission concentrations were recorded at 20°C but reached a maximum concentration of 316g/l in 36 days. Thus the emission processes appeared initially slower at 20°C, it changed by the 21 day and recorded important release. It should be noted that no CH₄ emissions were recorded at 4°C and this was expected.

4.2 Effects of Nitrate on N₂O, CO₂ and CH₄ Emissions

This experiment was conducted under 30°C with an initial addition of 25ml acetate and 10ml of different concentrations of NO₃⁻ (15mg/l & 30mg/l respectively) to each reactor set and thereafter fortnightly addition of 10ml 15mg/l and 30mg/l NO₃⁻ respectively throughout the experimental period of 36 days.

N₂O

Figure 4.6a shows the effects of NO₃⁻ on N₂O emission patterns over the experimental period. Prior to incubation, 336.2mg/l, 261.5mg/l & 113.1mg/l were recorded respectively for the AE-15, AN-15 and DE-15 (reactors administered with 15mg/l NO₃⁻). Conversely, a corresponding 69.0mg/l, 132.4mg/l & 363.3mg/l N₂O concentrations were recorded for reactors AE-30, AN-30 and DE-30 (reactors administered with 30mg/l NO₃⁻).

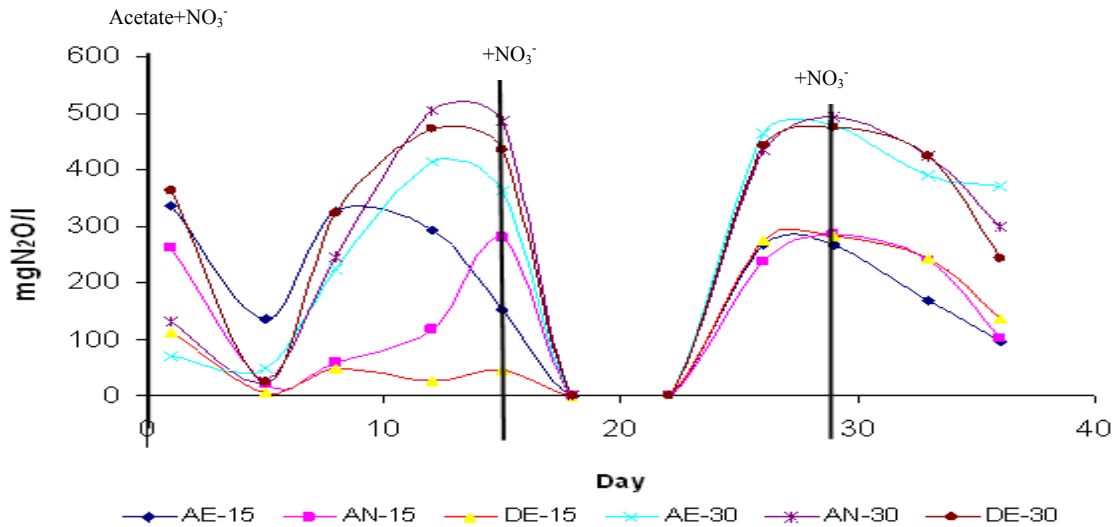


Figure 4.6a Effect of NO₃⁻ on N₂O emission

It was observed that though both reactor sets exhibited similar emission concentration patterns during the experimental period, there were much higher N₂O emissions associated with the higher NO₃⁻ (30mg/l) concentrations compared with the lower NO₃⁻ (15mg/l) concentrations. Surprisingly however, on addition of NO₃⁻ in all cases, N₂O responded with an initial drop before attaining a sharp increase in all except DE-15 which experienced a steady increase. By the fourth week the emission patterns in all reactors was so distinct with N₂O concentrations in AE-30, AN-30 & DE-30 almost doubled that for AE-15, AN-15 & DE-15. This suggests that increased NO₃⁻ concentrations increased N₂O emissions.

CO₂

The response of CO₂ emission to addition of NO₃⁻ at 30°C is depicted in Figure 4.6b. Generally, there are no distinct trends in CO₂ release patterns on addition of NO₃⁻. However, though emission concentrations for AN-30 & DE-30 were higher at the start of the experiment, by the fourth week emission concentrations for CO₂ in all reactors had almost levelled off from a low of 98.8g/l in AE-30 to a high of 143.9g/l in DE-15.

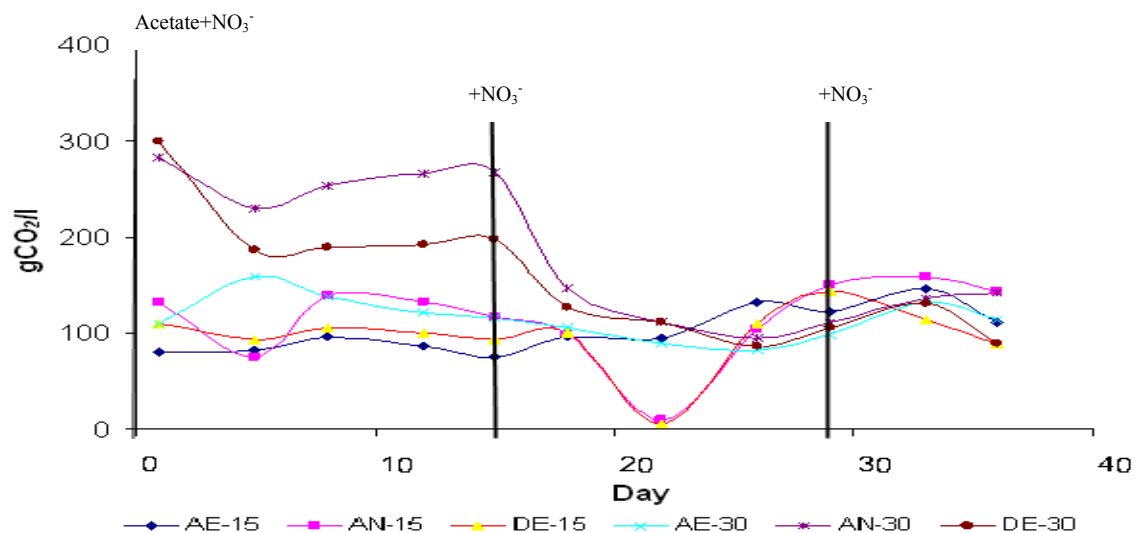


Figure 4.6b Effect of NO₃⁻ on CO₂ emission

CH₄

The influence of NO₃⁻ on CH₄ production is illustrated in Figure 4.6c. On addition of both acetate and NO₃⁻, CH₄ was not detected until after the second week when another dose of NO₃⁻ was added. Subsequently, a higher increase in CH₄ concentrations in AE-30, AN-30 and DE-30 was observed compared with AE-15, AN-15 and DE-15. Peak concentrations for AE-30, AN-30 and DE-30 (1424.6g/l, 1433.9g/l & 1290.2g/l) were almost double those for AE-15, AN-15 and DE-15 (768.9g/l, 779.7g/l & 496.5g/l) respectively) all reached between day 22 and 26. CH₄ concentrations then declined in all reactors for the rest of the experimental period.

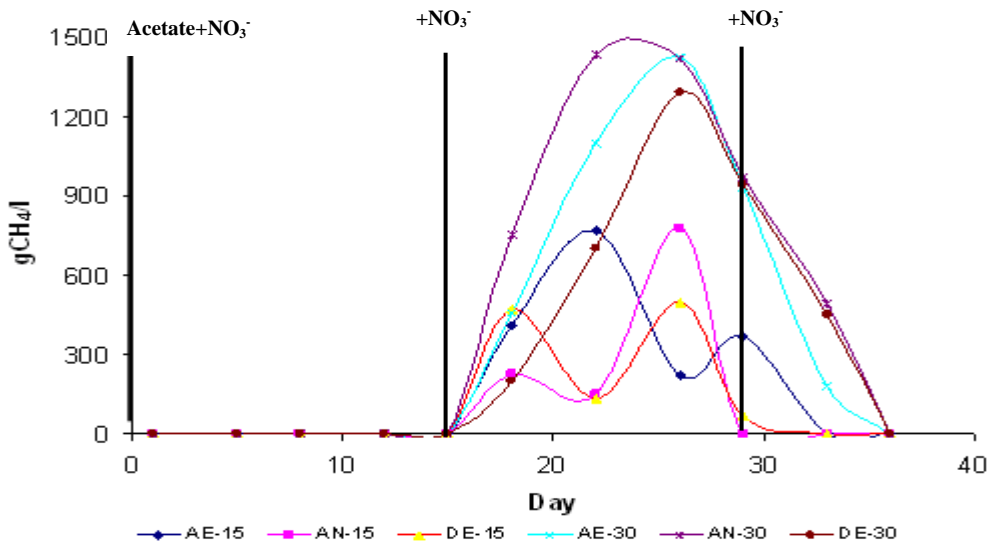


Figure 4.6c Effect of NO₃⁻ on CH₄ emission

While the decline may suggest consumption of CH₄ within the reactors, the concentration difference between reactors AE-30, AN-30 & DE-30 and AE-15, AN-15 & DE-15 may suggest that NO₃⁻ played a major role in CH₄ emissions.

Acetate, NH₄⁺, COD, NO₃-N and pH trends

The figures below indicate the influent and effluent acetate, NH₄⁺, COD NO₃-N concentrations and pH. They are presented as acetate (Figure 4.6d), NH₄⁺ (Figure 4.6e), COD (Figure 4.6f), NO₃-N (Figure 4.6h) concentrations and pH (Figure 4.6g). The trend in acetate showed over 99% utilisation of acetate from a high of 1389.99mg/l in the influent to a low of 0.22mg/l in the effluent.

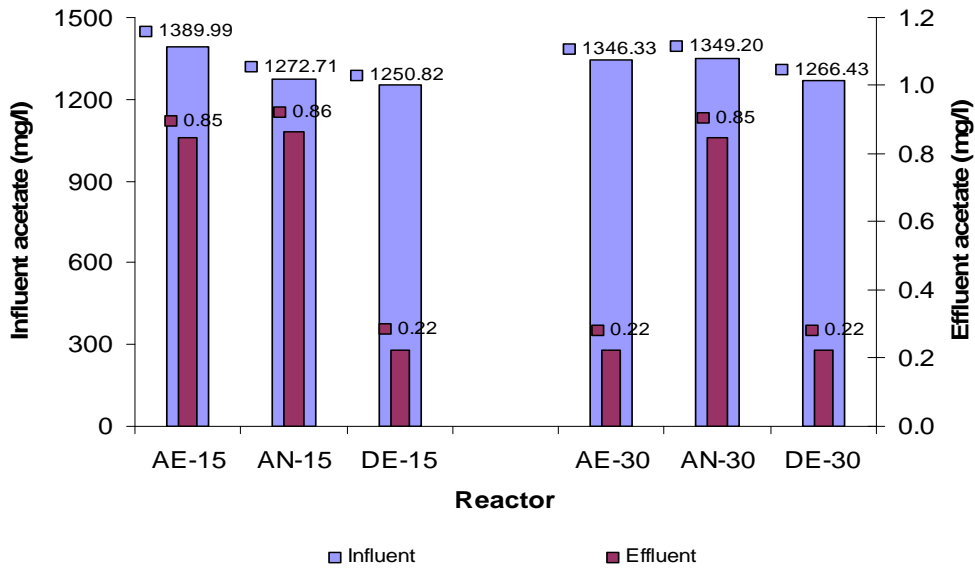


Figure 4.6d Acetate concentrations

A rise in NH_4^+ concentrations representing about 41.2%, 29.1% & 33.0% surge respectively for AE-15, AN-15 & DE-15 was recorded. Conversely, AE-30, AN-30 & DE-30 showed a corresponding percentage increase of 59.6%, 62.0% and 55.9% of NH_4^+ respectively.

