Resource recovery options in brewery effluent treatment using activated sludge and high rate algal ponds: Assessing environmental impacts

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Abstract

Wastewater treatment plants (WWTPs) are designed to clean effluents, but they also consume resources and produce waste. Various treatment technologies allow for the recovery of energy, nutrients and water from effluents turning this waste into products, which increases their sustainability and decreases the impact of WWTPs on the environment. There is a lack of literature which comprehensively compares the treatment performances, environmental impacts and beneficial downstream uses of the biomass generated by high rate algal pond (HRAP) and activated sludge (AS) treatment systems. This thesis aimed to compare (1) effluent treatment performance, (2) emissions and (3) downstream use of algae cultured in HRAP to sludge produced in AS and to obtain data to conduct a life cycle analysis (LCA) to compare the systems.

The focus was on adding value to the effluent treatment process, while identifying the associated environmental impacts and contributing to the first ever zero-waste brewery effluent treatment system. Furthermore, these data were used to provide a basis to critically review and contribute to improving the methods used in the LCA of effluent treatment systems; particularly since this was the first wastewater treatment LCA that compared AS and HRAP using data collected from the same temporal and geographic location and from a single effluent stream.

The electrical consumption, water emission and land application of waste biomass caused the major environmental impacts of both treatment systems. The HRAP had less than 50 % of the electrical energy consumption (0.11±0.01 kW/m^3 of effluent treated) compared to the AS system (0.29±0.11 kW/m^3) which resulted in the technology having a lower climate change, photochemical oxidant formation, freshwater and marine ecotoxicity and fossil fuel

depletion impact. It is imperative to understand the method of electrical energy (fossil fuel vs renewable) generation when conducting a LCA and deciding which technologies to use, since they have a major influence on the aforementioned impacts.

The biogas yield of algal and sludge substrates was similar with an average gas production of 241 ml/g volatile solids fed. Biogas from algae fed digesters had a significantly higher methane content (64.73±0.81 %) and lower carbon dioxide content (22.94±0.24 %) when compared to WAS fed digesters (60.08±0.18 % and 27.37±0.43 %) respectively due to it being a less oxidised substrate. Swiss chard plants (*Beta vulgaris*) fertilised with anaerobically digested (AD) algae or sludge had a significantly higher mean biweekly yield (5.08±0.73 kg/m^2) when compared to the inorganic-fertiliser control (3.45±0.89 kg/m^2; $p<0.0001$). No difference was observed in the soil's physical fertility when algae or sludge were applied to the soil ($p>0.05$). The HRAP produced more biomass (317.18±27.76 g/m^3) than the AS (83.12±64.91 g/m^3), which resulted in a significantly greater downstream production of biogas and fertiliser per volume of effluent treated. According to the LCA, this also resulted in the HRAP system having a higher terrestrial ecotoxicity, due to the greater volume of solids and thus heavy metals applied to the soil. This interpretation can be misleading, because the mass of heavy metals released into the environment is the same for both systems, with a greater portion being applied to the land in the HRAP scenario and discharged into fresh water in the case of AS. Future LCA models should clarify if these biomasses are going to be applied to a single piece of land or multiple sites as this will influence the risk of contamination via pollutant build up in the soil. The application of sludge or algae on soil increased the soil's sodium concentration and sodium absorption ratio from 774.80±13.66 mg/kg to 952.17±34.89 mg/kg and 2.91±0.04 to 3.53±0.13, respectively. Regulations on the application of algae or sludge on agricultural soils should be

altered to consider the limit values for sodium and future LCA's associated with effluent treatment facilities should incorporate the possibility of soil contamination through sodium build-up. This work also conceptualised the importance of reporting water emissions in wastewater treatment LCA in as much detail as possible, because this had a significant influence on the eutrophication impacts on water systems. Reporting water emissions as total nitrogen underestimated downstream eutrophication impacts compared with those using nitrogen-species concentration (ammonia, nitrite, nitrate etc). A marine eutrophication sensitivity co-efficient should be included in future LCA models which accounts for the probability of nitrogen and phosphorus emissions entering the coastal environment as well as the vulnerability of the marine environment to eutrophication.

Activated sludge systems are favourable for situations where space is limited, were there are inadequate options for biomass disposal (biomass not be used in agriculture or AD) and were electricity is generated from a renewable source; whereas, HRAP are more suitable under circumstances where electricity production relies on fossil fuel that carries a high environmental impact and where options are available to use the biomass for economic gain such as biogas and fertiliser production.

This thesis contributes towards a zero-waste brewery effluent treated process. The HRAP and AS treated effluent for reuse in the brewery or in agricultural irrigation. The solids were anaerobically digested, and the carbon was recovered as a biogas, while the digestate was applied as an agricultural fertiliser. This allowed for the recovery of water, nutrients and carbon.

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1,4 DB	1,4 dichlorobenzene
AD	Anaerobic digestion
AD-algae	Anaerobically digested algae
AD-sludge	Anaerobically digested sludge
AFP	Air filled porosity
AS	Activated sludge
BMP	Biochemical methane potential
CCI	Chlorophyll concentration index
CEC	Cation exchange capacity
CFC11	Trichlorofluoromethane
CHP	Combined heat and power unit
COD	Chemical oxygen demand
CSTR	Continuously stirred tank reactors
DDT	Dichlorodiphenyltrichloroethane
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
EBRU	Environmental Biotechnology Rhodes University
EC	Electrical conductivity
EPA	Environmental protection agency
GHG	Greenhouse gas
HRAP	High rate algal pond
HRT	Hydraulic retention rime
IAPS	Integrated algal ponding system
ISO	International Organization for Standardization
LCA	Life cycle analysis
LCFA	Long chain fatty acids
MLSS	Mixed liquor suspended solids
MWD	Mean weight diameter
NMVOC	Non-methane volatile organic compounds
NO_X	Nitrogen oxides
PFP	Primary facultative pond
PM10	Particulate matter <10 microns in diameter
PVC	Polyvinylchloride
SAB Ltd	South African Breweries Limited
SAR	Sodium absorption ratio
SGP	Specific gas production
SMP	Specific methane production
SRB	Sulphate reducing bacteria
SRT	Solids retention time
TA	Total alkalinity
TAN	Total ammonia nitrogen
TOC	Total organic carbon
TN	Total nitrogen
TS	Total solids
UASB	Up flow anaerobic sludge blanket
UDN	Unidentified dissolved nitrogen
VFA	Volatile fatty acids
VS	Volatile solids
WAS	Waste activated sludge
WWTPs	Wastewater treatment plants

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Chapter 1: Introduction

Shortages of water, energy and food are an increasing concern in South Africa and the rest of the world (Arnell 2004, Winter 2011, Turton *et al.* 2016). South Africa has faced power shortages and is currently experiencing water shortages (Winter 2011, Turton *et al.* 2016). The increase in the human population as well as the size and number of industries has led to more waste production, resulting in the pollution and degradation of most ecosystems (Hanjra and Qureshi 2010, Turton *et al.* 2016). In order to sustain the resources needed by the human population, while minimising environmental degradation via waste disposal, it is vital that we recover and reuse these resources contained in waste products.

Wastewater treatment plants (WWTPs) are critical in the preservation of human and environmental health (Mo and Zhang 2013). However, WWTPs consume a large portion of natural resources and produce waste products which need to be disposed of correctly. In the United States of America about 23% of municipal energy is consumed by WWTPs (Mo and Zhang 2013). These consumption rates are also expected to increase with increasing human population, economic growth and stricter regulations (Mo and Zhang 2013). Due to the critical services WWTPs provide and their high resource consumption, much research has been conducted on decreasing their resource consumption rates and increasing their sustainability (Lundin *et al.* 2004, Hellstrom *et al.* 2008, Mo and Zhang 2013). Wastewater treatment plants can increase their sustainability by increasing energy efficiency, performing resource recovery, decreasing waste production and reducing the use of non-renewable resources (Lundin *et al.* 2004, Hellstrom *et al.* 2008, Mo and Zhang 2013). Increasing energy efficiency is mainly done through equipment maintenance, replacement

with more energy efficient motors and evaluating energy consumption (Daw *et al.* 2012, Mo and Zhang 2013).

Resource recovery plays a major role in increasing the sustainability of WWTPs and is classified into three categories: energy generation, nutrient recycling and water reuse (Lundin and Morrison 2002, Mo and Zhang 2013). The organic content of wastewater can be utilised to produce energy via technologies such as combustion and anaerobic digestion (AD; Ward *et al.* 2008, Mo and Zhang 2013). Nutrient recycling recovers nitrogen and phosphorus from effluents in the form of biomass or fertilisers, which offsets the impact associated with the production of these products (Mo and Zhang 2013, Lundin *et al.* 2004). Moreover, treated wastewater can be reused for various purposes such as providing ecological benefits, reducing the demand for potable water and augmenting water supplies. The development of a zero-waste brewery effluent treatment system, which allows the recovery of resources contained within the effluent (water, nutrients and carbon), is a major achievement in sustainable effluent treatment.

1.1 Problem Identification

Ibhayi Brewery (SAB Ltd, Port Elizabeth, South Africa) has a partnership with Rhodes University where alternative, sustainable effluent treatment technologies are tested at an onsite experimental treatment facility.

The full volume of effluent is screened through a drum filter that removes solid wastes such as stones, plastics, glass, paper and labels from the waste stream, after which it is sent to an up-flow anaerobic sludge blanket reactor (UASB; Jones *et al.* 2016). After AD the effluent is

polished in an activated sludge system and then sent to the municipal sewer or used in non-production activities at the brewery (Mclean *pers. comm.* 2017). A portion of post-anaerobically digested (post-AD) effluent is also piped to an experimental wastewater treatment facility where technologies such as facultative ponds, high rate algal ponds, constructed wetlands and hydroponics are used to treat the effluent for reuse in aquaculture and crop irrigation.

The current activated sludge (AS) treatment process is costly and energy expensive (Simate *et al.* 2011, Power and Jones 2016); however, alternative technologies exist that could make the water and nutrients in brewery effluent available for reuse in downstream applications such as hydroponics, aquaculture and irrigation (Jones *et al.* 2016, Taylor *et al.* 2018). It is vital that research is done to develop technologies that are employed to reuse and recycle water, produce sustainable energy and produce fertiliser or food while using water efficiently (Simate *et al.* 2011, Power 2014).

All the treatment technologies used at Ibhayi Brewery have their advantages and disadvantages. For example, AD produces methane which is burned in a boiler to generate steam, but the process requires a relatively long hydraulic retention time (HRT; Angelidaki and Sanders 2004, Mclean *pers. comm.* 2017). Activated sludge treats the effluent quickly and utilises a small area of land but has a high electrical energy consumption (Tchobanoglous *et al.* 2003, Mclean *pers. comm.* 2017). Constructed wetlands and high rate algal ponds (HRAP) have low energy consumption but require large areas of land to treat effluent when compared to AS (Mo and Zhang 2013, Craggs *et al.* 2014). Both AS and HRAP treatment technologies produce a biomass which needs correct disposal, thus costing money.

Monitoring and conducting mass balances on each step in the effluent treatment process can be used to evaluate the process in terms of resource recovery. However, in reality, more than a single technology is used in the effluent treatment process and it is important to evaluate the resource recovery performance of the entire system. This can be used to determine what combination of treatment systems has the lowest resource consumption and the highest resource recovery.

Life cycle analysis (LCA) is a tool that has been developed and used in the past decade to identify the environmental impact of a product or process (Lundie *et al.* 2004, Chester *et al.* 2010). It considers all aspects involved in the life cycle of the process; for example, the construction phase, the materials consumed throughout its life and the density of the waste products produced. It is a good tool to use to evaluate which combination of effluent treatment technologies is the most sustainable.

There is a lack of literature that reviews the integrated energy, nutrient and water recovery of WWTP technologies, with the goal of demonstrating a zero-waste effluent treatment process and identifying their associated environmental impacts (McCarty *et al.* 2011, Mo and Zhang 2013). Individual resource recovery technologies have been evaluated and compared using LCA but literature is lacking with regards to the utilisation of combinations of these technologies (Mo and Zhang 2013). To add to this, some LCA studies on AS, HRAP and constructed wetlands only include the operational phases, while others include the construction and operational phase and even fewer include the construction, operational and end-of-life phase (Svanstrom *et al.* 2005, Hong *et al.* 2009, Brown *et al.* 2010, Mo and Zhang 2013). There are also large differences in the functional units and the impact categories used, which makes it difficult to compare different nutrient, energy and water

recovery technologies. This thesis aims, for the first time, to address these gaps in the LCA field and to identify a combination of brewery effluent treatment technologies that allow recovery of resources while minimising environmental impact. To achieve this, the following questions were answered:

i. What are the treatment efficiencies and nutrient removal mechanisms that occur during treatment in HRAP and AS technologies?
ii. What is the environmental impact associated with HRAP and AS treatment technologies?
iii. What contributes to the major environmental threats posed by HRAP and AS treatment technologies?
iv. Is it feasible to recover energy from waste activated sludge (WAS) and algal biomasses by AD?
v. Can anaerobically digested WAS and algal biomasses be used as an inorganic fertiliser replacement?
vi. Do sodium limits need to be included in the South African regulations for agricultural land application of wastewater treatment plant derived biomass?
vii. What combination of HRAP or AS effluent treatment technologies coupled with various biomass disposal options maximises resource recovery and minimises environmental impact?
viii. Can LCA provide a comprehensive unbiased comparison of the environmental impact of AS and HRAP treatment systems coupled with biomass disposal options?

1.2 Literature Review

This literature review will examine the treatment technologies used in the brewery effluent treatment process. The processes, and the advantages and disadvantages surrounding the treatment technologies, will be described. This will set the ground for conducting experiments on the different treatment technologies in order to obtain data which can be used to conduct a LCA of the entire treatment process and the different combinations available and which will provide a basis to provide a philosophical and critical review that will contribute to improving the methods used in the future.

1.2.1 Anaerobic digestion

Anaerobic digestion is an energy efficient and environmentally sustainable way of removing organic matter from waste streams as it allows for the recovery of energy from organic waste (Chynoweth *et al.* 2001, Angelidaki *et al.* 2003). It is a biological process that converts organic carbon into a gaseous mixture that is principally composed of methane and carbon dioxide and other trace gases (Figure 1.1; Mosey 1982, Lyberatos and Skiadas 1999, Chynoweth *et al.* 2001). These trace gases include nitrogen, nitrogen oxides, hydrogen, ammonia, hydrogen sulphide (H_2S) and other volatile compounds (Angelidaki and Sanders 2004). As the AD process produces methane and carbon dioxide, it combines wastewater treatment with the production of a valuable fuel source (Chynoweth *et al.* 2001, Angelidaki *et al.* 2003). Anaerobic digestion is currently becoming increasing popular due to escalation in conventional energy production costs (coal, oil and fossil fuel gasses) and because it is a cleaner source of renewable energy (Roberts 2015).

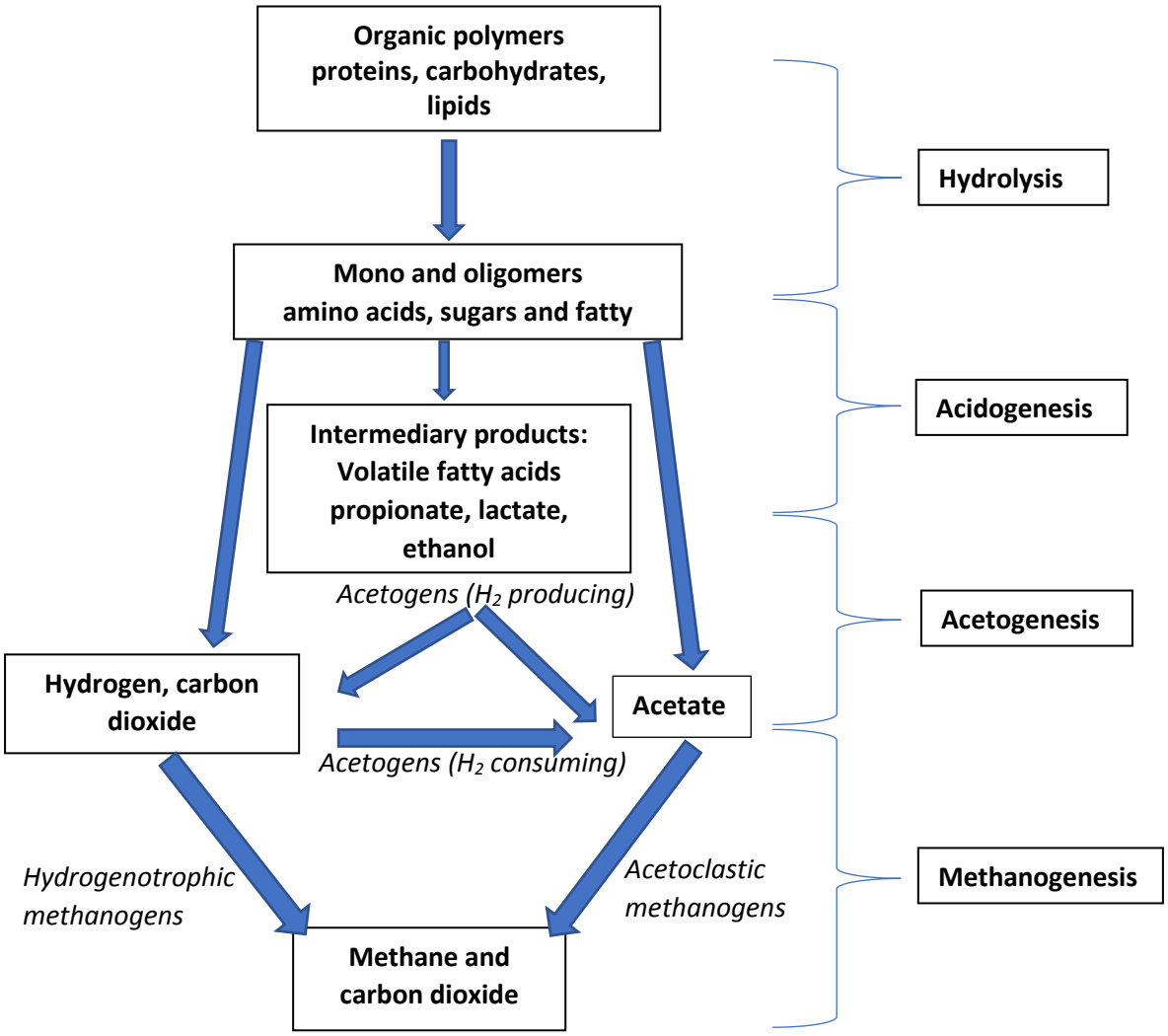

Figure 1.1: Flow diagram summarising the anaerobic conversion of organic matter into methane and carbon dioxide (Demirel and Scherer 2008).

Hydrolysis

The AD process happens through the concerted action of a highly integrated community of bacteria and archaea, in the absence of oxygen (Lyberatos and Skiadas 1999). These organisms live in a parallel and series symbiotic relationship which can be grouped into four main steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 1.1; Mosey 1982, Speece 1983, Lee *et al.* 2014). Complex organic matter is hydrolysed by fermentative bacteria to simple short chain molecules which are used by methane-producing archaea to produce methane and carbon dioxide (Figure 1.1; Speece 1983, Madigan *et al.* 2003). The

AD process produces biogas that has an average content of 55-65 % methane and 35-45 % carbon dioxide (Chynoweth *et al.* 2001, Cantrell *et al.* 2008). Factors such as feedstock composition, organic loading rate, pH, temperature, rate of mixing, HRT, dilution rate and design and operation of the reactor influence biogas production and composition (Lyberatos and Skiadas 1999, Angelidaki *et al.* 2003, Angelidaki and Sanders 2004).

Hydrolysis is the first step in the anaerobic conversion of organic matter into methane and carbon dioxide, where organic polymers (proteins, carbohydrates and lipids) are depolymerised into monomers and oligomers (amino acids, sugars and fatty acids) by hydrolytic bacteria (Speece 1983, Tchobanoglous *et al.* 2003). Hydrolysis takes place extracellularly by the action of enzymes such as cellulase, lipase and protease that are secreted by these bacteria (Angelidaki and Sanders 2004). The enzymes adsorb or attach to organic matter and depolymerise it (Jain *et al.* 1992, Vavilin *et al.* 1997, Angelidaki and Sanders 2004). It is therefore important to have good contact between biomass and substrate for hydrolysis to occur (Morgenroth *et al.* 2002, Angelidaki and Sanders 2004).

Hydrolysis is generally considered the rate-limiting step when particulate, insoluble substrates are digested, as acidogenic and acetogenic bacteria can only utilise dissolved organic compounds (Speece 1983, Bryers 1985). Biogas production is reduced when the solid's hydrolysis rate is higher than the HRT (Vavilin *et al.* 1997). Enzymatic depolymerisation of organic polymers is thought to follow Michaelis–Menten kinetics where reaction velocity is mainly influenced by substrate concentration (Angelidaki and Sanders 2004). Temperature also affects the rate of hydrolysis as it has an influence on enzyme kinetics, bacterial growth and the solubility of the substrate (Angelidaki and Sanders 2004). The influence of temperature on the rate of hydrolysis has been found to vary in accordance

with the Arrhenius equation, with an increase in hydrolysis coefficients from 0.1-0.2 per day under mesophilic conditions to 0.4-0.8 in the thermophilic range (Veeken and Hamelers 1999, Siegrist *et al.* 2002, Ho *et al.* 2014). Hydrolysis of fats hardly proceeds at temperatures between 15-20 ^{o}C. Temperature also influences the pKa of enzymes, which can change the charge and structure of enzymes, resulting in a change in catalytic efficiency, the amount of active enzyme and the binding of the substrates (Chaplin and Bucke 1990).

The effect of pH on hydrolysis is complex and most studies have found a pH of six to be optimal for hydrolysis (Morgenroth *et al.* 2002, Angelidaki and Sanders 2004, Vavilin *et al.* 2008). The influence of pH on hydrolysis rate is defined by the optimal pH for the enzymes present in the digester and the influence of pH on the solubility of the substrate (Angelidaki and Sanders 2004). The pH plays a major role in the hydrolysis of substrates that contain proteins as pH affects the solubility of proteins, with most protein becoming less soluble in acidic conditions resulting in decreased hydrolysis rates (Rollon 1999). However, various other studies have shown that when the pH is kept in the normal range for AD (6.8-8.0) it plays a minor role in the hydrolysis of proteins (Breure *et al.* 1986, Yu and Fang 2003, Vavilin *et al.* 2008).

The production and activity of enzymes responsible for hydrolysis can be inhibited by products produced during depolymerisation (Angelidaki and Sanders 2004). Amino acids, high inorganic phosphate levels and glucose can inhibit the microbial production of proteinases (Angelidaki and Sanders 2004). The production of cellulases is inhibited by high inorganic phosphate levels and high glucose levels while low glucose levels stimulate the production of cellulases (Jiang 2012). Free ammonium ions can also inhibit the hydrolysis of cellulose at concentrations above 990 mg/l (Vavilin *et al.* 1997). Lipase activity is reduced by

the accumulation of long-chain fatty acids at the lipid-water interface due to physical-chemical changes of the interface such as droplet size id-droplet size, ultrastructure and structure of triglycerides and of phospholipids (Angelidaki and Ahring 1992).

The physical state and structure of the substrate influences its accessibility to hydrolytic enzymes (Angelidaki and Sanders 2004). Particulate polymers have a lower hydrolysis rate than dissolved polymers because only a part of particulate polymers is accessible to the enzymes (Angelidaki and Sanders 2004). Song (2003) found that for a particulate substrate hydrolysis rate can be related to its surface area. This is because as the surface area of the substrate increases there is more area for a biofilm to generate, thus increasing the rate of hydrolysis. The formation of complexes by compounds can influence the accessibility of substrates (Angelidaki and Sanders 2004). Tong *et al.* (1990) demonstrated that the biodegradability of cellulose is severely decreased when it is incorporated in a lignocellulosic complex. Pre-treatment processes that break down particles and large organic complexes can increase the rate of hydrolysis, and ultimately methane production, when hydrolysis is the limiting step during AD.

Acidogenesis

After the complex organic matter has been hydrolysed into soluble monomers and oligomers, facultative fermentative acidogenic bacteria produce several simpler intermediates such as hydrogen, carbon dioxide, acetate, volatile fatty acids (VFA) and ammonia (Hill *et al.* 1987, Lyberatos and Skiadas 1999, Batstone *et al.* 2002). The acetate, hydrogen and carbon dioxide produced by acidogenic bacteria are used by methanogens to produce methane while the fatty acids, sugars and alcohols are used by acetogens to produce acetate and hydrogen (Hill *et al.* 1987, Demirel and Scherer 2008). Acidogenic

bacteria have a fast growth rate and rapidly convert soluble organics into VFA such as acetate, propionate, and butyrate (Mosey 1982, Hill *et al.* 1987). Acidogenesis is considered the easiest and fastest step during AD as neutral compounds are anaerobically oxidised to acids resulting in the release of protons which produce hydrogen gas.

Acetogenesis

A consortium of acetogenic bacteria oxidise the intermediate products produced during acidogenesis into acetate, hydrogen and carbon dioxide (Speece 1983, Lyberatos and Skiadas 1999, Yu and Fang 2003). There are two main groups of bacteria responsible for this process: obligate hydrogen-producing acetogens which convert fatty acids into acetate, and hydrogen-consuming homo-acetogens which produce acetate from carbon dioxide and hydrogen (Mara and Horan 2003). The hydrogen-producing acetogens are considered to be the dominant acetate producers (Mara and Horan, 2003). They exist in a symbiotic relationship with methanogens where the acetate, hydrogen and carbon dioxide result in a decrease in reactor pH and these products are metabolised by methanogens, resulting in pH buffering (Gerardi 2003). These micro-organisms only thrive in an environment where their metabolic products are consumed, as hydrogen accumulation is inhibitory to hydrogen-producing acetogens and acetate is inhibitory to methanogens (Gerardi 2003).

Acetogenesis is considered the most difficult step during AD as the Gibbs free energy change for the conversion of propionic and butyric acid to acetate is positive under standard conditions (Harper and Pohland 1986, Schink 1997). These reactions can only take place under a low hydrogen concentration and can only proceed when the hydrogen is taken up by the methanogens (Harper and Pohland 1986, Schink 1997). For example, the free energy change for the conversion of propionate to acetate only becomes negative when the

hydrogen partial pressure is below 10^{-4} atm (Figure 1.2; McCarty 1981). According to the Gibbs free energy change, the conversion of carbon dioxide and hydrogen to methane will only take place when the hydrogen concentration is above 10^{-6} atm (Harper and Pohland 1986). This results in a methanogenic hydrogen partial pressure niche where there is

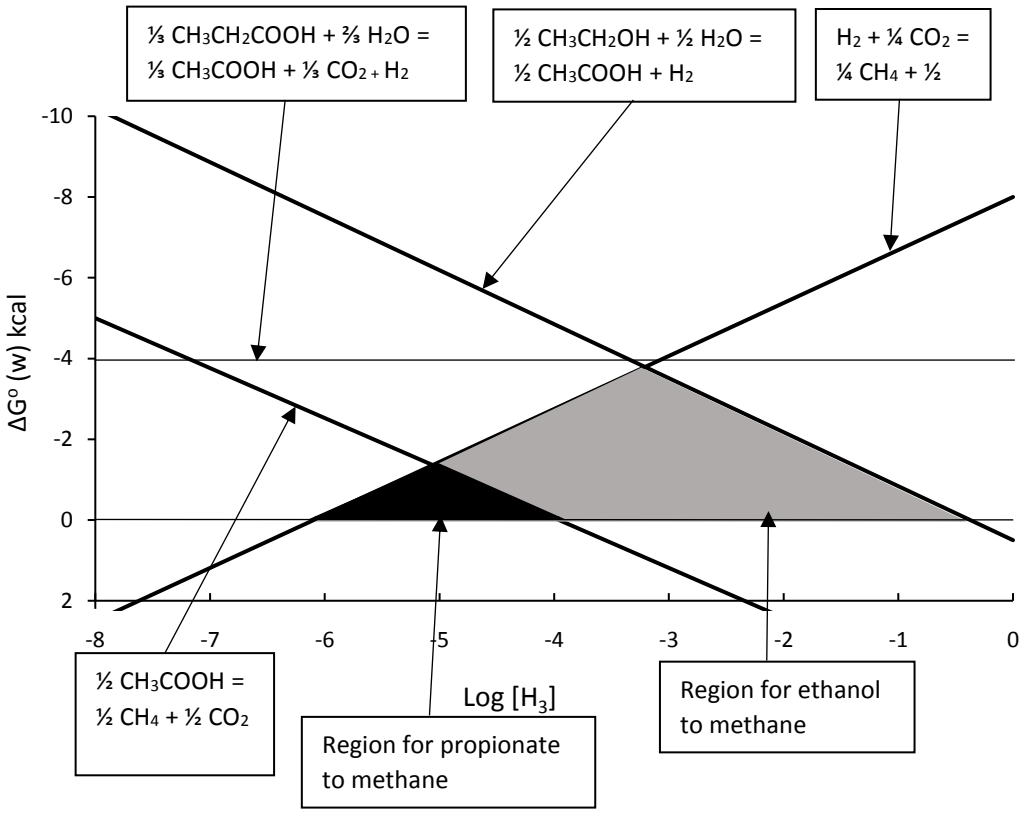

Figure 1.2: Effect of hydrogen partial pressure on the free energy change for the conversion of ethanol, propionate, acetate and hydrogen during methane fermentation (McCarty 1981).

sufficient energy for methanogens to convert acetate and hydrogen into methane and energy for acetogens to convert VFAs into acetate (Figure 1.2).

Methanogenesis

Methanogenesis is the final step in AD where archaea convert the carbon dioxide, hydrogen and acetate into methane and carbon dioxide (Speece 1983, Lyberatos and Skiadas 1999,

Madigan *et al.* 2003). This process is greatly reduced in the presence of oxygen and only happens under anaerobic conditions (Speece 1983, Lyberatos and Skiadas 1999). Methanogenesis is often considered the slowest step of AD due to the slow growth rate of methanogenic archaea, their low tolerance to environmental stress, the ease with which they are inhibited and the low specificity of each substrate for methanogens (Mara and Horan 2003, Gerardi 2003, Chen *et al.* 2008). The three main groups of methanogens are acetoclastic, hydrogenotrophic, and methylotrophic, these being classified by the substrate they use to produce methane (Gerardi 2003, Demirel and Scherer 2008). The acetoclastic methanogens convert acetic acid into methane and carbon dioxide (Mosey 1982, Hill *et al.* 1987, Demirel and Scherer 2008). Hydrogenotrophic methanogens reduce carbon dioxide with electrons obtained from oxidising hydrogen into methane and water, while methylotrophic methanogens consume substrates containing a methyl group, producing methane from the methyl group (Mosey 1982, Hill *et al.* 1987).

Acetoclastic methanogens reproduce more slowly than hydrogenotrophic methanogens but normally account for 70 % of the methane produced. The rest of the methane is produced mainly by hydrogenotrophic methanogens, with methylotrophic methanogens hardly contributing to methane production (Schink 1997, Batstone *et al.* 2002, Liden 2015). Despite hydrogenotrophic methanogens having the fastest growth rate, the limited supply of hydrogen in anaerobic digesters, and their lower affinity for substratefs limit their methane production (Schink 1997, Batstone *et al.* 2002). The conversion of carbon dioxide and hydrogen to methane maintains a low hydrogen partial pressure which is necessary for the conversion of VFA to acetate, keeping the AD process going (McCarty 1981, Schink 1997). During methanogenesis, alkalinity is generated via the production of OH^- which is needed to keep a stable pH in reactors (Schink 1997, Batstone *et al.* 2002). To ensure the successful

operation of anaerobic reactors, it is essential that a large population and diversity of methanogenic archaea are present as no one species or group utilises all the substrates available for methane production (Stadtman and Barker 1951, Gerardi 2003).

Environmental factors that influence anaerobic digestion

There are many "physical, chemical and physiological factors in the environment that affect bio-degradation of organic compounds, such as availability of the compounds, the availability of electron donors and acceptors, oxygen concentration, temperature, pH, moisture, salinity, sorption of chemicals to particulate material, concentration of the chemicals" (Angelidaki and Sanders 2004). Different factors might have different influences according the specific characteristics of the compound being degraded (Angelidaki and Sanders 2004).

Anaerobic digestion is carried out by different groups of bacteria/archaea that need to live in balance and need to share the limited amount of energy during the conversion process, resulting in their slow growth rate (Lyberatos and Skiadas 1999, Angelidaki and Sanders 2004). One of the major factors governing the biodegradation of polymers is the nature and availability of electron acceptors. Under aerobic conditions oxygen acts as an electron acceptor due to the high gain in energy when it accepts electrons (Angelidaki and Sanders 2004). Under anaerobic conditions there is an absence of oxygen and inorganic electron compounds such as carbon dioxide act as the electron acceptor. A small amount of energy is thus gained, and the energy released in the redox process as a result of electron transfer is used for maintenance and growth of the microbial population. This means that anaerobic degradation is a slower process than aerobic degradation (Angelidaki and Sanders 2004).