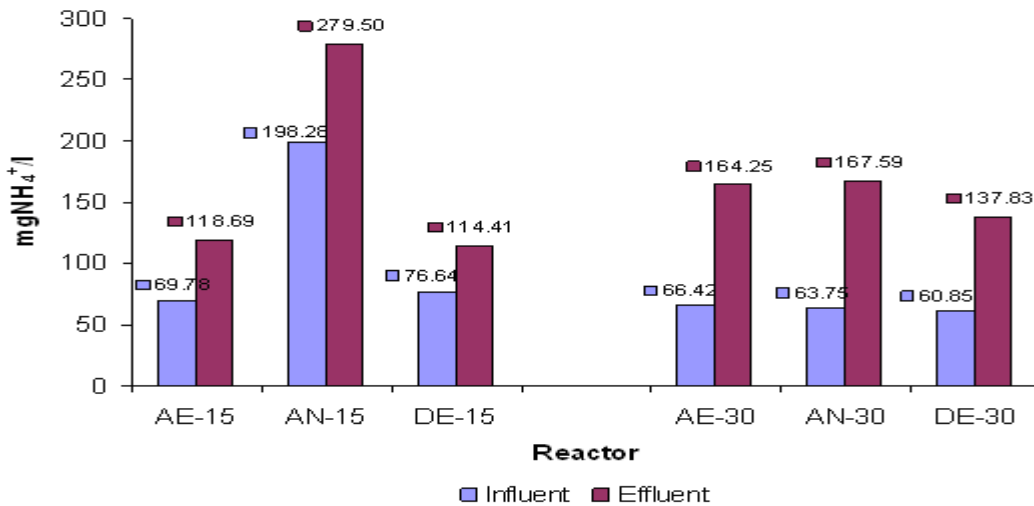


Figure 4.6e Ammonia concentrations

A large decline in COD that translated to an average of 68% reduction was computed with AE-30, AN-30 & DE-30 recording a slightly higher COD reduction

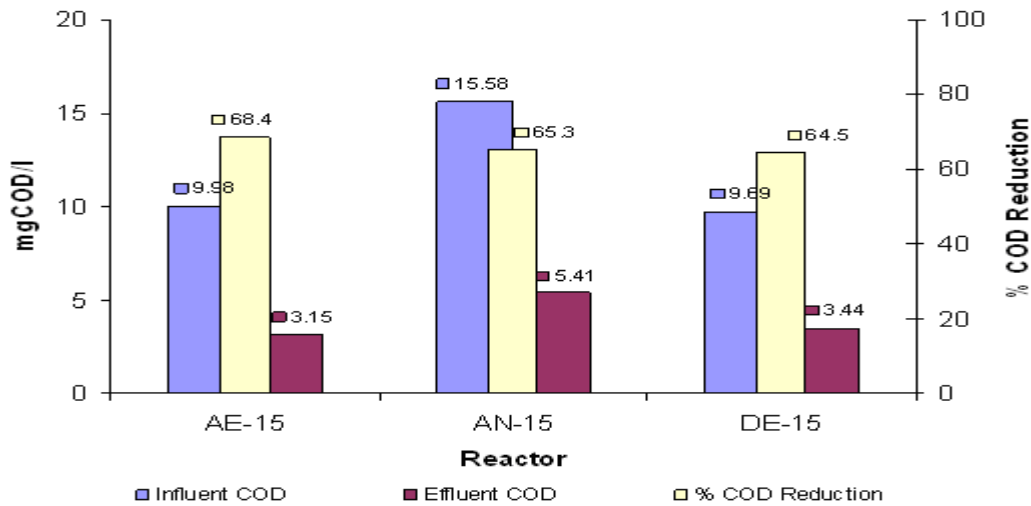


Figure 4.6f COD concentrations

The pH variation between influent and effluent ranged from a high of 8.3 in the influent to a low of 7.4 in the effluent (Figure 4.6g).

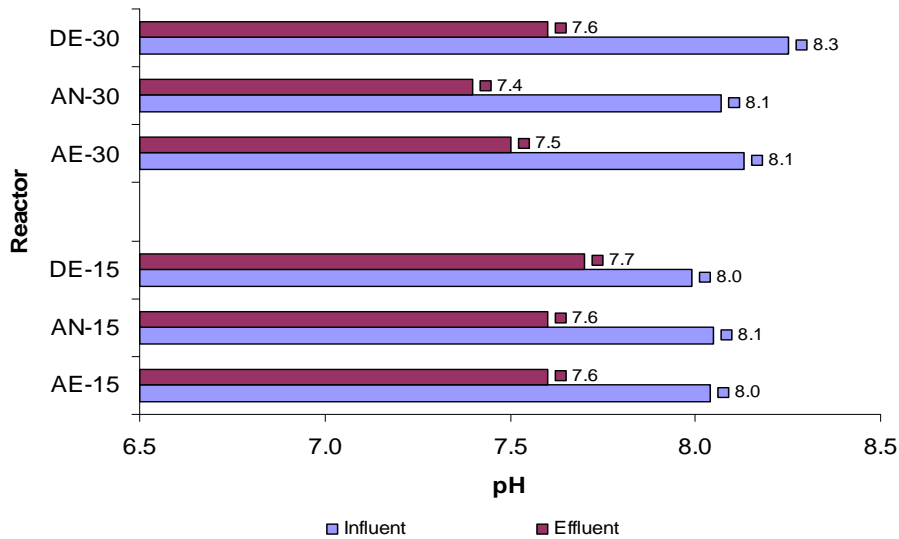


Figure 4.6g Influent & effluent pH

A general reduction in $\text{NO}_3\text{-N}$ was observed in the effluent of all the respective reactors (Figure 4.6h). Thus an average $\text{NO}_3\text{-N}$ reduction of 65% and 92% was computed for AE-15, AN-15 & DE-15 and AE-30, AN-30 and DE-30 respectively.

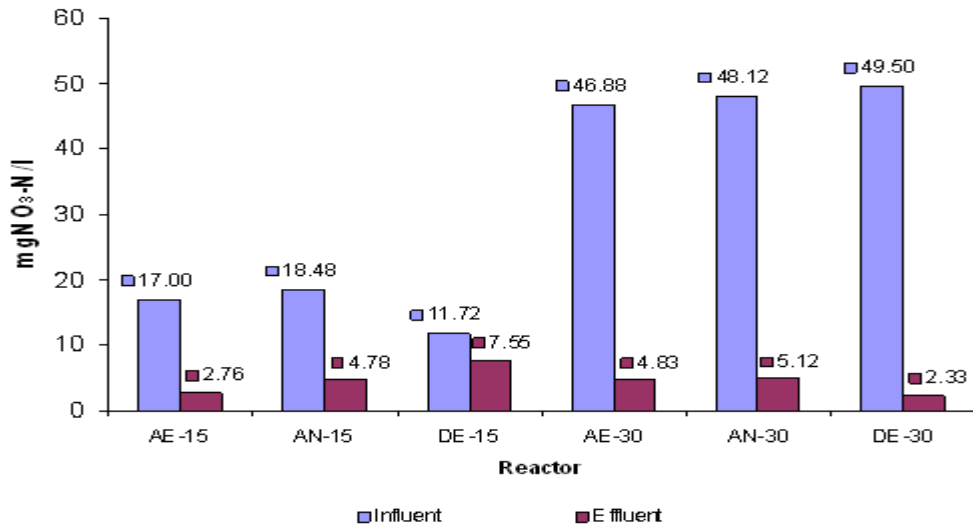


Figure 4.6h Nitrate concentrations

4.3 Effects of Ammonia on N₂O, CO₂ and CH₄ Emissions

N₂O

The effects of NH₄⁺ on N₂O release is presented in Figure 4.7a. AE-0, AN-0 & DE-0 contained no added NH₄⁺, while AE-NH₃, AN-NH₃ & DE-NH₃ were each treated with 30mg/l NH₄⁺ on day 13 and day 26. The trend analysis indicated that reactors AE-0 and AN-0 maintained a near 0 production with DE-0 showing an alternate rise and fall in N₂O release during the experimental period.

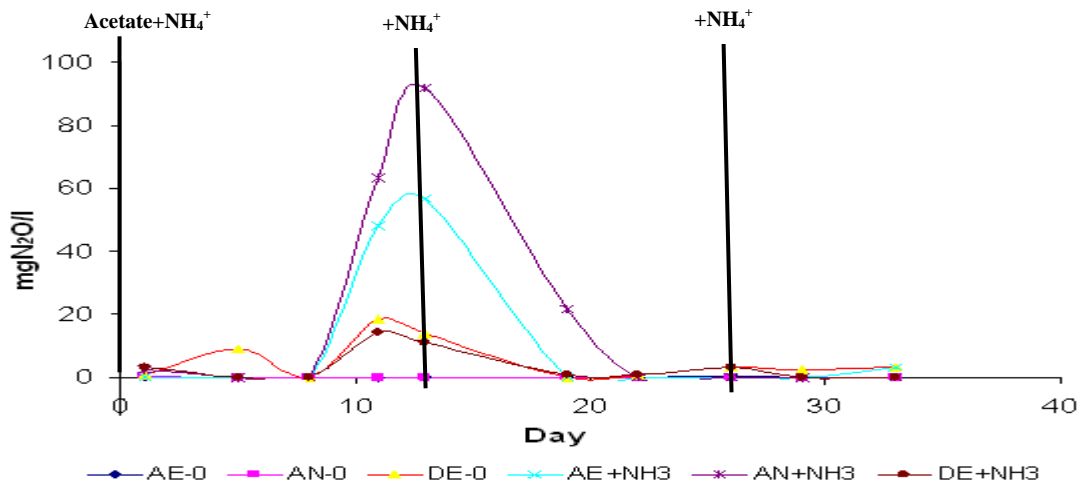


Figure 4.7a Effect of NH₃ on N₂O emission

AE-NH₃, AN-NH₃ & DE-NH₃ all recorded releases of N₂O with the maximum production occurring in AN-NH₃ and the least in DE-NH₃. It was however, observed that all peak N₂O measurements were recorded before addition of NH₄⁺ on day 13. A subsequent decrease in N₂O release in reactors AE-NH₃, AN-NH₃ & DE-NH₃ was observed though another dose of NH₄⁺ was added on day 26. This may imply that NH₄⁺ does not influence the release of N₂O in anoxic environment.

CO₂

Figure 4.7b illustrates the response of CO₂ emission to addition of NH₄⁺. As shown in the figure, there are no distinct trends in CO₂ release patterns on addition of NH₄⁺.

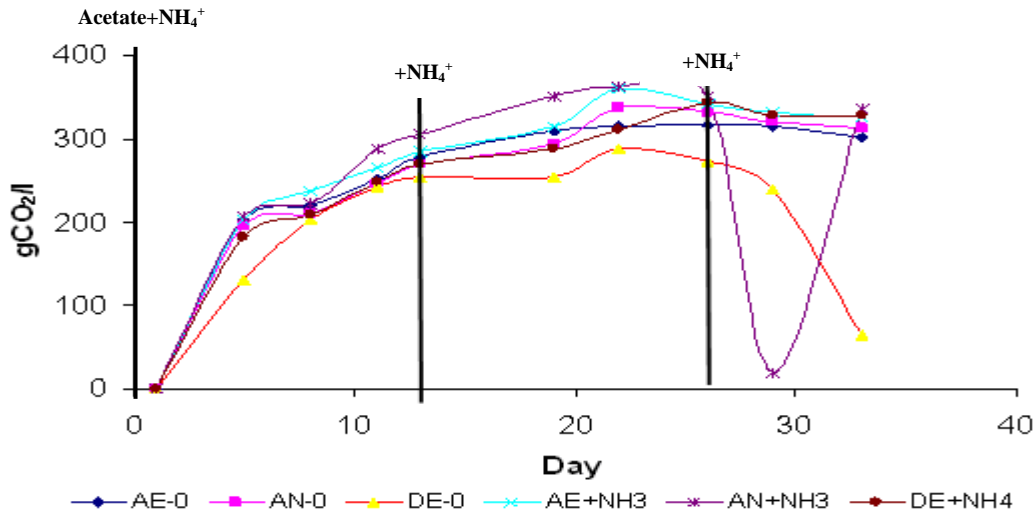


Figure 4.7b Effect of NH₃ on CO₂ emission

All reactors responded in the same way irrespective of NH₄⁺ treatment or not with CO₂ release in all reactors increasing steadily until day 21 when they remained flat for the rest of the experimental period. This probably is an indication that NH₄⁺ has no direct role on the release of CO₂.

CH₄

As shown in Figure 4.7c, CH₄ release patterns in all reactors were identical irrespective of addition of NH₄⁺ in reactors AE-NH₃, AN-NH₃ & DE-NH₃. CH₄ started after day 5 and rose steadily in all reactors in spite of the addition of NH₄⁺ in some reactors.

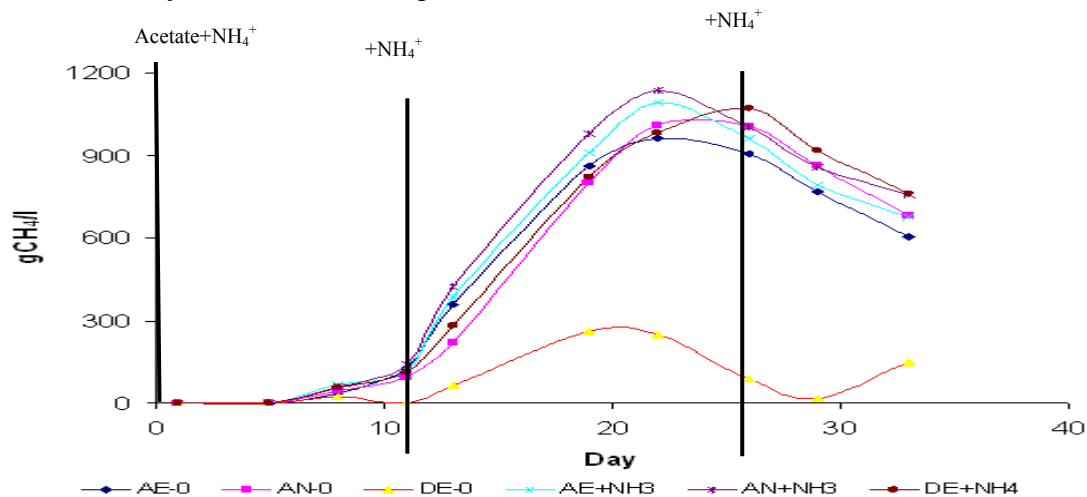


Figure 4.7c Effect of NH₃ on CH₄ emission

Peak concentrations were measured on day 22 in all reactors except DE-NH₃ which peaked on day 26. CH₄ concentrations recorded ranged from a low of 269.6g/l in DE-0 to a high of 1136.6g/l in AN-NH₃. It was however generally noted that CH₄

concentrations measured for AE-0, AN-0 & DE-0 were slightly lower than those measured for AE-NH₃, AN-NH₃ & DE-NH₃.

Acetate, NH₄⁺, COD, NO₃-N and pH trends

Figure 4.7d, Figure 4.7e, Figure 4.7f, Figure 4.7h and Figure 4.7g illustrate the trends in influent and effluent acetate, NH₄⁺, COD, NO₃-N concentrations and pH respectively for all reactors.

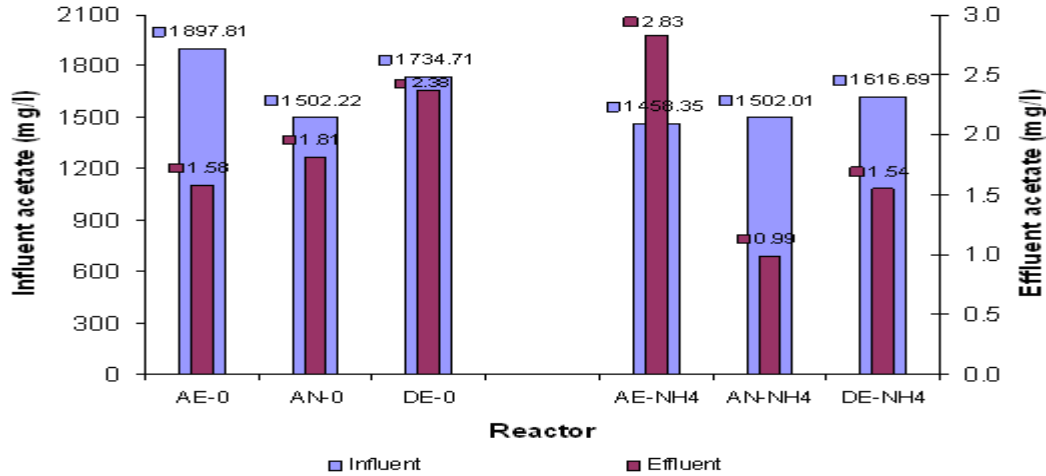


Figure 4.7d Acetate concentrations

All measurements showed over 99% decreases in acetate (Figure 4.7d). It recorded NH₄⁺ build up averaging 66% for NH₄⁺ treated reactors and 70% reactors without NH₄⁺ (Figure 4.7e).

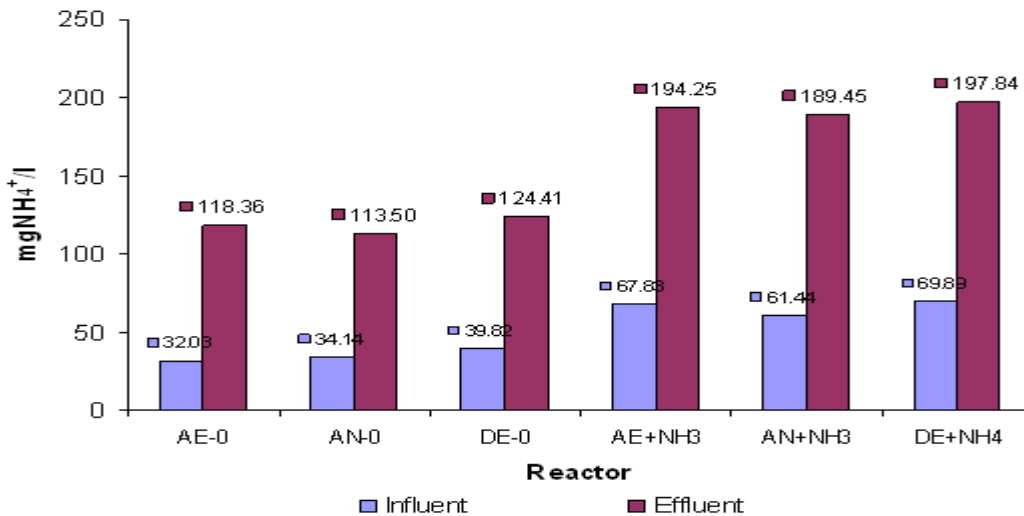


Figure 4.7e Ammonia concentrations

As indicated in Figure 4.7f over 68% in COD reduction was recorded in all reactors treated with NH₄⁺, while about 65% reduction occurred in the reactors without NH₄⁺ treatments.

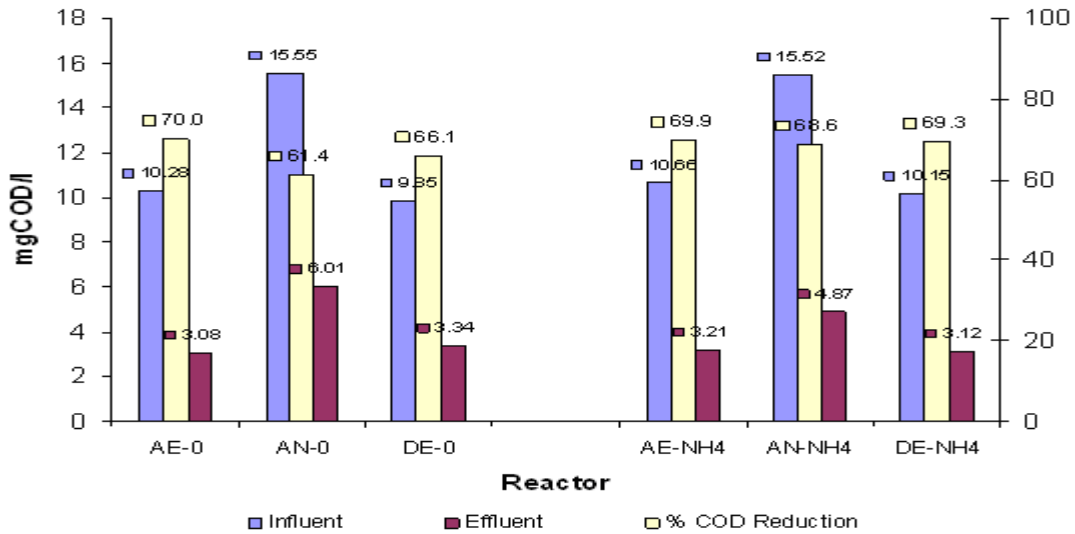


Figure 4.7f COD concentrations

An expected decrease in $\text{NO}_3\text{-N}$ was observed (Figure 4.7h) in all reactors. This ranged from 4.2% in AN-0 to 60.2% in DE-0 with AE-0 recording 10.5%. Similarly, the NH_4^+ treated reactors also recorded relatively reduced $\text{NO}_3\text{-N}$ values ranging from a high of 25.1% in AN- NH_3 to a low of 7.1% in DE- NH_3 with AE- NH_3 recording 10.4%.

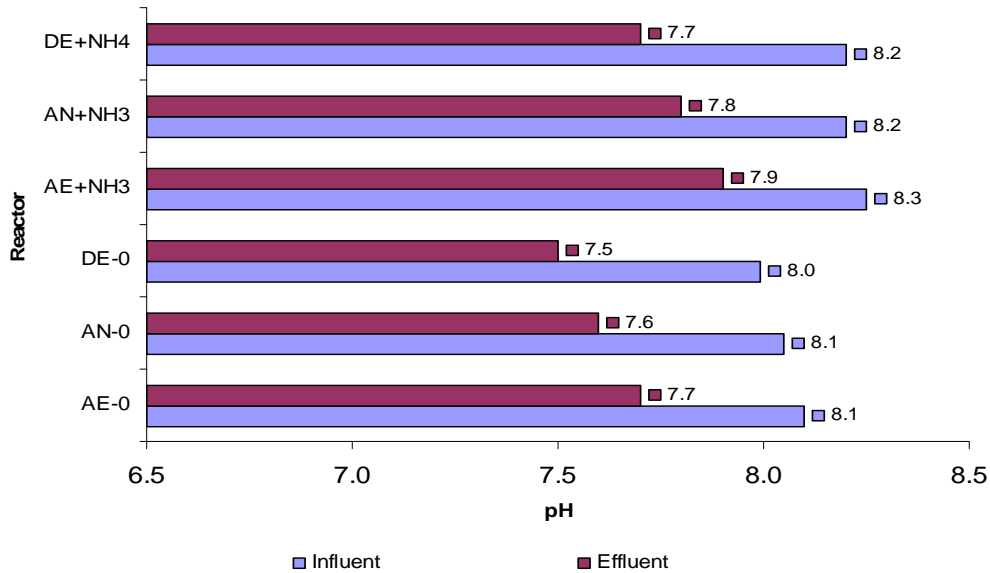


Figure 4.7g pH in influent & effluent

The pH fluctuations ranging from $7.5 < \text{pH} < 8.1$ in influent and effluent was also noted over the experimental period in all reactors (Figure 4.7g).

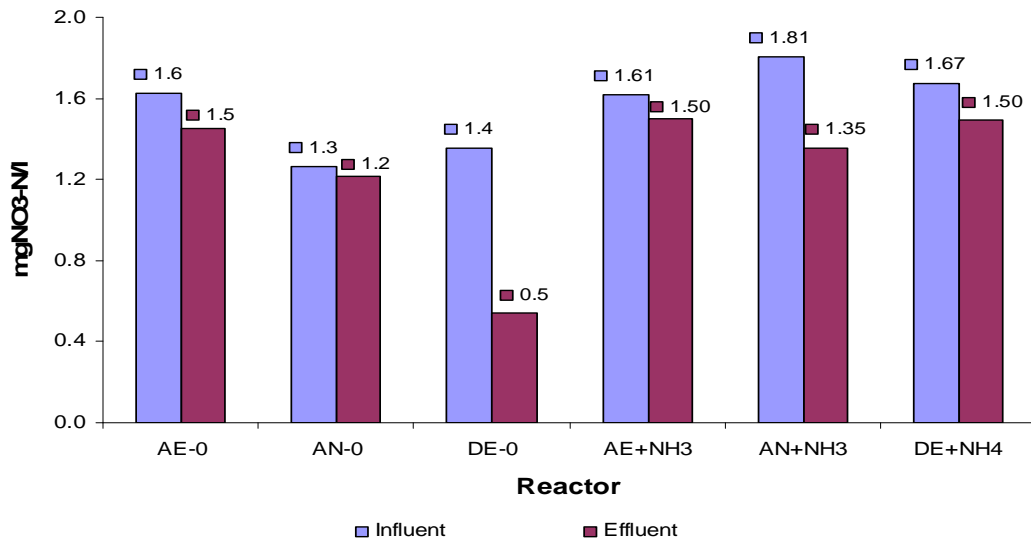


Figure 4.7h Nitrate concentrations in influent & effluent

4.4 Effects of shaking on N₂O, CO₂ and CH₄ Emissions

N₂O

Figures 4.8a & 4.8b illustrate the response of N₂O release to shaking. The trends in Figure 4.8a indicated that with the exception of DE-S which showed a sharp increase in N₂O after day 13 with peak concentration of 431.8mg/l, there was generally no release of N₂O in both AE-S & AN-S.