Hydrogen partial pressure affects the redox potential in the liquid phase (Mosey 1982, Lyberatos and Skiadas 1999). Acidogenic bacteria follow the glycolytic metabolic pathway and the factor that regulates the amount of fatty acids produced is the liquid phase redox potential (Lyberatos and Skiadas 1999). This results in high hydrogen pressure increasing redox potential in the liquid phase, reducing substrate utilisation and fatty acid production (Mosey 1982, Harper and Pohland 1986).

Temperature influences the growth, survival and metabolic activities of all micro-organisms (Angelidaki and Sanders 2004). In general, higher temperatures result in higher metabolic activities of micro-organisms, provided they are not high enough to kill the organisms. Temperature is the most important variable in controlling the rate of microbial metabolism in anaerobic environments (Tchobanoglous *et al.* 2003).

Anaerobic digestion is applied under three different temperature ranges, i.e. the psychrophilic (<20 ^{o}C), the mesophilic (25-40 ^{o}C) and the thermophilic (45-60 ^{o}C). The process can also happen in the hyper-thermophilic range (65-100 ^{o}C) but this is rarely used in practical operations (Gerardi 2003). The growth rate of micro-organisms responsible for AD increases within each temperature group, with the different temperature groups showing some overlap (Figure 1.3; McCarty 1981, Tchobanoglous *et al.* 2003). Anaerobic reactors that are run at lower temperatures have higher retention times, lower heating costs and a more stable micro-organism population, while reactors run at thermophilic temperatures have a much lower retention times, higher operational costs for heating and a less stable micro-organism population (Siegrist *et al.* 2002, Gavala *et al.* 2003). Most AD facilities operate at mesophilic temperatures and successful operations require that the

temperature be maintained in one of these ranges to prevent culture crashes (Speece 1983, Tchobanoglous *et al.* 2003).

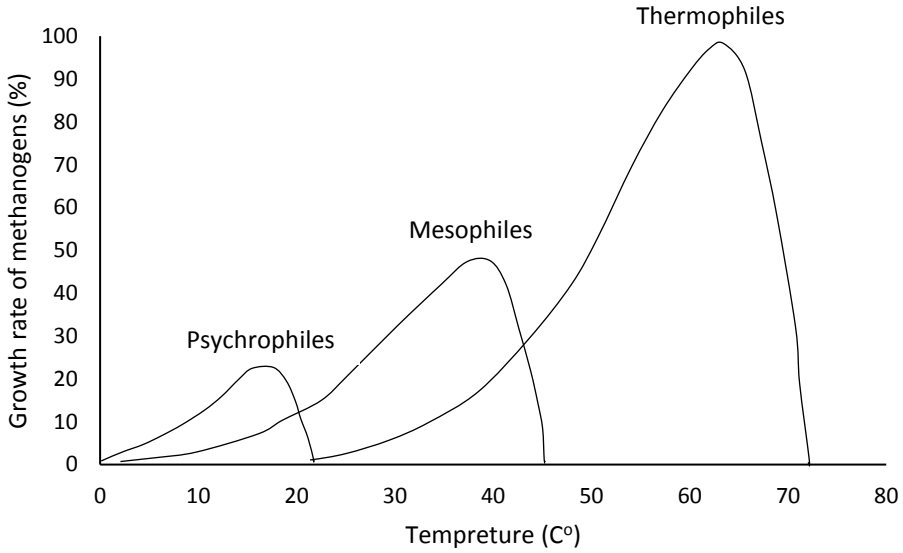

Figure 1.3: Relative growth rate of psychrophilic, mesophilic and thermophilic methanogens (van Lier *et al.* 1997).

Each group of micro-organisms in the AD process has its own optimum pH range, with acid producing bacteria having an optimal pH range of 5-7 and methanogenic archaea having an optimal range of 7-8 (Tchobanoglous *et al.* 2003, Chernicharo 2007). This results in an optimal combined pH range of 6.5-8.0 for anaerobic reactors (Tchobanoglous *et al.* 2003, Chernicharo 2007). Apart from its influence on the growth of micro-organisms, pH can also affect the dissociation of compounds such as ammonia, sulphide and organic acids, making them more/less toxic to micro-organisms (Kayhanian 1999, Tchobanoglous *et al.* 2003).

The pH operating parameters of digesters are commonly defined by the optimum range of methanogenic organisms rather than acidogenic organisms due to the slower growth rate of methanogens (Rajeshwari *et al.* 2000, Tchobanoglous *et al.* 2003, Buyukkamaci and Filibeli

2004). The domination of fermentative bacteria leads to excess VFA, which can lead to methanogenic inhibition and eventually failure (Rajeshwari *et al.* 2000, Buyukkamaci and Filibeli 2004). It is important to keep a balance between the rate of VFA production and the rate of methanogenesis, as methanogenic archaea are far more sensitive to the accumulation of VFA and the corresponding pH drop than the other micro-organisms involved in AD (Jiang 2012). If the rate of hydrolysis exceeds the rate of methanogenesis, accumulation of VFA and hydrogen will occur, causing a decrease in acetate and methane production which can lead to the irreversible acidification of the digester (Pavlostathis and Giraldo-Gomez, 1991). As such, pH and VFA concentration are good indicators of reactor stability (Tchobanoglous *et al.* 2003, Chernicharo 2007).

The pH in anaerobic digesters is mainly controlled by the bicarbonate buffer system. Bicarbonates and carbonates are formed from the production of carbon dioxide and the formation of soluble ammonia during the breakdown of proteins (Rozzi and Dipinto 1994, Pretorius 1994). The bicarbonate alkalinity generated from carbon dioxide and ammonia production is important as it balances the acidity generated from hydrolysis and is essential in maintaining a stable pH in reactors (Gallert *et al.* 1998, Hafner and Bisogni 2009). However, the production of hydrogen ions during AD normally exceeds the production of bicarbonate alkalinity, resulting in most treatment plants supplementing digesters with NaOH to stabilise digester pH (Rajeshwari *et al.* 2000). Monitoring pH and alkalinity is important in determining reactor stability as they can be an indicator of VFA concentrations and can be used to track corrective measures made to remediate them (Tchobanoglous *et al.* 2003, Chernicharo 2007).

The rate-controlling step during AD is predominately dependent on the nature of the feed (Speece 1983). Cellular biomass, greases, cellulose, and lignin degrade very slowly, and thus hydrolysis is normally the rate-limiting step of substrates where these make up the major components (Speece 1983). The digestion of feeds that contain a high content of insoluble organics are also limited by hydrolysis (Speece 1983). On the other hand, methanogenesis is normally the rate-limiting step for substrates that are mainly composed of soluble organic compounds due to the slower growth rate of methanogens compared to fermentative bacteria (Speece 1983).

The macro-nutrients needed by the micro-organisms responsible for AD include carbon, hydrogen, nitrogen, phosphorous, potassium, calcium, magnesium, sodium, iron and sulphur (Table 1.1; Mara and Horan 2003, Tchobanoglous *et al.* 2003). Apart from carbon, hydrogen and oxygen the two major macro-nutrients needed in the AD process are nitrogen and phosphorous with a required N/P ratio between 5-10:1 (Fricke *et al.* 2007, Weiland 2010, Jiang 2012). The general ratio of C/N/P/S for the AD process is 600:15:5:3 (Fricke *et al.* 2007, Weiland 2010). The overall stability of AD is influenced by the balance of these macro-nutrients, preventing the build-up of inhibitory products (Mara and Horan 2003).

Table 1.1: The effects and required concentration of some of the macro-nutrients needed by anaerobic micro-organisms (Mara and Horan 2003, Jiang 2012).

Nutrient	Required concentration (mg/l)	Effects on digestion
Ca	100-200	Essential for cell growth
Mg	75-150	Increases cell activity and facilitates granulation
Na	100-200	Increases cell activity
S	0.001-0.1	Used for protein synthesis

The C/N ratio of feeds has a major influence on the methane production rates, inhibition and operation during the AD process. The optimal C/N ratio for feedstocks is between 20-30, which results in a stable AD process (Kayhanian 1999, Zhang *et al.* 2007). If the C/N ratio is too low the metabolism of the substrate leads to an increase in ammonia formation which becomes toxic to anaerobic microbes (Hartmann and Ahring 2006). If the C/N ratio is too high the digester will be deficient in nitrogen, resulting in a decrease in anaerobic microbe growth (Jiang 2012). Since different wastes have various C/N ratios, the optimum C/N ratio can be achieved by co-digestion and this is recommended for waste streams that have a high or low C/N ratio (Callaghan *et al.* 1999, Alatriste *et al.* 2006, Bouallagui *et al.* 2009).

Micro-nutrients are required in low concentrations and are of vital importance in the formation and functioning of enzymes that are responsible for the breakdown of organic matter (Oleszkiewicz and Sharma 1990, Zandvoort *et al.* 2006). They are also involved in the microbial respiration process (Zandvoort *et al.* 2006). Micro-nutrients such as copper, molybdenum, nickel, selenium and tungsten have been identified as essential for enzymatic break down of organic matter while iron, copper, nickel and zinc are essential for methanogens to produce their maximum methane yield (Oleszkiewicz and Sharma 1990, Zhang *et al.* 2003, Worm *et al.* 2011). Although micro-nutrients are required in trace amounts, a deficiency will obstruct the AD process and can lead to inhibition and reactor failure (Zhang *et al.* 2003, Worm *et al.* 2011). The addition of these trace elements has been shown to increase the degradation of substrates and improve methane yields (Zhang *et al.* 2003, Worm *et al.* 2011, Roberts 2015). However, once over their tolerable concentrations all trace elements become potential toxicants and can inhibit microbial activities (Rajeshwari *et al.* 2000, Tchobanoglous *et al.* 2003). Rajeshwari *et al.* (2000) recommend

that the micro-nutrient in feeds should be about twice that required for cell growth. The elemental composition of methanogens is represented in Table 1.2 and can be used to calculate the micro-nutrient demands of anaerobic microbes (Rajeshwari *et al.* 2000).

Table 1.2: Elemental composition of methanogens per kilogram of dry biomass (Rajeshwari *et al.* 2000).

Macro-nutrients	Concentration in bacteria (mg/kg)	Micro-nutrients	Concentration in bacteria (mg/kg)
N	65000	Ni	100
P	15000	Co	75
K	10000	Mo	60
S	10000	Zn	60
Ca	4000	Mn	20
Mg	3000	Cu	10
Fe	1800		

Inhibition of anaerobic digestion

Ammonia is toxic to all micro-organisms at certain concentrations, especially in its unionised form (NH_3; Wurts 2003, Freeman 2005). In solution ammonia is in equilibrium between its unionised form (NH_3) and its ionised form (NH_4^+). The ratio between the ionised and unionised form is influenced by pH and temperature (Kayhanian 1999, Tchobanoglous *et al.* 2003). The percentage of unionised ammonia increases with pH, resulting in greater ammonia inhibition (Kayhanian 1999). Of the micro-organisms responsible for AD, methanogens are the most sensitive to ammonia inhibition and when this happens the concentration of VFAs will increase, accompanied by a decrease in pH. The decrease in pH will partly counteract the effect of ammonia due to the decrease in free ammonia, and this is known as the inhibited steady state (Tchobanoglous *et al.* 2003). Feedstocks with a low

C/N ratio will result in a large production of ammonia during acidogenesis and it is therefore advised to co-digest low C/N feedstock with high C/N feedstock to reduce ammonia production and inhibition (Alatriste *et al.* 2006, Hartmann and Ahring 2006, Bouallagui *et al.* 2009).

Sulphate reducing bacteria (SRB) are present in anaerobic microbial communities and utilise sulphate as the electron acceptor during the respiration of fermentative products, reducing sulphate to H_2S (Odom and Singleton 1993, Muyzer and Stams 2008). The two main groups of SRB are: heterotrophic SRB which use various intermediate compounds as a substrates (acetate, VFA, sugar, hydrogen etc.) and directly compete for acetate with acetoclastic methanogens; and autotrophic SRB which reduce carbon dioxide with electrons from hydrogen and complete with hydrogenotrophic methanogens (Lens and Kuenen 2001, Liamleam and Annachhatre 2007). Sulphur can be used as a substrate for SRB in its various forms of oxidation (thiosulphate, sulphite and sulphur) to produce sulphate and sulphide (Bak and Pfennig 1987, Böttcher *et al.* 2005). Sulphate reducing bacteria exist in large numbers within the anaerobic environment and use the same fermentative products as methanogens (Odom and Singleton 1993). Sulphate reduction is more energetically favourable than methanogenesis and will out-compete methanogenesis, resulting in SRB having a higher growth rate and lower half saturation value due to the kinetic advantage they have over methanogens (Speece 1983, Muyzer and Stams 2008). As the sulphate concentration in feedstocks increases so does the H_2S production which is accompanied by a reduction in methane production (Muyzer and Stams 2008). This is generally undesirable as H_2S can cause toxicity/inhibition to methanogens which can lead to digester acidification and eventually failure. Metals such as Ni, Co and Fe precipitate with S^{2-} and can lead to nutrient deficiencies in reactors (Odom and Singleton 1993). When biogas containing H_2S is

combusted, sulphur oxides are produced which results in reduced efficiency and damage to equipment (Odom and Singleton 1993, Tchobanoglous *et al.* 2003).

The bacteria and archaea responsible for AD require sulphur for growth, with optimal concentrations between 1-25 mg/l SO_4, while inhibitory effects are reported at concentrations above 50 mg/l SO_4 (Scherer and Sahm 1981, O'Flaherty *et al.* 1999). Hydrogen sulphide concentrations above 200 mg/l have been shown to be inhibitory and toxic to bacteria and archaea present in digesters (Koster *et al.* 1986, Parkin *et al.* 1990). There is a large variation in the reported concentrations of sulphate and H_2S that can cause inhibition and toxicity, and this is primarily due to operational design and the inoculum's natural tolerance limits. The pH of the digestate has the greatest effect on the toxicity of H_2S because pH influences the percentage of ionised H_2S. As the pH increases the percentage of the less toxic, ionised HS^- increases with HS^- accounting for the majority of sulphide at a pH between 8-9. At a pH between 7-8 (normal range to anaerobic reactors) 50 % of sulphide exists as the highly toxic H_2S, which is the only form able to pass through the cell membrane (Speece 1983, Chernicharo 2007). As the pH drops below seven the percentage of dissolved H_2S increases significantly, therefore it is vital to keep the pH at the upper limit when operating digesters containing high concentrations of sulphide.

Hydrogenotrophic methanogens are inhibited by nitrogen oxides such as nitrate, nitrite and nitric oxide (Tchobanoglous *et al.* 2003). Balderston and Payne (1976) found that nitrogen oxides inhibit methane production; however, the underlying cause for inhibition was unclear and could be due to inhibition of some component of the methanogenic enzyme complex itself. The effects of nitrogen oxides on AD are ambiguous and very complex, but a high concentration of nitrogen oxides in feeds has been found to decrease methanogenesis (Ahring 2003).

Heavy metals such as zinc, copper and cadmium can be toxic to acidogenic micro-organisms (Ahring and Westermann 1983). However, many of these elements and compounds can be tolerated in relatively high concentrations due to adsorption of the metal to organic compounds in the reactor (Ahring and Westermann 1983).

A sudden increase in the load of proteins, lipids and easily degradable substrates can cause reactor inhibition (Lu 2006). An increase in proteins will lead to an increase in ammonia concentration, while an increase in lipids will lead to an increase in long chain fatty acids (LCFA) which can inhibit the AD process as LCFA inhibit the metabolism of hydrolytic, acidogenic and methanogenic micro-organisms above certain concentrations (Lu 2006). A sudden increase in easily degradable substrates will be quickly hydrolysed into acids, resulting in a pH decrease in the digesters and the inhibition of methanogens which will further increase the VFA concentration in the digester and can lead to digester failure (Lyberatos and Skiadas 1999, Angelidaki and Sanders 2004, Lu 2006). It is therefore important to introduce new feeds gradually into anaerobic digesters to allow for the micro-organism community to adapt to them.

Theoretical methane yield

When determining the methane potential of different substrates, their chemical composition is of primary concern (Angelidaki and Sanders 2004). During anaerobic degradation of organic matter, carbon is converted into its most oxidised form, carbon dioxide, and its most reduced form, methane (Angelidaki and Sanders 2004). The ratio between the amount of carbon dioxide and methane formed depends on the oxygen state of the carbon in the substrate, with a positive relationship existing between the reduced carbon content of the substrate and methane production. The chemical composition of the

substrate can be used to calculate the maximum theoretical methane yield for the complete conversion of organic material to methane using Buswell's equation (Equation 1.1, Symons and Buswell 1933). Using the molar mass of carbon, hydrogen and oxygen the specific methane yield of a substrate can then be calculated using Equation 1.2 (Table 1.3; Symons and Buswell 1933, Angelidaki and Sanders 2004).

$$C_nH_aO_b + (n - a/4 - b/2)H_20 \rightarrow (n/2 + a/8 - b/4)CH_4 + (n/2 - a/8 + b/4)CO_2 \quad [1.1]$$

$$\text{Specific methane yield (l/g } VS_{fed}) = (n/2 + a/8 - b/4)x22.4 / (12n + a + 16b) \quad [1.2]$$

Table 1.3: Theoretical methane yield of various substrates (Angelidaki and Sanders 2004).