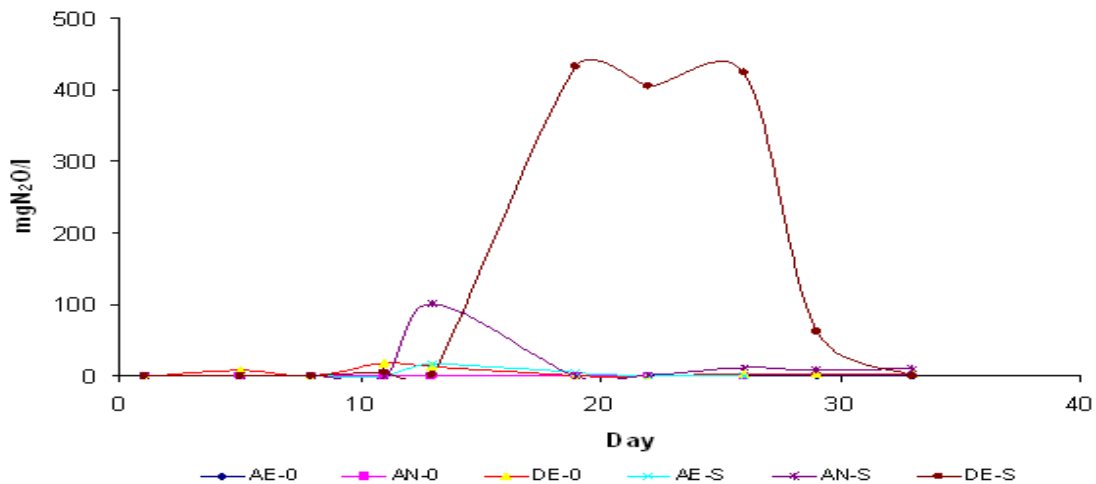


Figure 4.8a Effect of shaking on N₂O release

To a lesser extent AN-S reached a maximum concentration of 100.9mg/l on day 13 and thereafter fell to 0mg/l for the rest of the experimental period. N₂O response to non-shaking is similar in pattern without release of N₂O. It can generally be inferred that shaking has no direct effect on N₂O release. However, in the reactors with added NH₄⁺ as shown in Figure 4.8b, shaking indicated a possible influence on N₂O release. N₂O releases in the shaker setup AE-NH₃-S, AN-NH₃-S & DE-NH₃-S showed lower concentrations than those reactors (AE-NH₃, AN-NH₃ & DE-NH₃) that were not shaken.

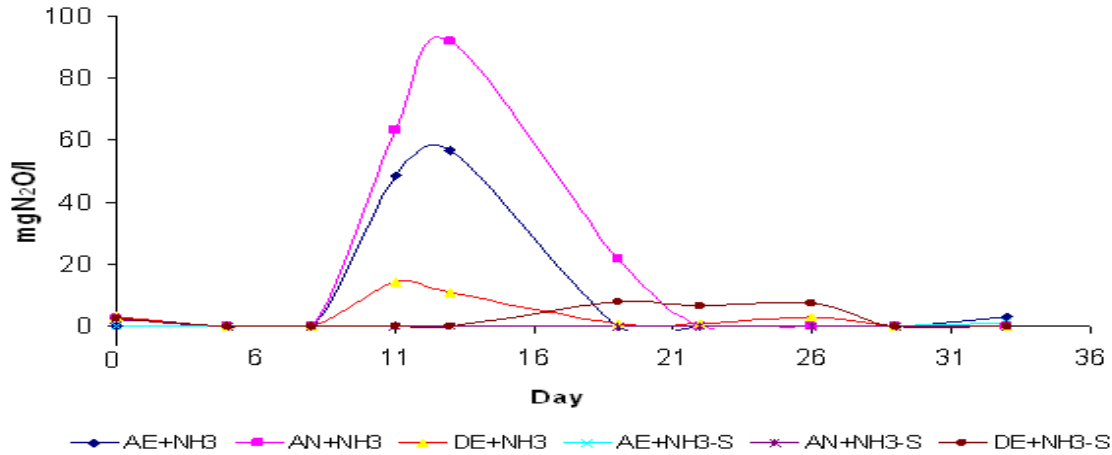


Figure 4.8b Effect of NH_4^+ & shaking on N_2O release

Thus while reactors AE-NH₃, AN-NH₃ & DE-NH₃ attained respective peak concentrations of 56.8mg/l, 91.8mg/l & 14.3mg/l, their counterparts under the shaker maintained a near 0mg/l release of N₂O except in DE-NH₃-S which attained peak N₂O concentration of only 7.5mg/l on day 19. This variation in concentrations between shaker setup and non-shaken could be a consequence of higher solubility of N₂O in the liquid phase linked to shaking.

CO₂

Depicted in Figure 4.8c is the response of CO₂ release to shaking. The trends showed a potential effect of shaking on CO₂ release.

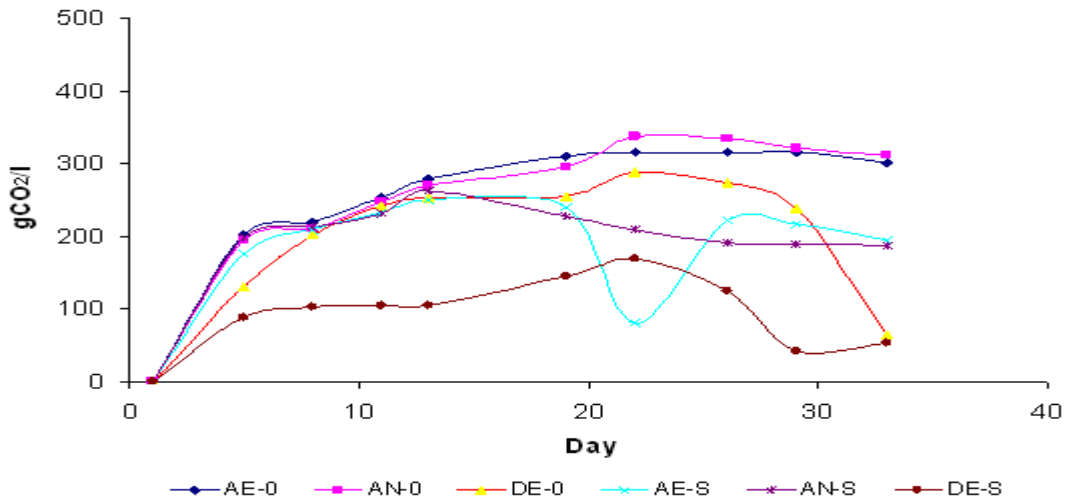


Figure 4.8c Effect of shaking on CO₂ release

Respective CO₂ maximum concentrations of 239.6g/l, 209.6g/l & 169.1g/l in reactors AE-S, AN-S & DE-S indicated shaking releases less CO₂ than the non-shaken reactors AE-0, AN-0 & DE-0 which recorded corresponding peak concentrations of 314.2g/l, 337.1g/l & 287.4g/l. Thus the corresponding lower concentrations recorded for reactors under shaker could be attributed to a possible higher solubility of CO₂ in the liquid phase as a result of shaking.

CH₄

The response of CH₄ release to shaking is depicted in Figure 4.8d. The trend patterns showed a possible influence of shaking on CH₄ release. Respective CH₄ concentrations in reactors AE-S, AN-S & DE-S indicated shaking releases less CH₄ than reactors AE-0, AN-0 & DE-0 which were not shaken.

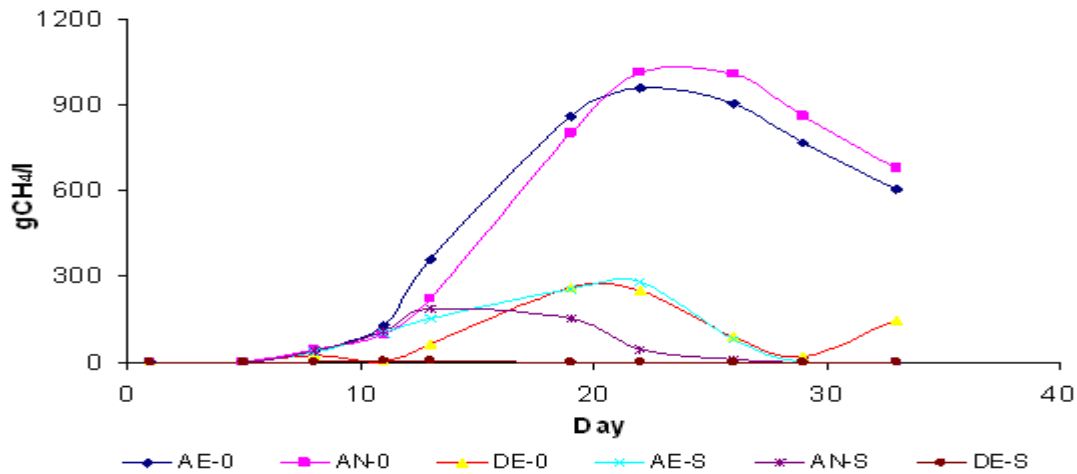


Figure 4.8d Effect of shaking on CH₄ emission

Maximum concentration values for AE-0, AN-0 & DE-0 ranged from a low of 261.7g/l in DE-0 to a high of 1012.1g/l in AN-0 with AE-0 recording 960.6g/l. Contrarily, reactors under shaker recorded lower release concentrations with peaks ranging from a low of 0 in DE-S to a high of 279.0g/l in AE-S with AN-S measuring 187.7g/l. This difference in concentrations between shaker setup and non-shaken could probably be linked to higher solubility of CH₄ in the liquid phase due to shaking.

Acetate, NH₄⁺, COD, NO₃-N and pH trends

Figure 4.8e, Figure 4.8f, Figure 4.8g, Figure 4.8i and Figure 4.8h illustrate the trends in influent and effluent acetate, NH₄⁺, COD, NO₃-N concentrations and pH respectively for all reactors

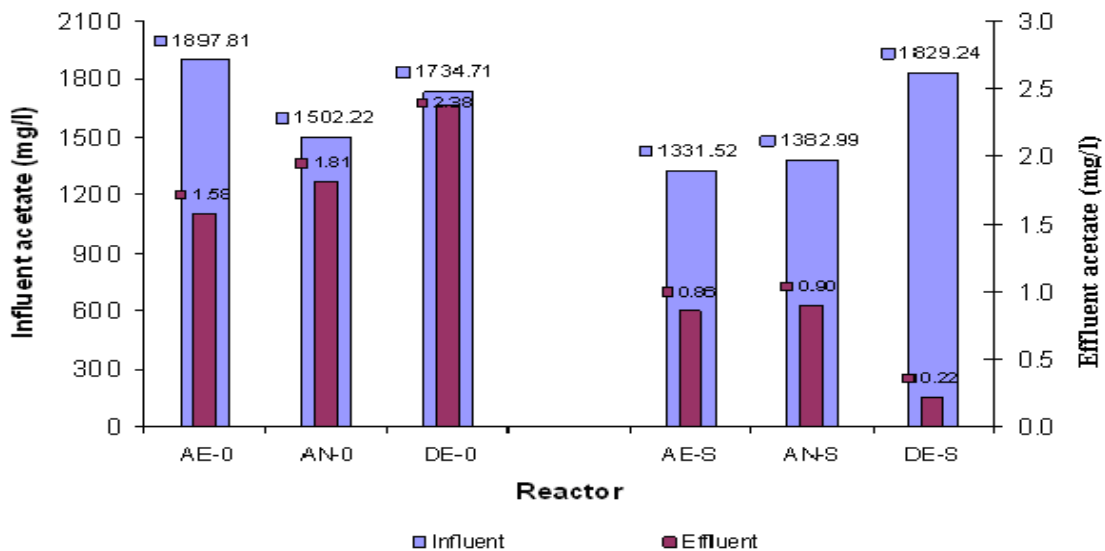


Figure 4.8e Acetate concentrations

The data showed more than 99% utilisation of acetate (Figure 4.8e). An average of 70% build-up in NH_4^+ was recorded for non-shaker reactors with AE-0 measuring the highest of 72.9% and DE-0 the lowest (68%). Conversely, an average of NH_4^+ increase of 56% was recorded for reactors that were shaken with reactor AN-S recording the highest increase of 64.2% and AE-S the lowest (42.4%) (Figure 4.8f).

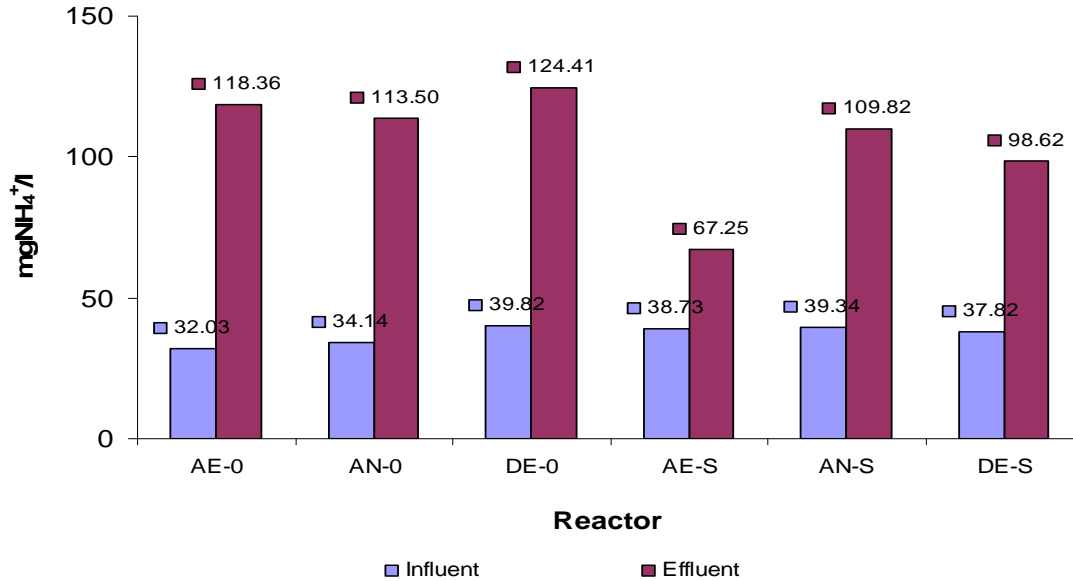


Figure 4.8f NH_4^+ concentrations

As indicated in Figure 4.8g an average of 68% in COD reduction was recorded in all reactors that were shaken with AE-S recording the highest percentage (71.1%) while AN-S and DE-S recorded 65.7% & 65.8% respectively. On the other hand an average of 65% reduction occurred in the reactors that were not shaken with the highest recorded by AE-0 (70%) and AN-0 and DE-0 recording a respective 61.4% & 66.1%..

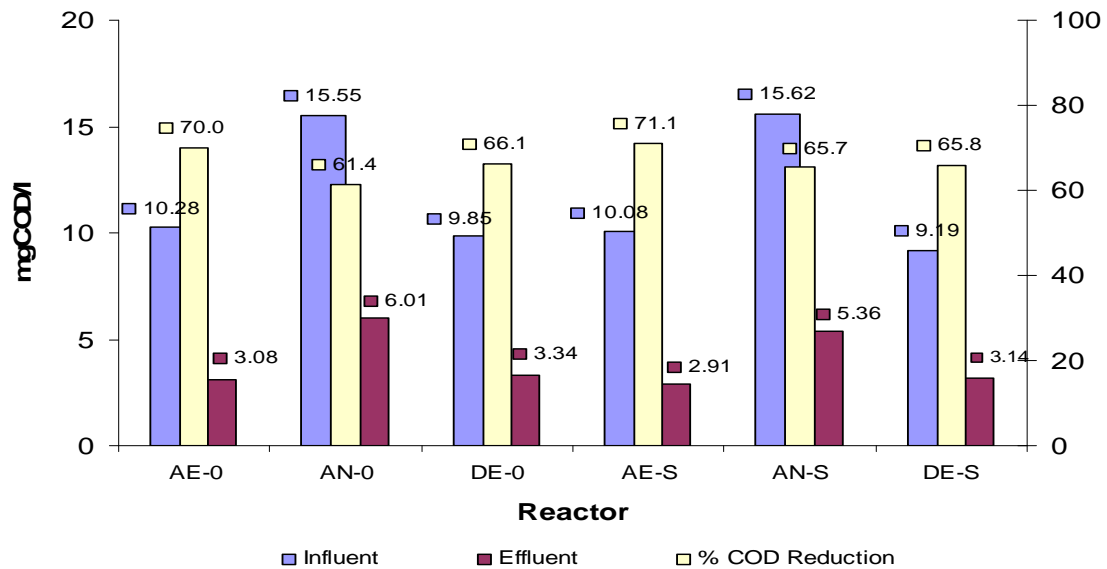


Figure 4.8g COD concentrations

As expected, a decrease in $\text{NO}_3\text{-N}$ was observed (Figure 4.8i) in all reactors. The reduction ranged from 4.2% in AN-0 to 60.2% in DE-0 with AE-0 recording 10.5%. Similarly, the reactors on the shaker also recorded relatively reduced reductions in $\text{NO}_3\text{-N}$ values ranging from a high of 87.4% in AN-S to a low of 2.4% in AE-S with DE-S recording 8.4%.

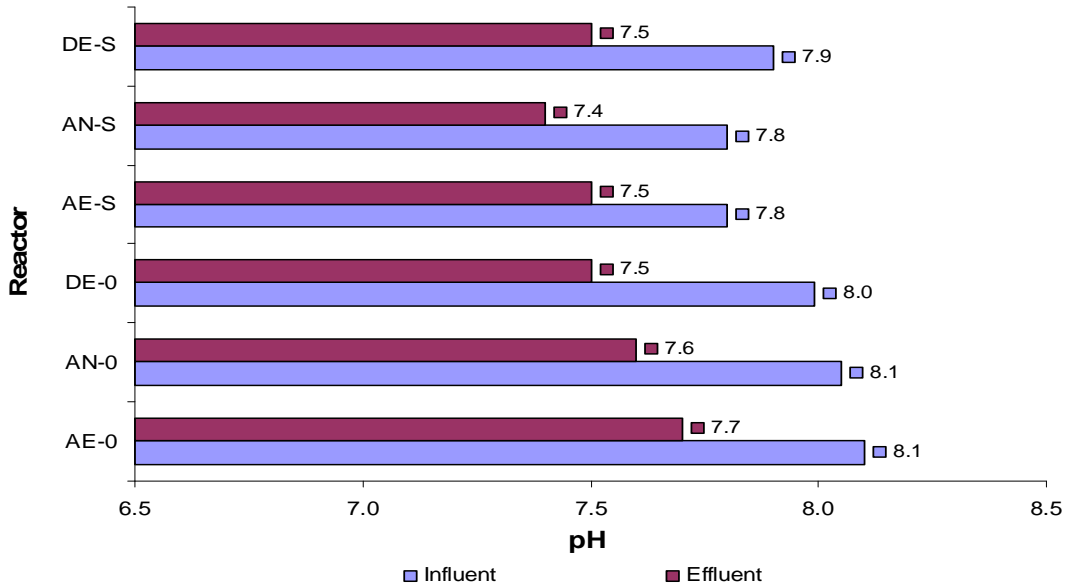


Figure 4.8h pH of Influent & effluent

In both shaker and non-shaker reactors, the pH variations ranged from $7.4 < \text{pH} < 8.1$ in effluent and influent over the experimental period (Figure 4.8h).

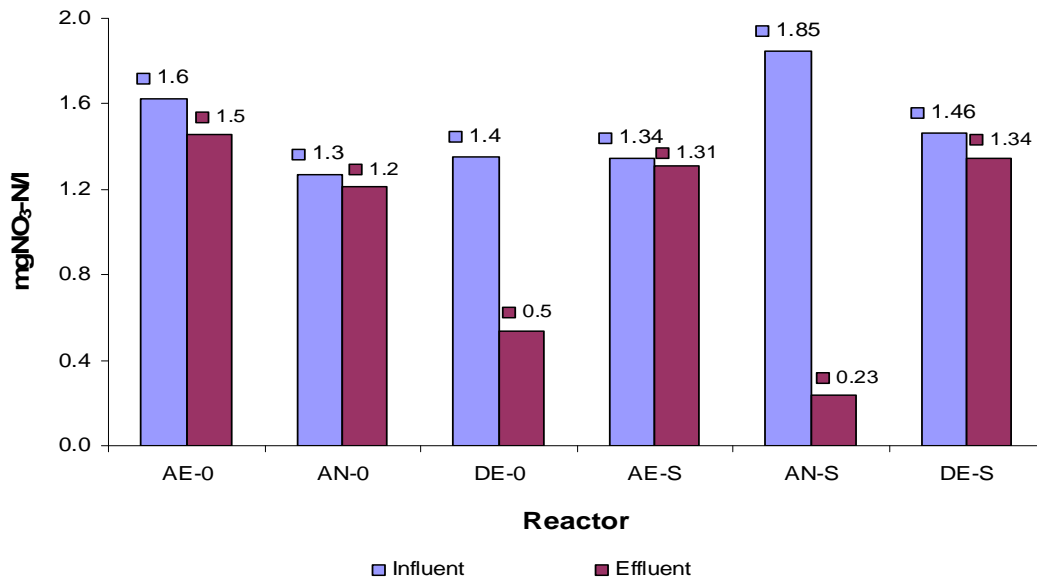


Figure 4.8i Nitrate concentrations

CHAPTER FIVE

5.0 DISCUSSION

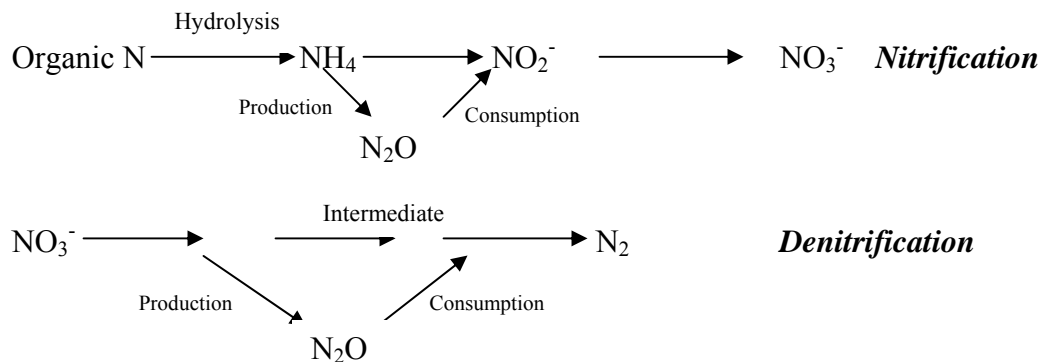
5.1 Effects of temperature on N₂O, CO₂ and CH₄ release

According to Liikanen et al., (2002), temperature and oxygen are among the most important factors regulating microbiological processes which result in greenhouse gases production and nutrient release.

The effect of temperature as indicated in *Figures 4.1a-c to 4.4a-c* shows a strong influence on the release of N₂O, CO₂ and CH₄. The experimental results established that higher temperatures (30°C & 37°C) released higher amounts of N₂O, CO₂ and CH₄ while lower temperatures (4°C & 20°C) recorded smaller emissions. This suggests that the hydrolysis and subsequent fermentation of organic matter increases with increasing temperatures. This observation is consistent with the explanation offered by Mata-Alvarez (2003) who affirmed that, from both thermodynamic and kinetic points of view, temperature influences anaerobic processes.

N₂O

N₂O is not an end product in itself, but an intermediate that builds up only when its production exceeds its consumption in both the nitrification and denitrification pathways. The relation is described below and may be influenced by temperature, O₂ or other factors. N₂O emissions under 37°C, 30°C, 20°C & 4°C as depicted in *Figures 4.1a, 4.2a, 4.3a & 4.4a* respectively showed that higher releases were associated more with 37°C & 30°C than with 20°C & 4°C. Earlier research by Stadmark and Leonardson (2007) also found that higher N₂O production occurred at higher temperatures in pond sediment. It was however noted that in this study, 30°C rather than 37°C was the favourable temperature for N₂O production.



Further more though dissolved oxygen (DO) was not monitored, N₂O releases under the various temperatures within the scope of this research was experimented in anoxic conditions and was found to be substantially higher at higher temperatures. This behaviour under anoxic conditions is a confirmation by Tallec et al., (2006) who noted that N₂O generation in municipal wastewater treatment facilities is usually insignificant except when the DO is positive but low (generally less than 1 mg/l).

N₂O response to NO₃⁻ addition was particularly interesting. As illustrated in *Figure 4.6a*, N₂O response at 30°C declined each time, with NO₃⁻ treatment and rose steadily a few days later. This may be attributed to the presence of other substances particularly H₂S which could inhibit complete heterotrophic denitrification leading to accumulation of N₂O hence the observed build up as shown in the process below.