Substrate type	Composition	CH_4 Yield (l/g VS_{fed})	CH_4 composition of biogas (%)
Carbohydrate	$(C_6H_{10}O_5)_n$	0.415	50
Protein*	$C_5H_7NO_2$	0.495	50
Lipids	$C_{57}H_{104}O_6$	1.014	70
Ethanol	C_6H_6O	0.730	75
Acetate	$C_2H_4O_2$	0.373	50
Propionate	$C_3H_6O_2$	0.530	58

Volatile solids (VS), * Nitrogen converted to ammonia.

The theoretical methane yield gives an idea of the potential biogas of substrates but in practical situations the biogas and methane yield will always be lower (Angelidaki and Sanders 2004). This is due to many factors such as: 5-10 % of the substrate is used for bacterial biomass production; some of the substrate is lost in the effluent; lignin and calcite containing compounds are not biodegradable; some organic material is inaccessible due to binding in particles or the structural composition of organic matter; and nutrient limitation (Angelidaki and Sanders 2004, Alzate *et al.* 2012). The carbon dioxide produced partly

dissolves in the liquor whereas methane mainly stays in the gas phase, resulting in a higher methane content of biogas than that predicted by the stoichiometric ratio (Angelidaki and Sanders 2004).

1.2.2 Activated sludge

Activated sludge (AS) is a process in biological wastewater treatment in which aerobic micro-organisms are cultured in suspension with the wastewater. The majority of micro-organisms in AS are facultative heterotrophic organisms which oxidize and mineralize the organic matter in wastewater into more stable products (Tchobanoglous *et al.* 2003, Mara and Horan 2003). It is the most widely used biological treatment of municipal and industrial effluent and has been in use for over 100 years (Mara and Horan 2003, Jenkins and Wanner 2014). The AS process consists of an aeration tank in which the micro-organisms are cultured and a sedimentation tank or clarifier which separates the solids from the treated effluent (Figure 1.4).

Aerators are used to supply oxygen to the micro-organisms in the aeration tank and keep them in suspension within the water column. The clarifier or sedimentation tank after the aeration tanks is responsible for separating the solids (biomass) from the treated effluent (Figure 1.4; Spellman 2000, Snyder and Wyant 2005). The settled sludge is then recirculated back to the aeration tank or is wasted, depending on the mixed liquor suspended solids (MLSS) concentration in the aeration tank (Figure 1.4; Spellman 2000, Snyder and Wyant 2005). A high concentration of micro-organisms is maintained in the aeration tank to increase the treatment performance and reduce the HRT of the reactor (Jenkins and Wanner 2014). However, if the MLSS concentration becomes too high this can result in

solids remaining in the effluent after clarification thus decreasing the quality of the treated effluent (Marais and Ekama 1976, Eckenfelder and Grau 1992, Jenkins and Wanner 2014). Desludging is carried out at intervals on AS plants to maintain desired MLSS concentrations (Jenkins and Wanner 2014).

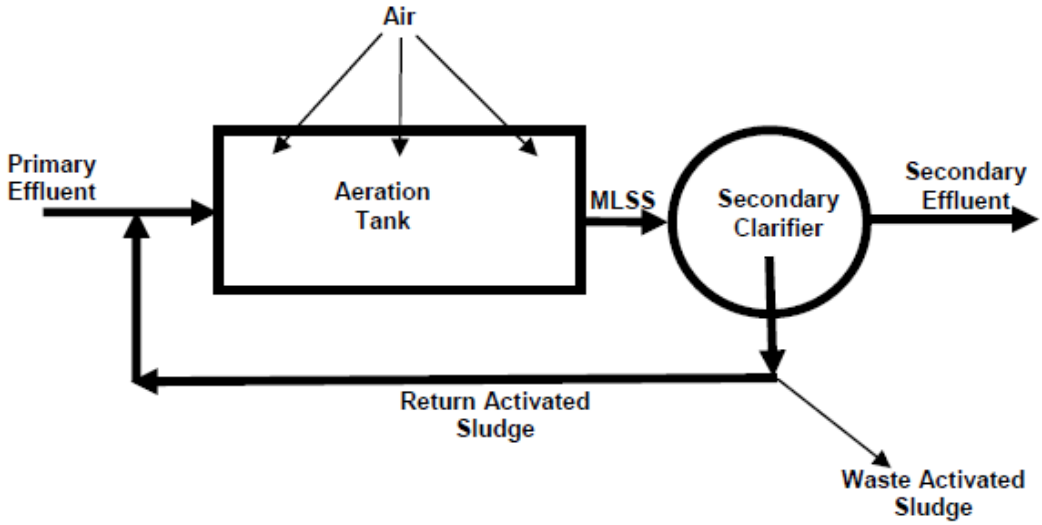

Figure 1.4: The activated sludge process, mixed liquor suspended solids (MLSS; Snyder and Wyant 2005).

The AS process performs four major treatment functions on wastewater: the oxidation or degradation of carbonaceous material; the oxidation or degradation of nitrogenous material; the removal of fine solids; and the removal of heavy metals (Marais and Ekama 1976, Eckenfelder and Grau 1992, Jenkins and Wanner 2014). The growth and maintenance of the large and diverse population of bacteria suspended in the aeration tank are mainly responsible for performing these functions (Jenkins and Wanner 2014). On average 0.7 kg of biomass is produced per kilogram of biological oxygen demand removed from the wastewater (Spellman 2000, Snyder and Wyant 2005). In summary, the AS process uses oxygen to convert the biodegradable components in wastewater into new biomass, carbon

dioxide, water and residual organic matter (Marais and Ekama 1976, Eckenfelder and Grau 1992).

The biological and process kinetics of the AS process are governed by the micro-organism composition, which in turn is influenced by the influent wastewater and the configuration of aeration tanks and their operation (Tchobanoglous *et al.* 2003, Jenkins and Wanner 2014). For example, if the main function of AS is nitrogen removal then an anoxic zone needs to be incorporated into the design to allow for denitrification (Spellman 2000, Tchobanoglous *et al.* 2003). The treatment performance of AS reactors is related to the growth rate of the micro-organisms, with increased treatment efficiencies at higher biomass growth rates (Mara and Horan 2003, Jenkins and Wanner 2014). Micro-organism growth rate is influenced by many variables, including the MLSS present in the reactor, substrate type, temperature, pH, operational procedures and the presence of toxins (Li *et al.* 2008, Rustum 2009, Jenkins and Wanner 2014).

Environmental factors that influence the activated sludge process

Aeration is used during AS to supply oxygen to the micro-organisms for their metabolism, which in turn will sustain the continuous degradation of organic matter and the nitrification process (Eckenfelder and Grau 1992, Mara and Horan 2003). A minimum of 1.5-2.0 mg/l of oxygen is required in the aeration tank to ensure the proper operation of the system. Low oxygen to the aeration tank is implicated in the growth of filamentous bacteria which causes settling challenges amongst other problems (Ritmann and McCarty 2001).

Activated sludge is a biological system and the activity and metabolism of sludge biomass are influenced by the temperature of the effluent (Mara and Horan 2003, Jenkins and Wanner 2014). In general, a 10 °C drop in temperature results in a 50 % decrease in sludge

biomass growth, resulting in a slower chemical oxygen demand (COD) removal and longer HRT. The biological activity of AS increases up to a maximum temperature of 90 °C, after which the activity drastically decreases (Tchobanoglous *et al.* 2003, Orhon *et al.* 2009). Most AS systems operate at mesophilic temperatures or ambient temperatures as the cost of heating the effluent outweighs the increased treatment performance (Snyder and Wyant 2005, Orhon *et al.* 2009).

A pH between 6.5 and 8.5 and an alkalinity between 1.0-1.5 mmol/l are required for optimal growth of AS biomass (Eckenfelder and Grau 1992, Ritmann and McCarty, 2001, Orhon *et al.* 2009). Growth and effluent treatment will occur out of this range, but at a reduced rate (Ritmann and McCarty 2001, Orhon *et al.* 2009). Oxygen uptake by the micro-organisms is optimal between pH 6.5-7.5 and most AS systems are operated within this range (Mara and Horan 2003, Jenkins and Wanner 2014). Sudden pH changes in wastewater are the most damaging to AS systems (Tchobanoglous *et al.* 2003, Snyder and Wyant 2005). The alkalinity and pH of effluent generally decrease during treatment in an AS system due to the nitrification process (Orhon *et al.* 2009, Jenkins and Wanner 2014). However, denitrification produces alkalinity, and the overall alkalinity change of the effluent is dependent on which of the above processes dominate during effluent treatment (Mara and Horan 2003, Orhon *et al.* 2009).

A wide range of heavy metals (e.g. cadmium, chromium, nickel, lead, mercury and arsenic at concentrations >1mg/l), herbicides, pesticides and various other organic and inorganic compounds are toxic to AS micro-organisms (Tchobanoglous *et al.* 2003, Jenkins and Wanner 2014). Toxicity can normally be noticed by an increase in the dissolved oxygen (DO) concentration of the aeration basin in conjunction with decreased treatment performance

(Tchobanoglous *et al.* 2003, Snyder and Wyant 2005). Deflocculation, and an increase in treated effluent turbidity, may also be signs of toxicity in the aeration basin as the organisms are impaired or destroyed by the toxic substance (Jenkins and Wanner 2014). An issue with the treatment of wastewater containing heavy metals is that these toxins become concentrated in the sludge biomass (Quan-ying *et al.* 2007, Lu *et al.* 2012). This leads to health issues regarding the disposal of the WAS, especially if it is put on agricultural lands (EPA 1999, Quan-ying *et al.* 2007).

Nitrogen removal

Nitrogen normally enters the aeration basin as ammonia or organically bound nitrogen. The organically bound nitrogen is broken down into ammonia via hydrolysis (Spellman 2000, Jenkins and Wanner 2014). Some of the dissolved nitrogen in the effluent is consumed by the bacteria and incorporated into the new biomass (Tchobanoglous *et al.* 2003). The ammonia in the effluent is converted to nitrite and then nitrate by bacteria in a process known as nitrification (Spellman 2000, Orhon *et al.* 2009). The nitrate can then be gassed out of the system as nitrogen gas in a process known as denitrification (Ritmann and McCarty 2001, Mara and Horan 2003). Denitrification occurs at low dissolved oxygen concentrations (<0.8 mg/l), therefore most AS systems have an anoxic zone where denitrification occurs. Nitrogen removal in AS systems has been reported to happen via ammonia out-gassing (especially if the effluent pH is greater than 8.5) and nitrogen oxides (NO_X) emissions, but the main removal mechanisms are incorporation into biomass and denitrification (Mara and Horan 2003, Jenkins and Wanner 2014).

Phosphorus removal

Phosphorus removal during the AS treatment process can happen through incorporation into micro-organism biomass and through chemical precipitation (Tchobanoglous *et al.* 2003, Jenkins and Wanner 2014). Up to 50 % of the incoming phosphorus may be incorporated into bacterial biomass and this percentage is mainly influenced by the incoming phosphorous concentration, with removal decreasing as incoming phosphorous concentration increases (Snyder and Wyant 2005, Orhon *et al.* 2009). Bacteria such as *Acinetobacter* spp. achieve phosphorus removal by storing and using ortho-phosphorus as an energy source (Wentzel *et al.* 1986).

Phosphate has also been shown to adsorb to the negatively charged outer layers of sludge biomass (Spellman 2000, Jenkins and Wanner 2014). Chemical precipitation of phosphorous is achieved by the addition of ferric chloride, aluminium sulphate or lime which combines with phosphorous to form a particle which is settled out in the clarifier (Snyder and Wyant 2005, Orhon *et al.* 2009). Iron or aluminium salts are normally added to the aeration basin of an AS system if biological assimilation is not sufficient to remove phosphate from effluent to within permissible limits (Snyder and Wyant 2005, Oikonomidis *et al.* 2010). Phosphate removal increased from 40 % to 80 % when Fe(II) was added to the aeration basin at 25 mg Fe per cubic meter of effluent fed (Oikonomidis *et al.* 2010). The chemical precipitation of phosphate with metal salts is dependent on pH with removal decreasing with increasing pH above five due to the greater formation of metal hydroxides at alkaline pH values (Gillberg *et al.* 1996). The addition of iron chloride salts has also been found to aid in the settling of flocs as they become larger and denser (Oikonomidis *et al.* 2010).

1.2.3 Integrated algal ponding systems

Algal aquaculture consists of monoculture systems used to cultivate specific strains of algae which are mainly used to produce products (polyunsaturated fatty acids, amino acids, pigments and natural dyes) for the pharmaceutical, nutraceutical, food and animal feed industries or mixed culture systems used for biofuel production (Borowitzka 1995, Luiten *et al.* 2003, Spolaore *et al.* 2006, Wang *et al.* 2016). Unlike aquaculture of unicellular algae for pharmaceutical and nutraceutical industries, where monocrops are produced and the production environment is manipulated to maximise algal production, the main purpose of mixed culture systems is effluent treatment (Oswald 1995, Craggs *et al.* 2014).

High rate algal ponds (HRAP) are shallow raceway-type ponds which are used either for effluent treatment purposes or for the aquaculture of mono-algal crops (Aguirre *et al.* 2011, Craggs *et al.* 2011). They consist of algae, bacteria and various other micro-organisms that are cultured in the wastewater and normally kept in suspension using paddle wheels (Aguirre *et al.* 2011, Craggs *et al.* 2011). The microbial community present in HRAP is dynamic and the species composition changes in response to changes in the effluent stream, particularly in effluent treatment where the culture is not manipulated to favour a monocrop (Mogane 2016, Jones *et al.* 2016). Currently, these mixed algal community complexes carry limited application and commercial value, due to the small size of many of the algal species and the difficulty of harvesting them (Craggs *et al.* 2011, Jones *et al.* 2016); the current research will focus on determining this value and adding value to the algal cultured in HRAP systems used for effluent treatment.

Wastewater treatment HRAPs are a passive treatment process that utilise gravity, solar energy and biological activity during treatment (Oswald 1995, Downing *et al.* 2002, Mambo

2014). They are low-technology driven and require less energy inputs and lower operational costs compared to conventional wastewater treatment systems (Oswald 1995, Craggs *et al.* 2011, Al-Balushi *et al.* 2012; Jones *et al.* 2016). High rate algal ponds are normally part of an advanced pond system comprising facultative ponds, algal ponds and settling ponds (Craggs 2005, Aguirre *et al.* 2011, Craggs *et al.* 2011), referred to as integrated algal ponding systems (IAPS; Rose *et al.* 2007, Jones *et al.* 2014).

In IAPS, wastewater first passes through a primary facultative pond (PFP) where settling, aerobic and anaerobic degradation of pollutants take place (Oswald 1995, Downing *et al.* 2002, Mambo 2014). The top layer (0-30 cm) of the PFP is aerobic while the lower layers are anaerobic (Oswald 1995, Downing *et al.* 2002, Mambo 2014). Wastewater decants out of the PFP into the HRAP where algae and aerobic micro-organisms oxidise pollutants into more stable products (Oswald 1995, Downing *et al.* 2002, Mambo 2014). Post-HRAP effluent then flows into settling ponds/clarifiers where the treated water is separated from the algal/bacterial biomass (Oswald 1995, Downing *et al.* 2002). The treated water can then be further treated using mechanical filtration for reuse purposes while the settled biomass can be used for fuel production or land application (Oswald 1995, Mambo 2014).

A symbiotic relationship exists in HRAP between the consortia of algae, bacteria and fungi (Figure 1.5; Oswald 1995). During the day autotrophic algae photosynthesize, releasing oxygen into the water, thus maintaining aerobic conditions within the ponds (Oswald 1995, Craggs 2005). The organic matter in the wastewater is aerobically degraded by heterotrophic bacteria and fungi, resulting in the release of carbon dioxide and ammonia which are assimilated into the biomass of the algae (Chen *et al.* 2003; Aguirre *et al.* 2011, Jones *et al.* 2016). The autotrophic algae present in HRAP synthesize organic carbon from

carbon dioxide, inorganic nutrients and sunlight, making them a technology with a low carbon footprint (Oswald 1995, Craggs 2005).