This position is supported by Manconia et al., (2006) who opined that, in the presence of sulphide, heterotrophic denitrification was incomplete, leading to N₂O accumulation instead of N₂ and also corroborated by Sørensen et al., (1980) who further asserted that specific action of sulphide caused a partial inhibition of NO reduction and a strong inhibition of N₂O reduction in denitrifying cells. Though, H₂S was not monitored in this research, the characteristic odour released from the reactors implicated its emission. Additionally, from *Figure 4.6a* it was observed that N₂O release was directly related to NO₃⁻ concentrations. Thus the emission concentration with 30mg/l treatment of NO₃⁻ was twice as high as the concentrations for 15mg/l treatments. This underscored the positive influence of NO₃⁻ on N₂O emissions as reported by Stadmark and Leonardson (2007) that increased NO₃⁻ concentrations had strong positive impact on the N₂O concentration.

The effects of NH₄⁺ on N₂O release as indicated in *Figure 4.7a* shows that NH₄⁺ treated reactors all recorded releases of N₂O. It was however, noted that all peak N₂O measurements were recorded before addition of NH₄⁺. A subsequent decrease in N₂O release was observed though another dose of NH₄⁺ was added. This may imply that NH₄⁺ does not influence the release of N₂O in anoxic environment. The anoxic conditions under which these experiment were conducted did not favour nitrification (NO₃⁻ production): a position supported by Liikanen and Martikainen (2003) whose work on CH₄ and N₂O fluxes in a eutrophic lake concluded that though higher concentrations of NH₄⁺ led to N₂O release, oxygen rather than NH₄⁺ was the key factor regulating the production of NO₃⁻ which enabled denitrification and production of N₂O.

Figure 4.8a indicates the effect of shaking on N₂O release. It can generally be inferred that shaking has no direct effect on N₂O release. However, in NH₄⁺ (30mg/l) treated reactors (*Figure 4.8b*), shaking indicated a considerable influence on N₂O recording lower emissions than those reactors that were not shaken. The relatively lower concentrations observed in the shaker setup could be attributed to increased solubility of N₂O in the liquid phase due to stirring thus making it less available in the gas phase. Furthermore, shaking could enhance the contact time between the gas and the liquid and thus speed up the amounts of gas that is retained in the liquid. It could also be attributed to the low agitation associated with low shaking speed (100rpm) under which the experiment was conducted since vigorous agitation was expected to free the gas from the liquid phase.

CO₂

The trend analyses for CO₂ are illustrated in *Figures 4.1b, 4.2b, 4.3b & 4.4b*. The observable patterns clearly point to strong influent of temperature in CO₂ releases. Stadmark and Leonardson (2007) reported higher net production of CO₂ at higher temperatures which confirms the findings of this research.

Conversely, no observable effects of increased NO_3^- concentrations were noted in CO_2 production as depicted in *Figure 4.6b*. Thus emission trends after NO_3^- treatment were observed to be relatively the same. The lack of response in CO_2 emissions could be attributed to the use of alternative oxidants as electron acceptors (Stadmark and Leonardson, 2007).

As shown in *Figure 4.7b*, there are no distinct trends in CO_2 release patterns when treated with NH_4^+ . There were similar CO_2 response patterns for all reactors irrespective of NH_4^+ treatment or not. This probably is an indication that NH_4^+ has no direct role on the release of CO_2 . It could therefore be deduced that though the carbon and nitrogen cycles occur simultaneously in the environment, they do not directly influence each other.

Figure 4.8c indicates shaking releases relatively less CO_2 than the non-shaken reactors. This could be due to a probable higher solubility of CO_2 in the liquid phase as a result of shaking thus making it less available in the gas phase.

CH₄

CH_4 response to temperatures, NO_3^- and NH_4^+ treatment and shaking are shown in *Figures 4.1c, 4.2c, 4.3c & 4.4c* (temperature), *4.6c* (NO_3^-), *4.7c* (NH_4^+) and *4.8d* (shaking).

According to Søvik et al, (2006), temperature, substrate supply, and degree of oxidation are the principal controls on CH_4 fluxes from all soils and wetlands. In this study, the temperature relationship indicated higher CH_4 emissions as temperature increases. It was therefore not surprising that no CH_4 releases were observed under 4°C . Most of the CH_4 was emitted were released within three weeks except at 20°C where emissions lasted for over six weeks. This could be due to the combined effects of temperature and substrate availability during the experiment. Thus the most important temperatures for CH_4 emissions were 37°C and 30°C . Methanogenic processes release CH_4 at optimum temperature of 37°C . It was therefore expected that higher CH_4 emissions should be associated with this temperature. This trend is in agreement with other research findings (IPCC, 2007) which reported that with increases in temperature, the rate of CH_4 production also increases thus making warmer climates more prone to CH_4 emissions than in temperate zones. The findings in this report further supported the IPCC (2007) and US EPA (2006) affirmation that below 15°C , significant CH_4 production is unlikely because methanogens are not active.

The effect of NO_3^- treatment on CH_4 emissions as depicted in *Figure 4.6c* pointed to a probable positive influence of NO_3^- on CH_4 releases. The detection of CH_4 only after a second dose of NO_3^- was surprising but nonetheless noticeable. Even more unexpected was the concentration difference which recorded higher concentrations (1424.6g/l, 1433.9g/l & 1290.2g/l respectively) for 30mg/l NO_3^- treatments. It is worth noting that these concentrations were almost double those for 15mg/l NO_3^- (768.9g/l, 779.7g/l & 496.5g/l respectively) treatments. While the later decline in CH_4 release may suggest consumption within the reactors, the obvious emission concentrations differences between 30mg/l treatments and 15mg/l treatments may imply NO_3^- played a major role in CH_4 emissions. This finding is contrary to Stadmark and Leonardson (2007) who reported no effect of NO_3^- on CH_4 releases.

As shown in *Figure 4.7c*, CH₄ release patterns in all reactors were identical irrespective of addition of NH₄⁺ in reactors. CH₄ concentrations recorded ranged between 269.6g/l and 1136.6g/l. It was however generally noted that though CH₄ concentrations measured for non-NH₄⁺ treatment were slightly lower than those measured for NH₄⁺ treatments, there was no clear cut distinction to implicate a positive influence of NH₄⁺ on CH₄ releases.

The effect of shaking on CH₄ emissions is presented in *Figure 4.8d*. The response patterns showed a possible influence of shaking on CH₄ release. Respective CH₄ concentrations in shaker reactors indicated less releases of CH₄ than reactors which were not shaken. A comparison of maximum concentration values showed non-shaken releases ranged from a low of 261.7g/l to a high of 1012.1g/l. On the contrary, reactors under shaker recorded lower release concentrations between 0mg/l and 279.0g/l. This observable concentration gradient between shaker setup and non-shaken could probably be linked to higher solubility of CH₄ in the liquid phase due to shaking.

5.2 The effects of substrate (acetate), COD, ammonia, nitrate, pH and sludge type on N₂O, CO₂ and CH₄ release.

The extent of CH₄ production depended primarily on the quantity of degradable organic material (acetate) in the reactors and the temperature. CH₄ production was substrate limited at higher temperatures and that the addition of acetate increased CH₄ as well as CO₂ production (Stadmark and Leonardson, 2007). This was observed in the difference between influent and effluent acetate concentrations (*Figures 4.1d, 4.2d, 4.5d & 4.4d*) which recorded over 99% utilisation in all reactors. This further signifies that at lower temperatures substrate (acetate) limitation was not a key factor for CH₄ production. Thus the over 99% reduction in acetate at 4°C may be due to its utilisation for other processes (e. g. carbon source for maintenance, reproduction etc) other than CH₄ production. The possible consumption of CH₄ as indicated in *Figures 4.1c, 4.2c & 4.3c* may not only be linked to substrate limitations, but possibly other processes as well (e. g. CH₄ oxidation).

COD reduction increased with increasing temperatures (*Figures 4.1f, 4.2f, 4.3f & 4.4f*). According to the US EPA, (2006) one of the principal factors in determining the CH₄ generation potential of wastewater is the amount of COD in the wastewater. Thus COD reduction is a direct indication of CH₄ emission. As observed in this research, COD reductions were higher in the systems with higher CH₄ emissions; recording an average reduction of 77.5% at 37°C, 66.9% at 30°C, 53.2% at 20°C and only 35.7% at 4°C. It is worth mentioning that since acetate contributed to the measured COD, it is not surprising that a reduction in acetate is accompanied by a corresponding reduction in COD concentrations in all reactors.

The build up of ammonia recorded in all reactors in this research (*Figures 4.1e, 4.2e 4.3e, 4.4e and 4.7e*) could be attributed to degradation of proteins and amino acids as a result of decay of/by microorganisms. According to Burton and Watson-Craik (1998) amino acids and other nitrogenous substances not required in bacterial growth are eliminated by two major processes: deamination and ammonification. Both processes lead to rise in ammonia concentrations as was observed in this research. Generally, the degradation of proteins and amino acids and their subsequent breakdown lead to increase in ammonia concentration and in anoxic environments ammonia tends to accumulate.

NO_3^- concentration changes recorded for influent and effluent (*Tables 4.1, 4.2, 4.3 and 4.4*) surprisingly indicated NO_3^- build up. The initial build up computed may be attributed to poor handling of samples or experimental artifacts that allowed oxygen intrusion into the samples thereby converting ammonia to NO_3^- . Subsequent analyses as indicated in *Figures 4.6h, 4.7h and 4.8i* however showed the contrary as major reductions (between 7.1%-92%) were measured in all reactors. These reductions as indicated in section 5.1 above were probably responsible for the increased N_2O emissions.

pH changes in the reactor influents and effluents (*Figures 4.1f, 4.2f, 4.3f, 4.4f and 4.7f*) points to relatively stable systems. The highest pH were all recorded in the influents while the lowest were recorded in the effluents ($8.1 > \text{pH} < 7.4$). The catabolism of proteins was generally expected to lead to a rise in pH since the resulting ammonia protonates with water to form ammonium. However, in the reactor conditions in this research, the pH increase was offset by the production of CO_2 during the experimental period.

Figures 4.5a-i show the response of sludge type to N_2O , CO_2 & CH_4 releases under different temperatures. The observable trends are indicative that at all temperatures, higher emissions of all the three gases are more identified with anaerobic sludge type with denitrifying sludge being the lowest emitter. This probably could be due to the sludge characteristics and conditioning that favour N_2O , CO_2 & CH_4 (particularly CH_4) production. Additionally under the same conditions more CH_4 is emitted than the other two gases. The trends in all sludge types which recorded sharp declines especially for N_2O and CH_4 is indicative of a probable consumption of the respective gases within the system and therefore pointing to the possibility of other processes at work as earlier stated above.

In summary the emissions of N_2O , CO_2 and CH_4 were found to be considerably higher with rising temperatures. This implies that biological processes respond positively to higher temperatures within a range where the enzymes are stable and retain full activity. The relatively lower emissions measured for N_2O at 4°C and 20°C were due to a slowdown of the denitrification and nitrification processes at lower temperatures. Furthermore, the lower emissions of CO_2 and CH_4 at 4°C and 20°C were also attributable to a slowdown of biological activities at lower temperatures. Additionally, activated sludge treatment system is an important source of GHGs with the anaerobic sludge having the highest potential for the release of the most important GHGs (N_2O , CO_2 and CH_4). It can be concluded that substrate availability coupled with anoxia in the reactors favoured CH_4 production and this is the major contributor to the atmospheric consequences of wastewater.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions are drawn within the limited scope of this research.

- The emissions of N_2O , CO_2 and CH_4 are regulated by a combination of physical, chemical and biological processes.
- The main mechanisms of N_2O , CO_2 and CH_4 releases are the microbiological degradation of organic matter.
- Elevated temperatures promote higher releases of N_2O , CO_2 and CH_4 .
- Higher nitrate concentrations positively influence the release of N_2O and CH_4 but have no influence on CO_2 release.
- Shaking at 100rpm leads to less N_2O , CO_2 and CH_4 emissions.
- Activated sludge wastewater treatment system is an important source of GHGs.
- Substrate availability coupled with a good temperature and anoxia in the reactors favoured CO_2 and CH_4 production and this is the major contributor to the atmospheric consequences of wastewater
- Anaerobic sludge has the highest potential for the release of the most important GHGs (N_2O , CO_2 and CH_4).

6.2 Recommendations

Based on the results, discussions and conclusions, the following recommendations are proposed.