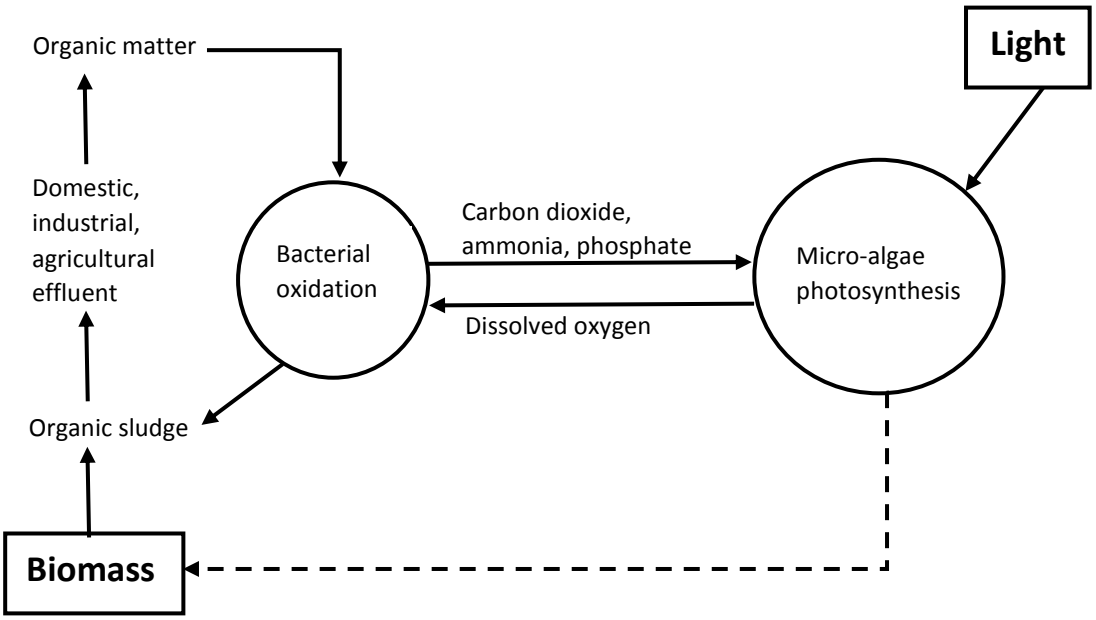

Figure 1.5: Symbiotic relationship between algae and bacteria in high rate algal ponds (Oswald *et al.* 1955, Munoz and Guieysse 2006, Sutherland *et al.* 2015).

The symbiotic relationship between algae and bacteria in HRAP is normally beneficial but can be inhibitory in certain circumstances (Bell and Lang 1974, Munoz and Guieyse 2006). Algae enhance bacterial growth by releasing extracellular compounds, such as carbohydrates and oxygen which are utilised by bacteria (Bell and Lang 1974, Munoz and Guieyse 2006). However, some algae species can produce microcystins and antibiotics which have growth inhibitory effects on the bacteria (Lopes and Vasconcelos 2011, Wang *et al.* 2016). Algae can also alter the environmental conditions of the liquor, such as increasing pH (due to photosynthesis) resulting in unfavourable conditions for bacterial survival (Munoz

and Guieyse 2006, Jones *et al.* 2016). On the other hand, bacteria can enhance algal growth via the production of carbon dioxide, ammonia, vitamins and phytohormones (Wang *et al.* 2016). However, bacterial cells can also release lytic substances that can kill algal cells (Munoz and Guieysse 2006, Wang *et al.* 2016). In general, the relationship between algal cells and aerobic micro-organisms in HRAP is beneficial to both (Bell and Lang 1974, Munoz and Guieyse 2006, Wang *et al.* 2016).

High rate algal ponds are effective at removing nutrients and decreasing the COD of wastewater, with most operating at a HRT of two to seven days (Oswald 1995, Craggs 2005, Munoz and Guieyse 2006, Mambo 2014, Jones *et al.* 2016). The assimilation of carbon, nitrogen and phosphorus by algae during photosynthesis occurs at a ratio of approximately 106:16:1 respectively (Fogg 1991). The two main factors affecting the HRT of HRAP are photoperiod and temperature, with a decrease in HRT as photoperiod and temperature increase (Oswald 1995, Cilliers 2012, Jones *et al.* 2016). High rate algal ponds are capable of removing more than 80 % of nitrogen, phosphorous and COD when operated under optimal conditions (Evans *et al.* 1997, Garcia *et al.* 2006, Jones *et al.* 2016). The treated water can be used for downstream fish culture, hydroponics, crop irrigation or reused by the primary water user (Jones *et al.* 2014, Power and Jones 2016, Taylor *et al.* 2018).

The design of HRAP mainly takes into account the organic load of effluent, the temperature and the available solar energy (Aguirre *et al.* 2011). The factors that can influence the performance of HRAP can be grouped into abiotic, operational and biotic. Abiotic factors include light intensity and quality, season, latitude, pH, temperature, and dissolved oxygen and carbon dioxide concentrations (Oswald 1995, Johnson 2010, Jones *et al.* 2016). Operational conditions include pond depth, HRT and turbulence (Oswald 1995, Johnson

2010, Jones *et al.* 2016). Biotic variables such as competition for resources, zooplankton grazing, pathogens and parasites can influence the performance of HRAP (Johnson 2010, Markou and Geogakakis 2011).

Environmental factors that influence high rate algal ponds

Algae, like other plants, use light as a source of energy during photosynthesis (Freeman 2005). Phototrophic organisms can utilise light with wavelengths between 400-770 nm (Richmond 2004). Light intensity varies during the day, and with season and latitude, therefore these factors play an important role in influencing the productivity and performance of HRAP (Richmond 2004, Freeman 2005, Munoz and Guieysse 2006). The highest HRAP treatment performance is observed in summer in low latitude regions and in the middle of the day, when the light intensity is highest (Richmond 2004, Freeman 2005, Munoz and Guieysse 2006). As latitude increases, and in winter, light has to travel farther through the atmosphere resulting in more light reflection and decreased photosynthesis (Richmond 2004, Freeman 2005, Munoz and Guieysse 2006).

Water turbidity affects the penetration depth of light needed for photosynthesis (Talbot *et al.* 1991, Richmond 2004). High rate algal ponds treating turbid wastewaters may require a longer HRT and shallower pond depth for efficient pollutant removal (Oswald 1995).

Algae undergo changes in their cell composition and physiological properties in response to different light intensities (Richmond 2004, Freeman 2005, Jones *et al.* 2016). Algae growth rates increase with light intensity up to an optimal intensity for that species in particular environmental conditions (Richmond 2004, Freeman 2005). Light intensities higher than the optimal intensity can lead to photoinhibition and decreased algal productivity (Talbot *et al.* 1991, Richmond 2004, Freeman 2005). High light intensities at low temperatures can also

result in photoinhibition. However, this is normally associated with increased temperatures, and photoinhibition of micro-algae grown under ambient light conditions is uncommon (Talbot *et al.* 1991). Increased temperatures is associated with a decrease in carbon dioxide solubility; at higher temperatures algal productivity is increased, resulting in increased carbon dioxide uptake by the algae (Richmond 2004, Freeman 2005). This can result in carbon dioxide depletion, and carbon dioxide addition is then required to maintain or increase algal productivity and HRAP treatment performance (Mashauri and Kayombo 2002, Kayombo *et al.* 2002, Sutherland *et al.* 2015). At water temperatures between 26-35 °C the limiting factor of algal productivity is normally dissolved carbon dioxide concentration, provided there are sufficient nutrients in the effluent. Subsequently, the addition of carbon dioxide will increase the productivity and treatment performance of HRAP (Mashauri and Kayombo 2002, Kayombo *et al.* 2002, Sutherland *et al.* 2015).

Most warm water micro-algae species grow and reproduce at temperatures between 15 and 35 °C (Richmond 2004, Griffiths 2010). Below 15 °C the growth of warm water algal populations is drastically decreased, with some dying (Richmond 2004). The growth rate of warm water algae increases with increasing temperature until a maximum growth rate is reached, which is normally around 35°C (Robarts and Zohary 1987, Park *et al.* 2011). A further increase in temperature results in a decrease in growth rate and algal productivity due to an increase in photorespiration and enzyme activity (Park *et al.* 2011). The growth rate of algae is thought to follow the biological temperature coefficient, with a 10 °C rise in temperature doubling algal growth (Richmond 2004, Munoz and Guieysse 2006). Nutrient removal and treatment performance of HRAP increase with an increase in temperature to an optimal temperature between 30-35 °C (Munoz and Guieysse 2006).

The alkalinity and pH of wastewater in HRAP follows a diurnal cycle where the pH reaches its highest at sunset and is lowest at sunrise. During the day, photosynthetic uptake of dissolved carbon dioxide is associated with the dissociation of bicarbonate and the production of hydroxyl ions, thus increasing the water alkalinity and pH to 8.5-11.0 (Tadesse *et al.* 2004, Jones *et al.* 2014). At night, respiration occurs, and this produces carbon dioxide which dissociates into carbonic acid causing the pH to drop to 6.8-8.0 (Wurts and Durborow 1992, Tadesse *et al.* 2004, Jones *et al.* 2014).

The dissolved oxygen concentration of the liquor in HRAP follows a diurnal pattern, with DO concentration peaking at sunset and bottoming at sunrise (Tadesse *et al.* 2004). During the day, photosynthetic activity produces oxygen which increases the DO concentration to supersaturated levels (8.0-11.0 mg/l; Tadesse *et al.* 2004, Mogane 2016, Jones *et al.* 2016). At night, respiration takes place which takes up oxygen resulting in a decrease in DO concentration below saturated levels (2.0-7.0 mg/l; Tadesse *et al.* 2004, Jones *et al.* 2016). The difference between sunset and sunrise DO concentration is influenced by algal biomass with a higher fluctuation in DO experienced at greater algal concentrations (Rubio *et al.* 1999).

Nitrogen removal

Organically bound nitrogen such as proteins and amino acids are hydrolysed in HRAP, resulting in ammonia production (Katipoglu-Yazan *et al.* 2012). Algae are able to assimilate ammonia; however, the majority of ammonia is oxidised to nitrite and then nitrate by *Nitrosomonas* and *Nitrobacteria* respectively (Mara and Horan 2003). Nitrate is then easily assimilated by the algal cell into its cellular biomass (Barker and Mills 1980, Raven *et al.* 1992).

Denitrification also occurs in HRAP, provided the conditions are favourable, where nitrate is reduced to nitrogen gas (Rodriguez-Caballero *et al.* 2014). The high pH (>9.0) in HRAP allows ammonia volatilisation from the surface of the pond and researchers have found that up to 70 % of nitrogen removal from wastewater can be due to ammonia volatilisation (Chen *et al.* 2003, Rose *et al.* 1996, Jones *et al.* 2016, Mogane 2016). In general, the majority of incoming nitrogen (40-60 %) is incorporated into algal biomass and the remainder is either removed through ammonia volatilisation and denitrification or remains in the effluent (Garcia *et al.* 2000, Park and Craggs 2011, Alcantara *et al.* 2015, Jones *et al.* 2016, Mogane 2016). The fate of nitrogen removal in HRAP is influenced by many factors, including its concentration, DO, pH, HRT and the species of micro-organisms used in the biological treatment process (Garcia *et al.* 2000, Tchobanoglous *et al.* 2003, Park and Craggs 2011).

Phosphorous removal

During treatment in HRAP, organic phosphorus is converted into soluble orthophosphates by aerobic bacteria (Larsdotter 2006, Powell *et al.* 2008). Soluble orthophosphates are then assimilated by micro-algae (Larsdotter 2006, Powell *et al.* 2008, Jones *et al.* 2016, Mogane 2016). The alkaline pH of HRAP liquor enables the adsorption of inorganic phosphate to algal and bacterial cells, which is removed via settling (Tadesse *et al.* 2004, Shilton 2006). Bacteria present in HRAP do assimilate orthophosphate but a greater proportion is accumulated in the algal biomass (Nurdogan and Oswald 1995, Rose *et al.* 1996).

Phosphate can precipitate out of solution with cations such as magnesium, calcium and aluminium present in the wastewater, when the pH is above 11.0 (Lasdotter 2006). However, the removal of phosphate is predominated by algal assimilation when the pH is below 11 (Lasdotter 2006). Mogane (2016) found algal assimilation to be the main

mechanism for phosphate removal from HRAP used to treat brewery effluent, with this removal rate significantly increasing when the water temperature was increased from 20 to 30 °C or when carbon dioxide was added to the effluent.

1.2.4 Anaerobic digestion of waste activated sludge and algal biomass

Research conducted on the AD of algae and WAS is mainly limited to biochemical methane potential (BMP) and specific methane production (SMP) assays, with limited literature on the continuous digestion of micro-algae and WAS (Yen and Brune 2007, Mussgnug *et al.* 2010, Gonzalez-Fernandez *et al.* 2011, Alzate *et al.* 2012, Park and Li 2012, Frigon *et al.* 2013, Schwede *et al.* 2013). To add to this, no research is available on the AD of brewery effluent grown algae or WAS, while only a handful of publications have investigated the introduction of effluent-grown algae (used to remove nutrients) in anaerobic digesters as a means of energy recovery from HRAP (Green *et al.* 1995, Chinnasamy *et al.* 2010, Alzate *et al.* 2012, Passos *et al.* 2013, Passos *et al.* 2015). The AD of micro-algae and WAS biomass is not straightforward due to several technical constraints, including low concentration of digestible biodegradable substrate, recalcitrant substrate constituents, cell wall degradability, low carbon to nitrogen ratio, ammonia toxicity and the effects of salinity and associated metal ions (Ward *et al.* 2014, Liden 2015).

Continuous digestion vs biochemical methane potential assays

Biochemical methane potential assays are relatively quick and easy to conduct to determine the maximum methane yield of a feedstock when compared to continuous digestion (Alzate 2012). The high ratio of inoculum to substrate prevents inhibitory effects such as VFA

accumulation and toxicity by certain compounds (Angelidaki and Sanders 2004, Alzate *et al.* 2012). However, BMP assays are unable to detect the effects of inhibition/toxicity on methane yield when the substrate is used in continuous digesters and commercial applications (Angelidaki and Sanders 2004, Park and Li 2012). The nitrogen and sulphide content of algae and WAS may cause inhibition of anaerobic micro-organisms during continuous digestion, but this may not be detected in BMP assays due to the lower concentration of substrate relative to inoculum (Angelidaki and Sanders 2004, Park and Li 2012, Roberts 2015). Biochemical methane potential assays can lead to misrepresentation of energy recovery during AD as they are generally run for long durations whereas continuous digestion is operated under much shorter retention times resulting in a decreased methane yield (Angelidaki and Sanders 2004). Park and Li (2012) found a 50 % reduction in methane production per VS added compared to reported BMP assay experiments when *Nannochloropsis* was subject to continuous digestion.

Algae

The methane potential of micro-algae falls within the reported yield for second generation biofuels (biofuels produced from non-food biomass) which has led to an interest in using algae as a feedstock for AD (Chandra *et al.* 2012, Ward *et al.* 2014). Ranges for methane production from BMP trials of micro-algae range from 0.024-0.600 l CH_4/g VS fed, with most studies observing a yield between 0.2-0.4 l CH_4/g VS (De Schamphelaire and Verstraete 2009, Gonzalez-Fernandez *et al.* 2011, Lakaniemi *et al.* 2011, Frigon *et al.* 2013). The highest methane yield of 0.6 l CH_4/g VS was recorded for a mixed algal feedstock that had undergone thermal pre-treatment (De Schamphelaire and Verstraete 2009). The large variation in methane production from micro-algae is due to the different inocula used, the

operating conditions of digesters and the operating conditions of algal cultures (Yen and Brune 2007, Mussgnug *et al.* 2010, Gonzalez-Fernandez *et al.* 2011, Alzate *et al.* 2012, Park and Li 2012, Frigon *et al.* 2013, Schwede *et al.* 2013). These conditions include aspects such as nutrient loading, retention time, photoperiod, temperature and environmental stress, and affect the composition of algal cells which in turn will influence the biogas production from these algae (Ward *et al.* 2014, Perez-Lopez *et al.* 2017). These give rise to the large variation in methane production values for algae, even from the same species.

Hydrolysis is normally the rate-limiting step during the AD of terrestrial autotrophs due to the resistance of their cell walls to hydrolysis, as this barrier protects the cell's contents from the fermentative bacteria (Liu *et al.* 2012). The lignocellulose part of the walls is especially resistant to hydrolysis, and feedstocks with a high lignocellulose content have a reduced methane yield and high HRT (Liu *et al.* 2012). Micro-algae do not contain lignocellulose and thus have been reported as being a suitable substrate for AD (Sialve *et al.* 2009, Heaven *et al.* 2011). However, this outer structure of micro-algal cells is resistant to hydrolysis and therefore its disruption is probably the main factor in improving the biodegradability of micro-algae (Markou *et al.* 2012). The cell wall issue is compounded by its high volume and surface area compared to the total cell volume in micro-algae (Klemm *et al.* 2005, Gonzalez-Fernandez *et al.* 2012).

Algal cell walls consist of several layers of glycoproteins, pectin, highly polymerised cellulose and hemicellulose with the diameter of fibres greater than in terrestrial plants (Klemm *et al.* 2005, Gonzalez-Fernandez *et al.* 2012, Ward *et al.* 2014). Physical, chemical and biological pre-treatments that break the cell wall have been successful in improving the digestibility of feedstocks but add to the cost of processing (Passos *et al.* 2013, Ward *et al.* 2014, Uggetti *et al.* 2017). The polymerised layer of cellulose in the wall is especially difficult to hydrolyse

and may pass through the digester without being fully broken down, resulting in a reduced methane yield (Gonzalez-Fernandez *et al.* 2012, Ward *et al.* 2014). Recalcitrant compounds (e.g. silica, uronic acid, lignin, polyaromatics, heteropolysaccharides, sporopollenin and algaenan) found in the wall and cytoplasm have a high resistance to biodegradation (Syrett and Thomas 1973, Gunnison and Alexander 1975, Gonzalez-Fernandez *et al.* 2012). *Chlorella* and *Scenedesmus* species have hemicellulose containing, carbohydrate-based walls which make them difficult to digest, and the pre-treatment process that resulted in the greatest cell disruption and disintegration resulted in the highest biogas yields (Mussgnug *et al.* 2010). Pre-treatments of algal feedstocks are done to improve their digestibility during AD. This results in an increase in processing costs but can increase the net energy production, improve the quality of the substrate and release nutrients that can be recovered downstream (Ward *et al.* 2014, Uggetti *et al.* 2017). Several methods exist, including biological, thermal hydrolysis, mechanical and chemical, with the main goal being to disrupt the algal cell wall to expose its contents to fermentative bacteria (Carrere *et al.* 2010). Samson and Leduy (1983) found a 26 % increase in soluble products from *Spirulina maxima* biomass after freeze/thawing, which can be expected to be accompanied by a decrease in HRT and increase the methane yield of digesters. Methane yield increased from 3 to 90 % when algal biomass had undergone thermal treatment ranging from 80-120 ^{o}C (Chen and Oswald 1998, Alzate *et al.* 2012, Gonzalez-Fernandez *et al.* 2012, Schwede *et al.* 2013). Passos *et al.* (2013) found a 78 % increase in SMP, after 45 days, when algae from a HRAP used to treat municipal sewage were subject to microwaves. Despite the increase in methane production, the high energy utilisation from microwave generation makes it an unfavourable method, and other lower energy solutions should be investigated (Passos *et al.* 2013). Pre-treatment methods can increase the methane yield of micro-algae, but the

energy cost of pre-treatment may exceed the energy gained from the extra methane produced, as has been observed by several authors (Yen and Brune 2007, Lee *et al.* 2012, Lee *et al.* 2013, Lu *et al.* 2013). It is therefore essential to calculate an energy balance to ensure that there is a net energy gain obtained from the cell destruction method (Passos *et al.* 2013, Uggetti *et al.* 2017).

It has been reported that algae cultures cannot be directly used for AD without concentrating the algal biomass first (Golueke and Oswald 1959, Green *et al.* 1995, Ward *et al.* 2014). This is due to the low VS in algal cultures which, when fed to digesters at sufficient rates, increase the HRT of the digesters causing bacterial washout (Ward *et al.* 2014). Therefore, some sort of algal biomass concentration step or digester design is required to increase digester SRT needed to ensure washout does not occur.

Anaerobic digesters such as anaerobic membrane reactors, anaerobic fluidised bed reactors, in pond digesters and UASB reactors that allow the decoupling of HRT and solid retention time are most suitable to digest algal biomass (Green *et al.* 1995, Zamalloa *et al.* 2012, Ward *et al.* 2014). An anaerobic membrane reactor fed *Phaeodactylum tricornutum* was able to maintain stability and reduce VS by up to 40 % when operated at a HRT of 2.5 d and a SRT of 10-20 days (Zamalloa *et al.* 2012).

The use of chemical coagulants (ion salts, aluminium salts or lime) have been found to increase the TS concentration of algal biomass when settled via gravity (Benemann *et al.* 1977, Harun *et al.* 2010). Mechanical dewatering steps such as a centrifuge, belt press, filter press and a screw drum can be used to increase the TS concentration of settled biomass (Golueke and Oswald 1959, Collet *et al.* 2011, Ward *et al.* 2014). However, all authors identified the high costs associated with the use of flocculation and the above-mentioned

mechanical harvesting techniques as a concern (Golueke and Oswald 1959, Harun *et al.* 2010, Collet *et al.* 2011, Ward *et al.* 2014).

The reported C/N ratio for algae ranges between 5-10, which is lower than the optimal range of 20-30 for AD (Yen and Brune 2007, Zhang *et al.* 2007, Ward *et al.* 2014). The low C/N ratio of algae generally leads to ammonia and VFA accumulation in digesters which reduces methanogenesis and can even cause digester failure (Yen and Brune 2007, Uggetti *et al.* 2017). The lipid concentration and C/N ratio of micro-algae can be increased by nutrient starvation prior to harvesting or light deficiency (Richardson *et al.* 1969, Wang *et al.* 2008, Gonzalez-Fernandez *et al.* 2012). Nitrogen deficiency in a culture medium can increase the C/N ratio of algal cells however, nutrient limitation has been found to limit the digestibility of micro-algae due to thickening of the cell wall (van Donk *et al.* 1997, Gonzalez-Fernandez *et al.* 2012). Algae can be added to high carbon-containing substrates to increase the C/N ratio to the desired levels to optimise methane production and digester stability; this is called co-digestion. Yen and Brune (2007) co-digested algal sludge with waste paper at various ratios and found that methane yield under co-digestion was higher than under single substrate digestion. However, straw is a valuable commodity and its use is not as sustainable as true waste product. They reported optimal methane production when the algae and waste paper were mixed together to achieve a C/N ratio between 20-25. There was an increase in cellulose degradation due to the extra nutrients supplied by the algae (Yen and Brune 2007).

Samson and Leduy (1983) found a two-fold increase in methane production when *Spirulina maxima* was co-digested with sewage sludge compared to the algae being digested alone. Blue-green algae co-digested with corn straw to achieve a C/N ratio of 20 increased the methane production by 63 % compared to corn straw alone, under batch conditions (Zhong

et al. 2012). Gonzalez-Fernandez *et al.* (2011) co-digested algal biomass grown on swine wastewater with swine manure at different proportions under batch conditions. They found a decreasing methane production as the algae to swine manure proportion increased and concluded that the recalcitrant nature of the algae was responsible for this. The co-digestion of algae with high C/N ratio feeds generally increases methane production and degradation rate as the algae supply nutrients that are not present in the high carbon-containing feeds such as paper waste (Yen and Brune 2007).

Waste activated sludge

Anaerobic digestion of WAS has the potential to produce sufficient gas to meet the energy requirements of the mechanical aerators used in the AS process (Tchobanoglous *et al.* 2003). The theoretical maximum methane yield of WAS can be as high as 0.53 l/g VS (Appels *et al.* 2008). Published methane production rates from the AD of WAS range between 80-600 ml/gVS fed, with most studies reporting gas production rates of 160-400 ml/gVS fed (Lin *et al.* 1997, Wook *et al.* 2000, Laffitte-Troque and Forster 2002, Bolzonella *et al.* 2005, Appels *et al.* 2008). The large variation in results is due to differences in substrate quality (variation in C/N ratio) and operational procedures (SRT and mesophilic or thermophilic digestion; Wook *et al.* 2000, Laffitte-Troque and Forster 2002, Bolzonella *et al.* 2005). A large scale continuously fed AD treating WAS can be expected to have a gas production between 150-250 ml/gVS fed (Bolzonella *et al.* 2005).

Anaerobic digestion of WAS involves the degradation of whole cells, and hydrolysis has been identified as the rate limiting step (Gavala *et al.* 2003, Tchobanoglous *et al.* 2003, Appels *et al.* 2008). Studies have thus been conducted on using a pre-treatment step to disrupt the

cell membrane and expose its contents to fermentative bacteria. A wide variety of biological, thermal hydrolysis, mechanical and chemical cell disruption methods have been shown to increase the methane yield, digestibility and volatile solids (VS) destruction of sludge biomasses during AD (Wook *et al.* 2000, Elliott and Mahmood 2007, Khanal et al 2007, Lin *et al.* 2009). These methods can increase the methane yield and digestibility of substrates but can also increase processing costs and operational energy consumption. When the energy consumption of the pre-treatment step is taken into account the net energy gained from increased methane production is normally lower (Tchobanoglous *et al.* 2003, Yen and Brune 2007, Lee *et al.* 2013, Lu *et al.* 2013).

Waste activated sludge has a relatively low VS content and a high water content (>96 %) which reduces gas production and VS destruction when fed directly into an AD (Tchobanoglous *et al.* 2003). Anaerobic digesters fed a low concentration of digestible substrate generally have a poor performance due to the decreased contact between anaerobic micro-organisms and digestible substrate (Tchobanoglous *et al.* 2003, Angelidaki and Sanders 2004). A solids concentration step is normally used, with the cheapest and most common process being gravity settling (Bolzonella *et al.* 1992, Tchobanoglous *et al.* 2003, Bolzonella *et al.* 2005).

The C/N ratio of WAS has been reported in the range of 5-12, with most studies reporting a range of 6-8 (Tchobanoglous *et al.* 2003, Bolzonella *et al.* 2005, Girault *et al.* 2012). This is below the optimal C/N ratio of 20-30 for substrates used in AD. The low C/N of WAS limits its feeding rate when used in AD as a high feeding rate will lead to increased ammonia production and eventually ammonia inhibition (Tchobanoglous *et al.* 2003, Angelidaki and Sanders 2004). A maximum TS feeding rate of 4.8 g/$L_{reactor}$/d has been identified, with most

AD receiving WAS being fed 1-2 g/$L_{reactor}$/d. (Tchobanoglous *et al.* 2003, Bolzonella *et al.* 2005). It is advisable to co-digest WAS with a high C/N ratio substrate, such as waste paper, if circumstances permit (Angelidaki and Sanders 2004, Yen and Brune 2007).

1.2.5 Biosolids from wastewater treatment plants

During biological effluent treatment processes such as AS and HRAP, pollutants in wastewater are assimilated by micro-organisms into biomass, while some adsorb to the cells (Tchobanoglous *et al.* 2003). This biomass, or biosolids, is separated from the treated water and needs to be correctly disposed of in order to minimise its impact on the receiving environment (EPA 1999). Tchobanoglous *et al.* (2003) state that the "management of the solids and concentrated contaminants removed by treatment has been, and continues to be, one of the most difficult and expensive problems in the field of wastewater engineering". The United States of America produces in excess of seven million dry tons of this biomass from WWTPs every year (EPA 1999). Wastewater biosolids are nutrient rich organic solids which have the potential to be used for energy, fertiliser or in soil amendment practices to improve agriculture production (Tchobanoglous *et al.* 2003, Guo 2012, Dobhal and Singhal 2017).

The composition and quality of biosolids varies greatly and is mainly dependent on the type of wastewater treated and the technologies used during treatment (EPA 1999, Lyberatos *et al.* 2004, Quilbe *et al.* 2005). Their characteristics determine the choice of disposal; for example, biosolids containing heavy metals are normally incinerated to produce energy while those that meet regulatory requirements for heavy metals and pathogens can be used for land application (DWAF 1997, EPA 1999).

Disposal is becoming more difficult due to more stringent regulations (EPA 1999, Tchobanoglous *et al.* 2003). Biosolids normally undergo treatment (stabilisation) before they are used or disposed of to ensure they meet regulatory requirements that protect public and environmental health (EPA 1999, Tchobanoglous *et al.* 2003). They are normally stabilised before disposal. Stabilisation refers to processes that decrease pathogen count, odour levels and VS concentration (EPA 1999, Tchobanoglous *et al.* 2003, Karanja 2011). The most common stabilisation methods include alkali stabilisation, AD, aerobic digestion, composting and/or heat drying with various steps and options (Table 1.4). Biosolids may also be dewatered before disposal. This includes the removal of water and is normally performed if the solids are going to be composted, landfilled or incinerated (EPA 1999, Tchobanoglous *et al.* 2003, Karanja 2011). Dewatering processes include air drying, vacuum filters, plate-and-frame filters, centrifuges and belt filter presses (EPA 1999).

Table 1.4. Stabilization technologies and associated disposal methods (EPA 1999).

Treatment process	Pros/Cons	Use/disposal method
Aerobic or anaerobic digestion	Reduce solid content Slow process	Soil amendment, organic fertiliser, forest and reclamation sites
Alkali stabilization	Increase solid content Fast process	Land application, landfill cover
Composting	Reduce solid content Slow process	Horticultural, nursery and landscape purposes
Heat-drying	Costly/energy expensive Fast process	Fertilisers, Incineration

Use and disposal practices

Landfill disposal involves the transport of treated sludge to a designated area where it is covered with landmasses (EPA 1999, Nilsson and Dahlstrom 2005). The regulations for landfill in South Africa are less stringent than land application as plants/crops are not

directly grown there. This disposal method is classified as temporary storage until it is decided how to handle the biomass (Nilsson and Dahlstrom 2005). Environmental issues associated with this practice include the leaching of heavy metals and nutrients into surrounding waterbodies and the release of methane and carbon dioxide from decomposition (EPA 1999, Nilsson and Dahlstrom 2005). Landfill disposal is discouraged due to concerns about air and ground-water pollution and difficulties in creating new sites (EPA 1999, Nilsson and Dahlstrom 2005).

Incineration of biosolids involves combustion at high temperatures where the generated heat can be used as an energy source (EPA 1999). Incineration greatly reduces biomass and produces a residual ash containing the inorganic components (EPA 1999). Incineration destroys virtually all pathogens, VS and toxic compounds although the products from incomplete combustion can be released into the atmosphere (EPA 1999, Nilsson and Dahlstrom 2005). Therefore, scrubbers and appropriate technologies need to be added to the incinerators to reduce air pollution. The ash produced from incineration contains all the heavy metals and still needs to be disposed of correctly. Incineration provides an opportunity to recover the energy stored in biosolids but is generally not recommended due to the air pollution potential it comes with (EPA 1999, Nilsson and Dahlstrom 2005).

Land application involves the incorporation of biosolids in or on soil and has been practised for centuries. It is the most common method for disposal and accounts for 41 % of WWTPs sludge disposal in the USA (EPA 1999). Biosolids serve in soil fertility enhancement and can supplement or replace commercial fertilisers as they contain nitrogen, phosphorous and various micro-nutrients such as copper, zinc, molybdenum, boron, calcium, iron, magnesium, and manganese (EPA 1999, Lyberatos *et al.* 2004, Quilbe *et al.* 2005, Karanja

2011). Their organic matter content can be used to increase the cation exchange capacity of the soil and to aid in amending soils that are carbon depleted due to agricultural practices (EPA 1999, Lyberatos *et al.* 2004, Quilbe *et al.* 2005, Karanja 2011).

In South Africa biosolids must meet certain requirements before they can be applied to the land. These regulations are based on the pathogen count and heavy metal concentration (DWAF 1997, EPA 1999). They are classed into different categories, depending on their stabilisation treatments, pathogen count and heavy metal concentration. This is then used to regulate the acceptable soil application rates and to determine whether they can be used for agricultural purposes or for non-agricultural purposes such as land reclamation from mining sites (DWAF 1997, EPA 1999). In South Africa biosolids applied to the land must be stabilised, must not cause odour or fly breeding, must contain no *Ascaris* ova or *Salmonella* organisms, have a maximum faecal coliform count of 1000 per 10 g dry sludge and be within heavy metal concentrations limits for crop production (DWAF 1997). Their application must also occur during planting, must be mixed or covered with soil, and they can only be applied at a nitrogen rate equal to or below that required by the crop (DWAF 1997). These regulations are designed to ensure that no harm comes to the public or the environment.

The land application of biosolids provides plants with macro- and micro-nutrients but the effects of contamination are of concern. Even if their heavy metal content is below regulated levels, continual application over a long period of time may lead to heavy metal build-up in the soil and eventually soil contamination (DWAF 1997, EPA 1999). Stabilization processes need to guarantee that pathogens are removed from the biomass so as not to allow their spread into the soil and surrounding ecosystems (Avery *et al.* 2005). Another issue with regard to land application is nutrient runoff and leaching (Quilbe *et al.* 2005). The

leaching rate of nutrients into ground water is affected by many variables, including the stabilization process, the nutrient content of the biosolids, slope and soil characteristics, tillage management and rainfall (Lyberatos *et al.* 2004, Quilbe *et al.* 2005). Lyberatos *et al.* (2004) concluded that biosolid application is very beneficial in nutrient deficient soils but should be avoided in nutrient rich soils that receive strong rainfall, due to increased nutrient leaching.