- Sludge samples from a similar wastewater treatment system should be studied to observe the patterns and compare with the results of this study.
- Further investigation should be conducted on the influence of H_2S on N_2O build up so as to know the actual concentration of H_2S at which N_2O reduction is inhibited.
- The NO_3^- , NH_4^+ and total N_2 concentrations should be monitored on regular basis within the experimental period to closely follow and possibly determine the N_2O balance.
- Study the effect of varied shaking speeds on the release of N_2O , CO_2 and CH_4 .
- Varying effects of pH on N_2O , CO_2 and CH_4 should be studied to assess pH influence on N_2O , CO_2 and CH_4 releases.
- The role of oxygen on N_2O , CO_2 and CH_4 should be investigated.
- The possibility of anaerobic oxidation of methane in wastewater treatment sludge using nitrate as electron acceptor should be pursued to help explain the probable consumption of N_2O and CH_4 in this study.

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APPENDIX

Appendix 3.1A List of equipment

No	Equipment	Use
1	500ml and 2000ml Glass bottles and rubber corks	Reactors and calibration
2	Glass flasks, beakers and measuring cylinders	Measurements during analyses
3	Plastic syringes and syringe needles	For sampling of gases from reactor headspace
4	COD tubes	For COD measurements
5	Incubators (ovens, warm/cold rooms),	To provide optimum temperature for experimental setup and analyses
6	Chromatographs	To analyse and quantify gases drawn from reactor headspace
7	Spectrophotometer	For colorimetric analyses of COD and NH_4^+
8	Weighing machine	For quantitative analyses of substance and chemicals
9	Pipettes and burettes	For volumetric measurements
10	Shaker	For shaking sludge at 100rpm

Appendix 3.1B List of chemicals

No	Chemical	Use
1	Ammonium chloride	Nutrient/ COD determination
2	Disodiumpentacyano nitrosylferrate (III)	Ammonia determination
3	Formic acid	Acetate determination
4	Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$)	COD determination
5	Mercury sulphate	COD determination
6	Potassium hydrogen phthalate	COD determination
7	Potassium nitrate	Nutrient
8	Silver sulphate	COD determination
90	Sodium acetate	Bacteria substrate
10	Sodium citratedihydrate	Ammonia determination
11	Sodium dichloroisocyanurate	Ammonia determination
12	Sodium hydroxide	Ammonia determination
13	Sodium salicylate	Ammonia determination
14	Sulphuric acid	COD determination

Appendix 4.1 N₂O Emissions from Sludge Types at Different Temperatures

mgN ₂ O / l												
Aerobic AE: Sludge			AN: Anaerobic Sludge				DE: Denitrified Sludge					
Day	4°C			20°C			30°C			37°C		
	AE-1:4°C	AN-1:4°C	DE-1:4°C	AE-2:20°C	AN-2:20°C	DE-2:20°C	AE-3:30°C	AN-3:30°C	DE-3:30°C	AE-4:37°C	AN-4:37°C	DE-4:37°C
1	0.270	1.521	0.547	0.474	0.613	0.487	0.283	0.400	0.673	0.268	0.371	0.875
2	1.808	16.013	6.442	6.714	11.408	3.996	0.515	5.716	0.107	0.000	0.000	0.000
3	2.375	15.806	8.394	2.165	7.152	1.354	8.171	7.787	2.764	0.000	0.041	0.000
4	2.661	13.880	4.766	2.276	4.310	0.893	1.779	2.689	0.000	0.000	0.075	0.000
7	1.781	3.107	0.134	0.000	4.135	0.000	0.000	0.000	0.000	0.215	0.450	0.000
8	0.400	2.076	0.363	0.000	0.000	0.000	0.000	0.043	0.000	0.277	0.472	0.159
9	0.226	1.753	0.492	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	0.000	2.563	0.340	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11	0.000	2.208	0.179	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	0.000	2.452	0.115	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	0.000	0.694	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
17	0.000	1.279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
18	0.000	0.633	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21	0.000	0.772	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
22	0.000	0.735	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
23	0.000	0.725	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
24	0.000	0.829	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
25	0.000	0.869	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.076	2.088
29	0.000	0.695	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.957	0.857	4.118
30	0.000	0.740	0.000	0.000	0.000	0.000	0.000	0.000	0.000	47.347	1.940	10.528
36	0.000	0.424	0.000	0.000	0.000	0.000	3.937	16.812	10.199	5.200	6.504	21.429
37	0.000	0.518	0.000	0.000	0.000	0.000	8.314	73.739	17.020	107.080	181.845	66.851
39	0.000	0.383	0.000	0.000	0.000	0.000	11.430	131.261	31.523	113.746	151.434	85.176
40	0.000	0.497	0.000	0.000	0.000	0.000	17.937	131.065	38.063	79.217	94.432	62.155
43	0.000	0.351	0.000	0.000	0.000	0.000	58.938	60.740	68.726	34.710	112.092	38.685
44	0.000	0.524	0.000	0.000	0.000	0.000	131.449	107.420	104.305	20.435	94.390	29.396
46	0.000	0.373	0.000	0.000	0.000	0.000	1111.004	31.878	716.348	17.914	172.207	29.888
47	0.000	0.903	6.818	1.193	0.000	0.790	1194.737	27.333	823.900	19.169	210.187	28.083
50	0.000	0.161	1.066	0.000	0.000	0.000	1342.584	101.842	1216.630	12.560	462.029	28.337
52	0.000	0.302	0.685	0.000	0.000	0.000	1325.750	457.598	1224.798	9.466	606.038	19.607
54	2.461	1.859	1.342	0.000	0.000	0.000	1329.550	917.917	1230.557	14.039	722.859	14.713
56	0.709	0.934	1.420	0.000	0.000	0.000	1275.229	1226.016	1157.928	17.692	890.016	14.335
58	1.557	1.232	2.483	0.000	0.000	0.000	1253.480	1272.073	1113.115	16.346	899.272	18.962
60	1.290	1.097	2.167	0.724	0.689	0.489	1250.138	1263.125	1089.447	27.136	664.139	13.548
63	1.501	1.133	2.987	0.824	0.861	0.810	1210.008	1232.964	1079.676	109.719	169.384	24.851
65	1.783	0.095	2.731	0.652	0.684	0.382	1223.959	1216.529	1088.589	216.005	43.553	37.084
67	1.769	1.080	4.032	0.568	0.799	2.139	1230.442	1221.591	1101.201	419.357	21.985	49.695
70	3.385	2.972	5.964	0.784	1.089	2.388	1231.307	1211.070	832.541	1065.138	60.062	35.050

Appendix 4.2 CO₂ Emissions from Sludge Types at Different Temperatures

Day	AE: Aerobic Sludge			AN: Anaerobic Sludge			DE: Denitrified Sludge			gCO ₂ /L		
	4°C			20°C			30°C			37°C		
	AE-1:4°C	AN-1:4°C	DE-1:4°C	AE-2:20°C	AN-2:20°C	DE-2:20°C	AE-3:30°C	AN-3:30°C	DE-3:30°C	AE-4:37°C	AN-4:37°C	DE-4:37°C
1	4.125	8.023	2.871	3.230	7.573	2.675	2.876	7.797	2.580	3.212	7.797	2.667
2	6.088	8.762	3.095	19.899	37.119	21.238	34.813	48.478	30.611	49.296	91.080	61.969
3	4.516	7.924	2.720	42.930	52.966	43.610	84.017	105.516	79.567	131.191	168.021	115.961
4	3.185	7.986	2.958	62.292	72.489	63.932	108.411	130.101	97.918	145.766	179.888	131.592
7	13.626	26.149	24.731	94.493	131.184	85.870	125.584	149.456	121.434	211.765	243.115	190.963
8	17.847	26.315	28.323	103.799	150.436	94.876	139.527	158.023	135.331	222.390	254.240	201.384
9	18.957	25.696	31.554	107.682	159.255	100.015	144.177	167.454	143.555	232.691	255.608	208.663
10	19.586	24.039	32.957	109.966	153.289	103.543	152.172	169.994	141.657	233.676	249.227	203.538
11	19.753	24.442	35.808	116.511	88.433	109.727	155.667	177.634	147.597	234.726	244.848	210.543
14	20.798	24.492	44.309	129.883	165.648	124.415	178.156	196.970	177.868	245.928	257.414	218.586
15	22.173	25.977	27.109	136.525	173.243	133.393	181.644	198.590	178.412	219.499	235.318	202.095
17	22.225	25.872	50.299	148.921	181.850	143.916	196.048	214.198	192.453	241.968	271.171	220.987
18	24.377	28.400	54.147	153.205	187.306	149.697	193.756	211.407	185.455	228.256	253.197	213.628
21	23.250	25.422	56.406	166.750	197.913	162.626	201.742	221.902	193.866	254.463	266.285	235.385
22	23.074	24.474	55.292	171.720	203.511	168.230	202.772	197.677	194.888	253.635	269.780	230.925
23	23.166	24.037	53.730	175.268	205.486	171.270	199.334	221.556	193.341	240.532	260.508	212.026
24	23.036	24.280	55.672	184.278	213.536	178.666	200.219	222.925	194.702	242.684	256.844	220.382
25	23.755	24.556	55.764	188.639	218.332	185.527	201.008	228.982	198.107	222.840	249.615	212.382
28	24.357	25.370	58.175	201.724	228.748	195.804	207.250	265.966	203.939	233.933	255.662	216.795
29	24.387	25.542	56.299	-0.559	233.246	201.682	207.690	269.902	207.444	231.567	246.771	212.262
30	23.285	24.171	52.122	236.460	223.163	192.679	198.998	249.426	197.443	210.615	225.024	197.993
36	46.331	25.450	68.491	211.175	237.861	208.078	185.194	201.463	175.014	207.367	206.073	189.562
37	45.903	25.440	68.074	229.223	244.391	227.266	187.094	208.854	185.268	210.665	219.775	189.400
39	48.172	24.243	64.098	237.084	264.710	242.348	179.101	198.714	174.581	198.386	202.160	181.920
40	45.259	23.810	60.329	234.846	258.182	232.452	164.225	185.940	165.342	189.816	189.107	173.288
43	57.725	22.803	37.681	232.594	251.098	226.912	148.366	170.569	166.238	179.679	194.204	165.534
44	36.791	21.984	57.595	238.679	264.357	236.413	155.478	167.215	177.873	179.975	197.903	167.011
46	34.505	22.206	57.023	242.577	260.133	236.729	215.581	154.304	218.491	180.301	201.217	158.979
47	34.388	22.576	57.073	241.480	247.767	269.922	212.227	148.003	220.952	175.877	201.399	158.909
50	31.238	21.798	54.640	236.271	250.406	232.689	217.787	165.678	216.071	169.163	199.757	153.008
52	31.350	22.484	56.411	242.791	260.066	239.846	214.929	228.450	219.566	169.768	211.123	155.065
54	31.940	22.890	57.362	230.985	250.590	233.450	204.249	223.507	214.402	151.085	197.732	140.020
56	33.801	23.474	60.245	239.316	253.981	236.987	194.936	228.333	197.478	151.349	198.379	136.955
58	34.833	25.246	62.551	242.605	258.242	239.229	191.483	234.477	189.803	145.319	187.410	127.713
60	34.311	24.474	60.989	249.214	236.000	235.435	181.119	228.345	182.373	145.192	173.997	115.200
63	31.806	22.805	59.571	239.082	229.574	227.579	159.747	226.808	170.407	170.638	169.437	111.431
65	33.997	24.300	61.566	231.830	242.595	230.975	146.966	214.810	159.635	183.000	159.270	107.105
67	34.811	23.738	59.145	234.149	244.525	233.836	145.371	211.426	154.645	191.955	156.284	104.901
70	35.161	22.492	56.257	233.393	242.970	230.276	125.211	190.209	150.274	202.891	154.414	106.971