Waste activated sludge contains nitrogen and phosphorus and can be used as a fertiliser replacement or supplement (Lyberatos *et al.* 2004, Quilbe *et al.* 2005). The origin and stabilisation treatment process affect the quality and availability of nutrients for plant growth (Lyberatos *et al.* 2004, Quilbe *et al.* 2005). In general WAS contains 3-8 % and 2-4 % nitrogen and phosphorus respectively (Sommers 1977, EPA 1999). It is important to apply biosolids to soils at the same rate or lower than the utilization rate of the crop, to inhibit leaching of nutrients into ground and surface water (Quilbe *et al.* 2005).

The factors that affect the utilization rate of nitrogen in soils are dependent on temperature, rainfall, immobilization, ammonia and denitrification processes (Lyberatos *et al.* 2004, Quilbe *et al.* 2005). The available nitrogen in WAS varies from 45-85 % (Coker *et al.* 1987). Coker *et al.* (1987) found that initially 25 % of nitrogen in AD WAS was available to plants, in the form of ammonia, and the subsequent availability of nitrogen to plants was due to the decomposition of the sludge in the soil. Soon *et al.* (1978) concluded that nitrogen in sludge is 50 % less available to plants when compared to ammonium nitrate fertiliser. The availability of nitrogen in WAS to plants is influenced by the stabilization steps used, the environmental conditions of the soil and the crop grown (Lyberatos *et al.* 2004, Quilbe *et al.* 2005).

Literature sources give the availability of phosphorus in WAS as varying between three and eighty percent (Carliell and Wheatley 1997). The availability of phosphorus in WAS is dependent on the treatment process used to remove orthophosphate from the wastewater and the degree of sludge stabilization (Johannesson 1999). For example, AD WAS has a higher phosphorus availability than heat dried WAS sludge (Lyberatos *et al.* 2004).

1.2.6 An introduction to Life cycle analysis

Life cycle analysis (LCA) is the "cradle-to-grave" assessment of the environmental, social and economic aspects and potential impacts associated with a product, process, or service (Lundie *et al.* 2004, Mo and Zhang 2013). This analysis evaluates the impacts associated with the construction, inputs, releases and disposal of a process, product or service (Lundie *et al.* 2004, Mo and Zhang 2013). It has become standard in the study of environmental impacts such as energy consumption, greenhouse gas emissions and criteria pollutants (Chester *et al.* 2010). A LCA is one of the most all-round tools used to assess the potential impacts of a product or system which has a standardized international structure (Lundin and Morrison 2002, Lundie *et al.* 2004, ISO 2006). It offers the ability to evaluate the impact of all aspects of wastewater treatment and energy and nutrient recycling technologies (Lundin and Morrison 2002, Lundie *et al.* 2004, Mo and Zhang 2013).

A LCA involves four steps: (1) goal and scope definition, (2) inventory analysis, (3) impact assessment and (4) interpretation (Figure 1.6; Chester *et al.* 2010). The goal of the study, its functional unit, the system boundary and allocation procedure must be clearly defined in the first step, which includes defining the scope of the project (ISO 2006, Guinee *et al*. 2002). The functional unit describes the function of a particular product or system being

studied, to which inputs and outputs can be related, and thus allows the comparison of different systems (ISO 2006, Guinee *et al.* 2002). An example of a functional unit could be 100 km driven when comparing tyres made from different composites. The system boundary of a LCA defines what processes will be included/excluded during the study. The chosen system boundary needs to be carefully described as the exclusion of components can have major influences on the results and interpretation of a LCA (ISO 2006, Guinee *et al.* 2002). Allocation involves the "partitioning of input/output flows of a process to the product system under study" (ISO 2006). For example, chlorine, caustic soda and hydrogen are produced as by-products during salt electrolysis; these products are also produced by separate industries/processes. As such these by-products reduce the need for a separate industry to manufacture them and indirectly decrease the environmental impact associated with the primary production method. Avoided allocation or allocation is used to model the reuse of by-products that are normally produced by a separate industry. Avoided allocation includes making an inventory of the standard process used to produce the same output and subtracting these emissions from the emission of the system that is being studied/produces the by-products (Guinee *et al.* 2002, ISO 2006). Allocation can also be performed by dividing impacts where the impacts of a process are shared between the product and by-products on a mass or economic basis (ISO 2006).

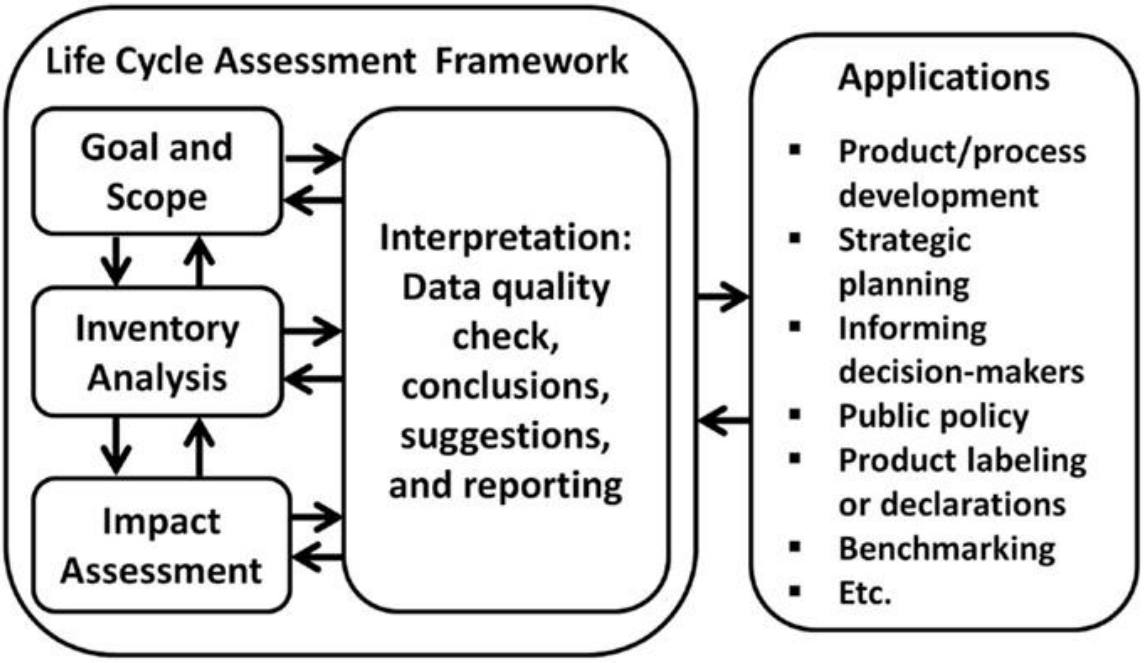

Figure 1.6: Four steps of life cycle analysis (Guinee *et al.* 2002, ISO 2006).

Inventory analysis involves the identification of flows to and from the environment of the system being studied (ISO 2006). Typical inventory flows include inputs such as water, energy and raw materials and outputs to the air, land and water (ISO 2006). The impact assessment of a LCA identifies the impacts, resource use and emissions of the system under study (ISO 2006, Chester *et al.* 2010), and contains four steps: (1) classification, (2) characterisation, (3) normalisation and (4) weighting. During classification all emissions are sorted into classes such as NO_x and carbon dioxide, for example (Figure 1.7; ISO 2006). Classified emissions (e.g. ammonia, nitrate, heavy metals etc.) are then multiplied by a factor which reflects their contribution to a midpoint environmental impact such as global warming or freshwater eutrophication during the characterisation step (Figure 1.7; ISO 2006).

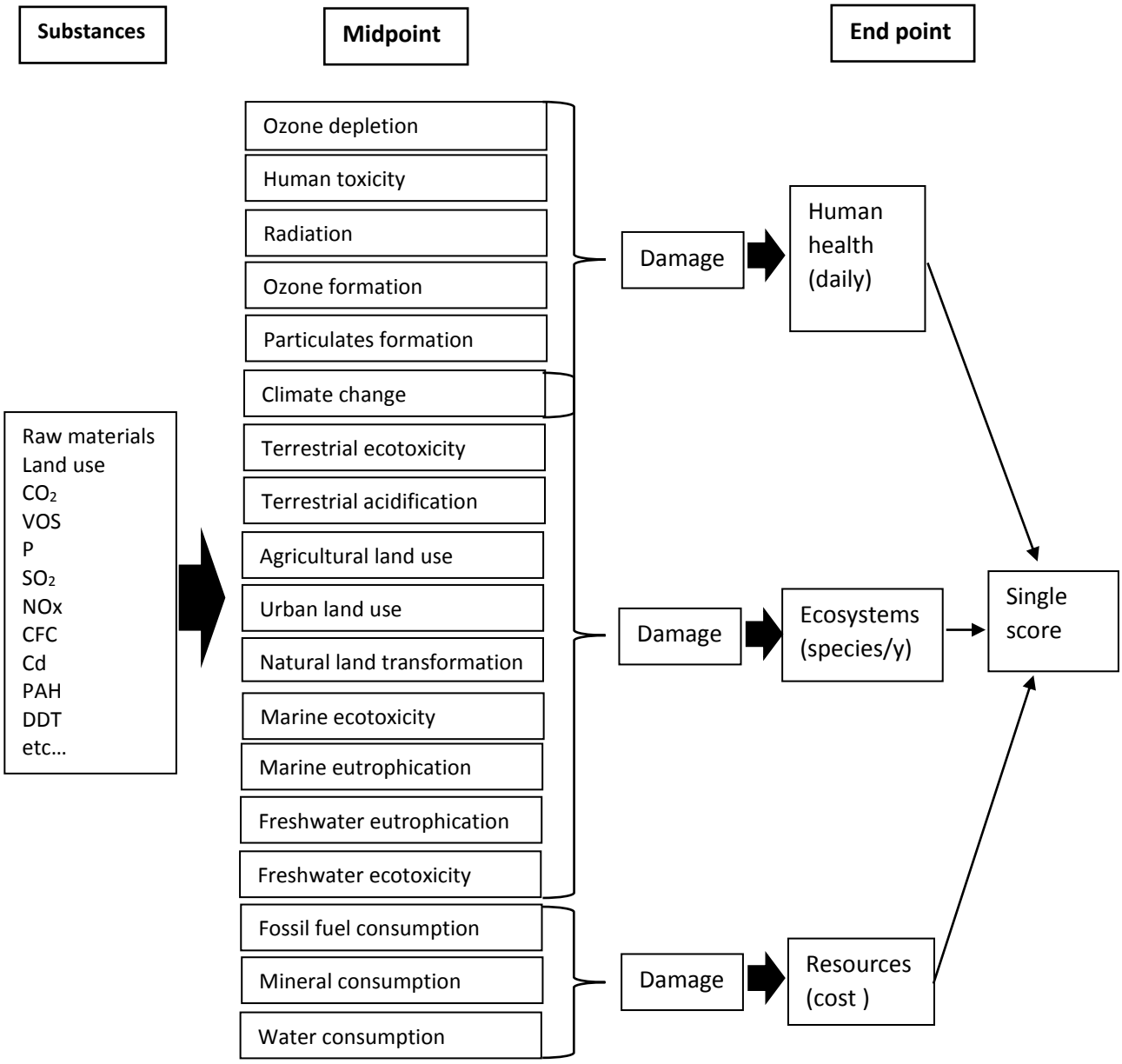

Figure 1.7: Relationship between life cycle impact parameters (left) midpoint (middle) and endpoint (right) impact categories. Volatile organic solids (VOS), phosphorus (P), nitrogen oxides (NOx), chlorofluorocarbons (CFC), polycyclic aromatic hydrocarbons PAH, dichlorodiphenyltrichloroethane (DDT); (ReCiPe 2008).

The ISO 14040 standards include predetermined factors used to determine the impact of certain emissions on 18 identified midpoint environmental impact categories (Figure 1.7; ISO 2006). These midpoint impact categories can be further combined to produce three endpoint impact categories; however, endpoint categories are broad and the interpretation

of results is normally done on midpoint impacts (Lundin and Morrison 2002, ISO 2006). Impacts can then be normalised by comparing them to a reference value such as the impact on a citizen over a year (ISO 2006). This allows the identification of those impact categories of the greatest concern.

Interpretation of a LCA involves analysing results, reaching conclusions, explaining limitations and providing recommendations (ISO 2006). The conclusions of a LCA should be transparent and in accordance with the goal and scope of the study (Guinee *et al.* 2002, ISO 2006). Sensitivity and uncertainty analysis are used to determine the robustness of conclusions and results. These analyses are used to deal with uncertain data used in the LCA model to determine key issues for further analysis and how a change in a model parameter can influence the overall results of the study (ISO 2006). Sensitivity analysis determines the effect the assumptions made in the study have on the final results. For example, how will changing the allocation method, system boundary or choice of data used influence the conclusions of the study? Uncertainty analysis deals with variation of the data used in the model; how robust and reliable are the conclusions of a study in relation to possible variation assigned to inputs and emissions (ISO 2006).These analysis aid in identifying which conclusions in a LCA are reliable and consistent and which are greatly influenced by inventory data and assumptions made.

1.2.7 Life cycle analysis of wastewater treatment systems

The number and size of WWTPs are continually on the increase due to greater volumes of wastewater production and more stringent discharge limits (Lundin *et al.* 2000). Wastewater treatment plants seem to be highly efficient when assessed on specific water

quality parameters, but they consume natural resources and produce waste products (Lundin *et al.* 2000, Mo and Zhang 2013). With the need for ecological sustainability in the 21st century, the goals of WWTPs need to include: minimizing the use of natural resources, water and energy, reducing waste generation and enabling the recovery of energy and plant nutrients (Lundin *et al.* 2000, Mo and Zhang 2013). A LCA systems approach enables the assessment and comparison of changes in wastewater treatment practices and technologies in terms of the resource use, environmental load and emissions they produce (Lundin *et al.* 2000, Mo and Zhang 2013). The majority of LCA studies on conventional effluent treatment technologies conclude that reducing their environmental impact can be achieved by minimizing sludge production and through the recovery of nutrients, energy and water (Roeleveld *et al.* 1997, Lundin *et al.* 2000, Mo and Zhang 2013).

Environmental impact scores from LCA studies on wastewater treatment and sludge handling technologies can be very different. This is mainly due to differences in systems boundaries, impact categories, allocation choices and sludge handling options. Impact categories vary between studies, with most including energy and broader categories such as human toxicity, ecotoxicity, land use, water use, and air and water emissions (Lundin *et al.* 2000, Houillon and Jolliet 2005, Brown *et al.* 2010, Mo and Zhang 2013). Some studies only include the operational phase (Hospido *et al.* 2005, Houillon and Jolliet 2005, Brown *et al.* 2010), while others include the construction and operational phases (Peters and Lundie 2001, Hong *et al.* 2009, Peters and Rowley, 2009). The system boundaries should be selected according to the goal of the study (Lundin *et al.* 2000). If chemical and biological treatment systems are compared, the environmental impact of the production of chemicals needs to be incorporated in the analysis (Lundin *et al.* 2000). When comparing different

biological treatment methods, it is important to include the environmental burden, waste biomass handling and construction phases (Lundin *et al.* 2000, Hong *et al.* 2009).

Studies comparing constructed wetlands or HRAP to conventional AS systems report a decrease in global warming potential, aquatic toxicity and resource consumption, but most fail to include the end use of the generated biomass (Siracusa and La Rosa, 2006, Zhou *et al.* 2009, Roux *et al.* 2010, Mo and Zhang 2013). The end use of generated biomass and nutrient recovery through algae need to be included to fully assess the environmental impact of the entire treatment system. The avoidance of fossil-based fertilizer production from land application of biosolids is only considered in some studies (Lundin and Morrison 2002, Peters and Rowley 2009, Brown *et al.* 2010). As a result, conclusions are different; Houillon and Jolliet (2005) reported that land application was favourable for sludge disposal while Peters and Rowley (2009) preferred incineration.

A common difference between LCA studies on biological wastewater treatment systems is whether the generated biomass is regarded as a resource or a waste product (Lundin *et al.* 2000). Most studies view sludge as waste and do not consider the option of recycling nutrients from sludge (Lundin *et al.* 2000, Siracusa and La Rosa 2006, Zhou *et al.* 2009, Mo and Zhang 2013). A comprehensive LCA should include more than just the operation and construction of the wastewater treatment systems and should also include interactions with surrounding technical systems through power generation, agriculture, fertiliser production and other related material flows (Lundin *et al.* 2000, Mo and Zhang 2013).