Appendix 4.3 CH₄ Emissions from Sludge Types at Different Temperatures

AE: Aerobic Sludge			AN: Anaerobic Sludge			DE: Denitrified Sludge			gCH ₄ /L			
Day	4°C			20°C			30°C			37°C		
	AE-1:4°C	AN-1:4°C	DE-1:4°C	AE-2:20°C	AN-2:20°C	DE-2:20°C	AE-3:30°C	AN-3:30°C	DE-3:30°C	AE-4:37°C	AN-4:37°C	DE-4:37°C
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76	21.05	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.39	67.98	22.88
7	0.00	0.00	0.00	0.39	0.00	0.00	36.45	73.54	28.69	276.70	316.01	211.43
8	0.00	0.00	0.00	7.97	0.03	6.59	60.44	103.07	42.99	285.86	365.81	233.86
9	0.00	0.00	0.00	18.90	0.00	0.00	82.33	140.82	61.16	441.56	572.01	390.61
10	0.00	0.00	0.00	29.80	35.77	24.58	106.50	174.87	77.37	529.32	655.05	454.61
11	0.00	0.00	0.00	40.81	50.01	34.45	124.38	201.18	93.53	607.64	726.39	601.32
14	0.00	0.00	0.00	89.85	119.20	80.98	194.27	318.00	151.42	730.56	917.13	747.00
15	0.00	0.00	0.00	112.07	154.99	103.70	192.79	324.97	159.14	545.29	726.21	605.08
17	0.00	0.00	0.00	151.54	211.16	144.24	205.82	365.00	154.33	563.47	799.58	576.24
18	0.00	0.00	0.00	164.02	229.32	160.00	209.54	340.17	143.36	459.84	621.75	469.39
21	0.00	0.00	0.00	204.42	293.68	219.65	183.91	329.07	117.27	455.78	664.86	4.60
22	0.00	0.00	0.00	225.09	307.24	211.05	157.33	155.88	83.22	379.49	595.55	379.78
23	0.00	0.00	0.00	235.11	321.41	243.80	109.23	282.38	60.92	297.93	502.00	272.18
24	0.00	0.00	0.00	239.12	329.70	255.55	69.97	246.64	36.07	252.49	383.28	200.26
25	0.00	0.00	0.00	242.51	328.63	260.53	45.26	243.87	32.91	171.43	308.84	137.98
28	0.00	0.00	0.00	248.60	353.53	293.23	36.78	435.55	43.77	112.17	264.08	87.78
29	0.00	0.00	0.00	260.63	362.23	299.20	26.94	446.47	37.32	62.25	163.57	45.13
30	0.00	0.00	0.00	236.20	349.80	282.46	38.41	423.76	33.54	16.28	89.10	13.99
36	0.00	0.00	0.00	300.16	401.60	311.96	0.00	0.00	0.00	3.88	26.14	0.00
37	0.00	0.00	0.00	305.88	379.09	316.95	0.00	0.00	0.00	0.00	0.00	0.00
39	0.00	0.00	0.00	290.68	350.11	289.91	0.00	0.00	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	268.51	322.33	263.02	0.00	0.00	0.00	0.00	0.00	0.00
43	0.00	0.00	0.00	240.35	285.60	227.34	0.00	0.00	0.00	0.00	0.00	0.00
44	0.00	0.00	0.00	226.07	266.92	207.74	0.00	0.00	0.00	0.00	0.00	0.00
46	0.00	0.00	0.00	218.90	241.73	209.26	0.00	0.00	0.00	0.00	0.00	0.00
47	0.00	0.00	0.00	230.67	236.00	259.13	0.00	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	176.95	194.57	179.23	0.00	0.00	0.00	0.00	0.00	0.00
52	0.00	0.00	0.00	167.05	194.42	161.94	0.00	0.00	0.00	0.00	0.00	0.00
54	0.00	0.00	0.00	146.61	172.03	140.59	0.00	0.00	0.00	0.00	0.00	0.00
56	0.00	0.00	0.00	126.74	153.76	123.13	0.00	0.00	0.00	0.00	0.00	0.00
58	0.00	0.00	0.00	128.80	142.90	117.95	0.00	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	122.76	119.71	113.57	0.00	0.00	0.00	0.00	0.00	0.00
63	0.00	0.00	0.00	93.39	93.73	86.10	0.00	0.00	0.00	0.00	0.00	0.00
65	0.00	0.00	0.00	79.09	77.16	70.24	0.00	0.00	0.00	0.00	0.00	0.00
67	0.00	0.00	0.00	69.71	67.01	59.02	0.00	0.00	0.00	0.00	0.00	0.00
70	0.00	0.00	0.00	65.09	63.54	48.10	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 4.4 Influent and Effluent COD Concentrations

Reactor	Influent	Effluent	% COD Reduction
AE-1	10.22	7.25	29.0
AN-1	15.30	12.33	19.4
DE-1	9.90	4.10	58.6
AE-2	10.08	5.03	50.1
AN-2	15.21	5.25	65.5
DE-2	8.75	4.91	44.0
AE-3	11.16	3.30	70.4
AN-3	15.75	5.27	66.5
DE-3	9.87	3.58	63.8
AE-4	10.27	2.82	72.6
AN-4	15.49	3.11	79.9
DE-4	9.17	1.83	80.0
AE-15	9.98	3.15	68.4
AN-15	15.58	5.41	65.3
DE-15	9.69	3.44	64.5
AE-30	10.66	3.21	69.9
AN-30	16.25	5.27	67.6
DE-30	9.25	2.78	69.9
Non-Shaking			
AE-0	10.28	3.08	70.0
AN-0	15.55	6.01	61.4
DE-0	9.85	3.34	66.1
AE+NO ₃ ⁻	10.27	4.03	60.7
AN+NO ₃ ⁻	13.12	3.97	69.7
DE+NO ₃ ⁻	9.17	3.11	66.0
AE+NH ₃	10.66	3.21	69.9
AN+NH ₃	15.52	4.87	68.6
DE+NH ₃	10.15	3.12	69.3
Shaking			
AE-S	10.08	2.91	71.1
AN-S	15.62	5.36	65.7
DE-S	9.19	3.14	65.8
AE+NO ₃ ⁻ -S	9.67	2.97	69.3
AN+NO ₃ ⁻ -S	10.12	3.33	67.1
DE+NO ₃ ⁻ -S	13.35	3.26	75.6
AE+NH ₃ -S	11.12	4.38	60.6
AN+NH ₃ -S	10.27	2.8157	72.6
DE+NH ₃ -S	15.49	3.11433	79.9

Appendix 4.5 Influent and Effluent Ammonia Concentrations

Reactor	Influent	Effluent	% NH ₃ Increase
AE-1	54.39	137.76	60.5
AN-1	51.79	91.35	43.3
DE-1	15.91	154.64	89.7
AE-2	31.67	294.73	89.3
AN-2	57.74	264.35	78.2
DE-2	35.21	307.38	88.5
AE-3	36.27	67.72	46.4
AN-3	49.96	209.28	76.1
DE-3	36.84	89.66	58.9
AE-4	37.38	273.63	86.3
AN-4	39.22	273.42	85.7
DE-4	48.31	336.29	85.6
AE-15	69.78	118.69	41.2
AN-15	198.28	279.5	29.1
DE-15	76.64	114.41	33.0
AE-30	66.42	164.25	59.6
AN-30	63.75	167.59	62.0
DE-30	60.85	137.83	55.9
Non-Shaking			
AE-0	32.03	118.36	72.9
AN-0	34.14	113.5	69.9
DE-0	39.82	124.41	68.0
AE+NO ₃ ⁻	48.83	128.76	62.1
AN+NO ₃ ⁻	37.44	92.52	59.5
DE+NO ₃ ⁻	32.79	114.35	71.3
AE+NH ₃	67.83	194.25	65.1
AN+NH ₃	61.44	189.45	67.6
DE+NH ₃	69.89	197.84	64.7
Shaking			
AE-S	38.73	67.25	42.4
AN-S	39.34	109.82	64.2
DE-S	37.82	98.62	61.7
AE+NO ₃ ⁻ -S	37.43	134.27	72.1
AN+NO ₃ ⁻ -S	31.44	127.59	75.4
DE+NO ₃ ⁻ -S	36.69	127.88	71.3
AE+NH ₃ -S	60.42	193.62	68.8
AN+NH ₃ -S	63.75	173.48	63.3
DE+NH ₃ -S	60.85	198.87	69.4

Appendix 4.6 Influent and Effluent Nitrate (NO₃-N) Concentrations

Reactor	Influent	Effluent	% NO ₃ -N Reduction
AE-1	0.47	<0.1	
AN-1	<0.10	<0.1	
DE-1	0.18	0.39	
AE-2	0.16	0.27	
AN-2	<0.10	0.48	
DE-2	<0.10	0.22	
AE-3	<0.10	3.39	
AN-3	<0.10	0.24	
DE-3	<0.10	38.81	
AE-4	0.12	4.26	
AN-4	0.20	0.03	
DE-4	<0.1	0.45	
AE-15	17.00	2.76	83.8
AN-15	18.48	4.78	74.1
DE-15	11.72	7.55	35.6
AE-30	46.88	4.83	89.7
AN-30	48.12	5.12	89.4
DE-30	49.50	2.33	95.3
Non-Shaking			
AE-0	1.62	1.45	10.5
AN-0	1.27	1.21	4.2
DE-0	1.35	0.54	60.2
AE+NO ₃ ⁻	41.72	0.40	99.0
AN+NO ₃ ⁻	44.08	1.40	96.8
DE+NO ₃ ⁻	43.21	1.14	97.4
AE+NH ₃	1.61	1.50	7.1
AN+NH ₃	1.81	1.35	25.1
DE+NH ₃	1.67	1.50	10.4
Shaking			
AE-S	1.34	1.31	2.4
AN-S	1.85	0.23	87.4
DE-S	1.46	1.34	8.4
AE+NO ₃ ⁻ -S	37.57	1.50	96.0
AN+NO ₃ ⁻ -S	31.50	0.82	97.4
DE+NO ₃ ⁻ -S	33.56	0.47	98.6
AE+NH ₃ -S	1.45	0.87	39.9
AN+NH ₃ -S	1.84	1.74	5.7
DE+NH ₃ -S	1.36	1.10	19.5

Appendix 4.7 Influent and Effluent Acetate (CH₃COO⁻) Concentrations

Reactor	Influent	Effluent	% Acetate Reduction
AE-1	957.32	1.98	99.8
AN-1	918.60	2.00	99.8
DE-1	305.94	1.62	99.5
AE-2	1070.66	0.92	99.9
AN-2	906.34	0.76	99.9
DE-2	305.94	0.66	99.8
AE-3	891.55	0.22	99.9
AN-3	807.22	0.66	99.9
DE-3	300.35	0.22	99.9
AE-4	841.87	0.76	99.9
AN-4	876.75	0.22	99.9
DE-4	316.64	0.22	99.9
AE-15	1389.99	0.85	99.9
AN-15	1272.71	0.86	99.9
DE-15	1250.82	0.22	99.9
AE-30	1346.33	0.22	99.9
AN-30	1349.20	0.85	99.9
DE-30	1266.43	0.22	99.9
Non-Shaking			
AE-0	1897.81	1.58	99.9
AN-0	1502.22	1.81	99.9
DE-0	1734.71	2.38	99.9
AE+NO ₃ ⁻	1637.20	1.65	99.9
AN+NO ₃ ⁻	1525.00	2.25	99.9
DE+NO ₃ ⁻	1641.18	0.78	95.3
AE+NH ₃	1458.35	2.83	99.8
AN+NH ₃	1502.01	0.99	99.9
DE+NH ₃	1616.69	1.54	99.9
Shaking			
AE-S	1331.52	0.86	99.9
AN-S	1382.99	0.90	99.9
DE-S	1829.24	0.22	99.9
AE+NO ₃ ⁻ -S	1635.63	0.90	99.9
AN+NO ₃ ⁻ -S	1846.58	2.53	99.9
DE+NO ₃ ⁻ -S	1642.78	0.85	99.9
AE+NH ₃ -S	1724.94	0.22	99.9
AN+NH ₃ -S	1394.37	0.68	99.9
DE+NH ₃ -S	1728.35	0.67	99.9

Appendix 4.8 Influent and Effluent pH

Reactor	Influent	Effluent
AE-1	7.7	7.2
AN-1	7.7	7.2
DE-1	7.9	7.2
AE-2	7.9	6.9
AN-2	7.6	6.9
DE-2	7.9	7.0
AE-3	7.7	7.2
AN-3	7.7	7.4
DE-3	7.9	7.1
AE-4	7.7	7.1
AN-4	7.7	7.4
DE-4	7.6	7.4
AE-15	8.0	7.6
AN-15	8.1	7.6
DE-15	8.0	7.7
AE-30	8.1	7.5
AN-30	8.1	7.4
DE-30	8.3	7.6
Non-Shaking		
AE-0	8.1	7.7
AN-0	8.1	7.6
DE-0	8.0	7.5
AE+NO ₃ ⁻	8.1	7.5
AN+NO ₃ ⁻	8.1	7.6
DE+NO ₃ ⁻	7.7	7.4
AE+NH ₃	8.3	7.9
AN+NH ₃	8.2	7.8
DE+NH ₃	8.2	7.7
Shaking		
AE-S	7.8	7.5
AN-S	7.8	7.4
DE-S	7.9	7.5
AE+NO ₃ ⁻ -S	7.8	7.5
AN+NO ₃ ⁻ -S	8.0	7.7
DE+NO ₃ ⁻ -S	7.7	7.4
AE+NH ₃ -S	7.9	7.5
AN+NH ₃ -S	8.1	7.8
DE+NH ₃ -S	8.0	7.6