To date, there are only two published LCA studies which compare HRAP to AS treatment technologies (Garfi *et al.* 2017, Arashiro *et al.* 2018). Both papers used thermotical inventory data collected from various published papers and compared systems situated in different

climates (Garfi *et al.* 2017, Arashiro *et al.* 2018). The LCA study conducted by Arashiro *et al.* (2018) had different destinations for the biomass produced where sludge from the AS system was incinerated while the algae biomass was used for methane production followed by land disposal. Both sludge and algae can potentially be used as a feed stock for AD digestion (Bolzonella *et al.* 2005, Ward *et al.* 2014) and could have been included in both scenarios. The current study aims to address this gap by conducting a LCA and comparing a HRAP to an AS system used to treat the same effluent, in the same location with the same biomass disposal options using data collected simultaneously from both systems, which will provide a basis to critically review and contribute to improving the methods used in the field of LCA on nature-based wastewater treatment technologies.

1.2.8 Conclusion

Wastewater production is continually on the increase due to the growth of the human population, industrialisation and urbanization. It poses an environmental and human health risk if not treated correctly, prior to its disposal into the environment. Current effluent treatment technologies are effective at removing pollutants from wastewater to suitable discharge standards; however, they consume resources and produce waste products. With the need for ecological sustainability in modern times, the goals of WWTPs need to include minimizing the use of natural resources, water and energy, reducing waste generation and enabling the recovery of energy and nutrients.

Various treatment technologies allow the recovery of energy, nutrients and water from effluent. There is a lack of literature which comprehensively compares the treatment performances, environmental impact and beneficial downstream use of waste biomass

generated from AS and HRAP effluent treatment systems. A LCA systems approach enables the assessment and comparison of changes in wastewater treatment practices and technologies in terms of resource use, environmental load and emissions produced. This analysis will also allow the comparison of different combinations of effluent treatment technologies coupled with various waste biomass handling options in terms of overall environmental impact. This can be used to aid decision makers in constructing sustainable wastewater treatment systems.

Individual resource recovery technologies have been evaluated and compared using LCA but literature is lacking with regard to the sequential utilisation of these technologies used to produce a zero-waste effluent treatment system. To add to this, some studies of HRAP, constructed wetlands and biosolids applications only include the operational phase, while others include the construction and operational phases, and relatively few include the construction, operational and end-of-life phases. There are also large differences in the functional units and the impact categories used, which makes it difficult to compare different nutrient, energy and water recovery technologies. The data used in LCA studies of WWTPs are often theoretical, collected from different regions and from different waste streams. This results in differences and reduces the robustness of the conclusions that can be drawn from the studies. In order to increase the robustness of LCA studies on WWTP, different technologies that treat the same effluent need to be monitored, coupled with conducting experiments on various combinations of sludge handling technologies.

The data used in LCA studies of WWTPs are often theoretical, collected from different regions and from different waste streams, and although the current literature is comprehensive (Dixon *et al.* 2003, Machado *et al.* 2007, Memon *et al.* 2007, Fuchs *et al.*

2011, Garfi *et al.* 2017, Arashiro *et al.* 2018), the impact that the different effluent sources have on LCA conclusions is unknown. These differences might compromise the robustness of the conclusions that can be drawn from the studies; or they might have little bearing on the conclusions. This study will, for the first time, use LCA to compare different effluent treatment technologies that produce different by-products, but that treated the same effluent stream, and will therefore contribute towards better understanding the impact of effluent characteristics/differences in LCA.

1.3 Aims and Objectives

This thesis set out to investigate a combination of effluent treatment and sludge handling technologies that allow maximum recovery of resources while minimising environmental impact, with a focus on adding value to algae cultured in algal treatment facilities and comparing this to sludge produced in an activated sludge effluent treatment system. A LCA was conducted, evaluated and used to identify the main environmental impacts associated with these technologies. This was achieved by addressing the following objectives, which were to:

ix. evaluate nutrient recovery, nutrient transformation and biomass balances of HRAP and AS (Chapter 2);

x. evaluate the energy recovery from the AD of waste activated sludge (WAS) and algal biomasses (Chapter 3);

xi. evaluate the use of anaerobically digested WAS and algal biomasses as a fertiliser replacement (Chapter 4); and

xii. to use the information from the previous three experiments to conduct a thorough LCA to determine the most suitable combination of effluent treatment and sludge handling technologies which maximise resource recovery and minimise environmental impact (Chapter 5).

Chapter 2: The first empirical comparison of activated sludge and high rate algal ponding technologies used to treat brewery effluent

2.1 Introduction

South Africa is currently faced with energy and water shortages (Winter 2011, Turton *et al.* 2016), yet it discards large volumes of effluent every day. Effluents contain resources such as water, organic pollutants, nitrogen and phosphorous (Tchobanoglous *et al.* 2003) that could potentially be used in food and energy production. It is vital that we develop effluent treatment technologies that not only produce reusable water but provide an opportunity for the recovery of nutrients and carbon for downstream reuse in, for example, the fertiliser and biofuel industries (Park *et al.* 2011). Even though wastewater treatment technologies reduce the environmental impact of effluent, they have an environmental impact themselves by producing waste products and consuming natural resources during operation (Lopsik 2013). Therefore, treatment performance, technical and economic aspects as well as environmental impact need to be taken into account when selecting the most suitable technology (Molinos-Senante *et al.* 2014, Garfi *et al.* 2017).

Activated sludge (AS) is a commonly used technology for the biological treatment of municipal and industrial effluents (Tchobanoglous *et al.* 2003). The AS process involves the cultivation of aerobic micro-organisms in suspension with the wastewater in an aeration tank. The aeration tank is followed by a sedimentation tank or clarifier, which separates the bulk of micro-organisms from the treated effluent (Jenkins and Wanner 2014).

The facultative heterotrophic micro-organisms present in the aeration tank oxidize and mineralize organic matter in wastewater into more stable products such as carbon dioxide, water and biomass (Tchobanoglous *et al.* 2003, Jenkins and Wanner 2014). The AS process

performs four major functions of wastewater treatment: oxidation or degradation of carbonaceous material; oxidation or degradation of nitrogenous material; removal of fine solids; and removal of heavy metals (Marais and Ekama 1976, Eckenfelder and Grau 1992, Spellman 2000).

Activated sludge is commonly used in water treatment technology due to their efficiency, which is primarily a result of the high growth rate of the aerobic micro-organisms (Chan *et al.* 2009). Consequently, AS systems have a short HRT and are able to treat large volumes of effluent in a small space compared to anaerobic, artificial wetlands or algal treatment systems (Chan *et al.* 2009). However, activated sludge systems consume large quantities of energy which is used by the agitators/aerators needed to keep the sludge in suspension and maintain the high dissolved oxygen levels needed by the biologically active sludge (Chan *et al.* 2009, Park *et al.* 2011). On average, a drop in biological oxygen demand (BOD) of one kilogram by AS produces about 0.5 kg of dry weight excess sludge (Lui 2003). The drawback of AS systems is that they require skilled operators, have a high rate of energy consumption and are therefore costly to operate, and the sludge needs to be disposed of (Massoud *et al.* 2009, Garfi *et al.* 2017).

High rate algal ponds (HRAP) provide opportunities for low-energy wastewater treatment combined with algal biomass production (Park *et al.* 2011, Craggs *et al.* 2014). These systems consist of shallow raceway ponds (0.3-0.5 m deep) where a paddlewheel is used to keep the algae in suspension with the wastewater and to maintain photosynthetic oxygenation and dissolved oxygen concentration (Park *et al.* 2011, Craggs *et al.* 2014). A consortium of algae, bacteria and other micro-organisms grow in the raceways, and which assimilate nutrients and degrade and oxidise organic matter (Park *et al.* 2011, Craggs *et al.*

2014). During photosynthesis, the micro-algae provide oxygen to aerobic bacteria present in the ponds (Park *et al.* 2011, Garfi *et al.* 2017). High rate algal ponds are identified as having "low energy consumption, simple operation and lower capital and operating costs compared to conventional systems" (Garfi *et al.* 2017). High rate algal ponds are also considered ideal for the recovery of resources from effluents; for example, harvested algae can be used to produce biofuels (Craggs *et al.* 2014, Montingelli *et al.* 2015, Uggetti *et al.* 2017), nutritional fatty acids and proteins (Luiten *et al.* 2003, Spolaore *et al.* 2006) and pharmaceutical products (Borowitzka 1995).

There is much literature on both HRAP and AS systems describing the treatment efficiencies, carbon and nitrogen balances, energy consumption, and waste product production (Garcia *et al.* 2000, Park and Craggs 2011, Craggs *et al.* 2014, Alcantara *et al.* 2015, Montingelli *et al.* 2015, Uggetti *et al.* 2017). However, only six publications were found that compared the land utilisation, energy or resource consumption and environmental impact of HRAP and AS systems (Garcia *et al.* 2006, Craggs *et al.* 2011, Park *et al.* 2011, Craggs *et al.* 2014, Alcantara *et al.* 2015, Garfi *et al.* 2017). Of these six publications, five make vague statements in comparing the systems, e.g.: HRAP "operational costs are less than one fifth those of activated sludge systems", and "the capacity of micro-algae to simultaneously remove carbon, nitrogen and phosphorus via mixotrophic assimilation represents an important advantage in comparison with aerobic activated sludge"; however, there is minimal to no data to support these statements (Park *et al.* 2011, Alcantara *et al.* 2015). Garfi *et al.* (2017) conducted a life cycle analysis (LCA) on the environmental impact of HRAP and AS looking mainly at their overall carbon emissions and environmental impact. However, they used data from various authors and did not compare expected treatment performances or water quality parameters of a single effluent treated by these technologies. The general consensus

is that HRAP consume less energy, produce less carbon dioxide emissions and require unskilled operators when compared to AS systems (Craggs *et al.* 2011, Park *et al.* 2011, Craggs *et al.* 2014). However, AS systems require 50 times less surface area and are not vertically limited when compared to HRAP systems, which have a maximum depth of 0.6 m (Park *et al.* 2011). No study has directly monitored the treatment performance, nutrient dynamics and emissions of HRAP and AS systems used to treat the same effluent, under the same set of environmental conditions and at the same time.

At Ibhayi Brewery in Port Elizabeth, South Africa there is a fully operational, commercial-scale AS and a fully operational pilot-scale HRAP system which treats post-anaerobically digested (post-AD) brewery effluent for reuse in non-production activities at the brewery. This provided an opportunity to monitor the treatment performance, the waste produced, the fate of carbon and nitrogen and the energy consumption of both systems. This information can be used to help wastewater treatment plant design-engineers develop a better understanding of how these systems compare when deciding which technology to use.

2.2 Aims and objectives

Ibhayi brewery aims to better the sustainability and environmental impact of its effluent treatment process. There was a need to identify and quantify the emissions and treatment capacity of HRAP and AS treatment processes that treat the same effluent, under similar environmental conditions. The aim of this experiment was to make the first empirical comparison of HRAP and AS technologies in treating brewery effluent, in the same temporal and geographical space. This was achieved by comparing the water treatment efficiency,

energy consumption, nutrient dynamics and microbial productivity of HRAP and AS. The objectives of this experiment were to:

- compare the biomass produced by high rate algal ponding and activated sludge systems;
- estimate the carbon dioxide emissions from high rate algal ponding and activated sludge systems;
- determine the main nitrogen removal processes in activated sludge and high rate algal ponding systems; and
- evaluate the effluent treatment efficiencies of high rate algal ponding and active sludge systems.

2.3 Materials and Methods

2.3.1 Experimental system

The full volume of brewery effluent (1100 m^3/d) was screened through a drum filter (Autrex Industrial Screening, Serial no. A 140/02, Model no. R 015) to remove solid wastes such as stones, plastics, glass, paper and bottle labels, after which the screened effluent was sent to an anaerobic digester. This digester had a total volume of 3000 m^3, operated on a mean throughput rate of 1100 m^3 per day and was operated by a commercial effluent treatment company (Proxa Pty Ltd, South Africa). After AD a portion of the effluent stream was sent to a pilot scale experimental effluent treatment facility where a primary facultative pond (PFP) and a HRAP system was used to treat the effluent for further use in aquaculture and agriculture. The rest of the AD treated effluent was sent to a full-scale AS system after which the treated water was re-used in non-production activities at the brewery, such as bottle washing.

High rate algal ponds

Effluent entering the HRAP had undergone AD and stabilisation in PFP (volume of 75 m^3) operated at a HRT of four days. In the PFP, further anaerobic and aerobic degradation took place depending on the position in the water column (Chapter 1, Section 1.2.3). Effluent from the PFP decanted into a splitter box, which divided the effluent into two streams, supplying two identical parallel HRAP systems (Figure 2.1). Each system consisted of two ponds in series, pond 1 and pond 2 (Figure 2.2). The ponds were made of green polyvinylchloride liner supported by a galvanised metal frame (Figure 2.2). The first pond of each system was 25 cm deep, with a surface area of 14.8 m^2 and a volume of 3700 l. Gravity fed effluent decanted from the first pond to the second pond, which was 11.5 cm deep, with a surface area of 15 m^2 and a volume of 1700 l. Each pond had a stainless-steel paddle wheel which continuously stirred the effluent and kept the algal cells in suspension (Figure 2.2). Paddle wheels moved HRAP effluent at an approximate velocity of 4.15 m/s and 6.10 m/s in ponds 1 and 2 respectively. Both algal systems were fed 1800 l/d of effluent which equated to a HRT of three days. A HRT of three days was used as Jones *et al.* (2016) found this to be the shortest HRT without compromising the treatment efficiency of the algal ponds. The HRAPs were fed continuously during daylight hours (08:00-17:00), while feed stopped during the remaining 15 h.

Post-HRAP effluent, from both systems, decanted into a 500 l sump, situated below ground level. From there, effluent was pumped into two elevated algal settling cones using a submersible pump (Figure 2.2). Valves at the bottom of the settling cones were opened weekly to drain the settled algal slurry into a slurry collection tank. The clarified post-HRAP effluent was collected in a 1000 l tank and used to culture a variety of fishes such as tilapia

(*Oreochromis mossambicus*), catfish (*Clarias gariepinus*) and guppies (*Poecilia reticulata*; production of which did not from part of this trial).

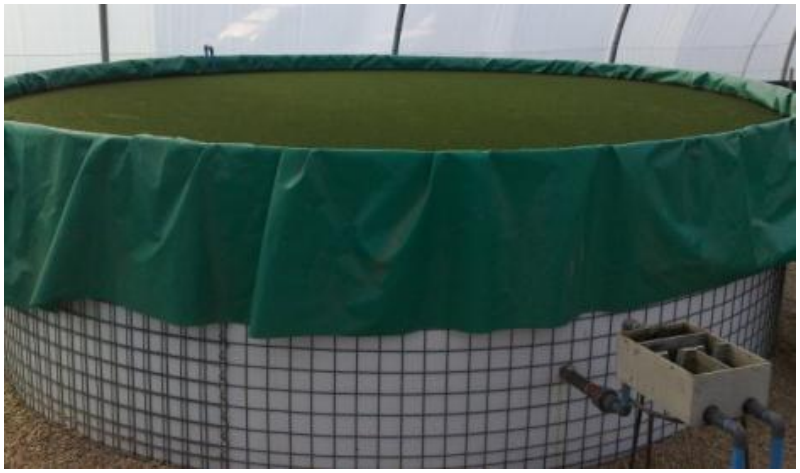

Figure 2.1: Primary facultative pond and splitter box.

Figure 2.2: High rate algal ponds used to treat anaerobically digested brewery effluent in the foreground (A), with algal settling cones on the left (B) and the primary facultative pond in the centre (C).

Activated sludge

The majority of the brewery's post-AD effluent (about 800 m^3/d) was piped into a rectangular aeration basin (height 2.5 m, surface area 128 m^2, volume 320 m^3) which contained two vertical 28 kw powered mechanical aerators (Wag 280UT08, Zest WEG Group Pty Ltd, South Africa; Figure 2.3). The remaining 300 m^3 of post-AD effluent was disposed to the municipal sewer. The aeration basin was operated at a HRT between 0.4 and 0.5 days. The dissolved oxygen (DO) concentration in the aeration tank was automatically maintained between 0.5 and 1.2 mg/l. When the DO decreased below 0.5 mg/l both agitators were automatically switched on to aerate the medium. When the DO increased above 0.8 mg/l a single agitator was operated, whereas if the DO concentration was above 1.2 mg/l both agitators were automatically switched off. For approximately 90 % of the time during this study, one agitator was running, and this resulted in maintaining the DO at about 0.8 mg/l.

Post-AS effluent decanted into a clarifier, operated at a HRT between 0.3-0.4 days, which removed the suspended solids through settling (Figure 2.3). Clarified effluent was then passed through micro-media filters, ultra-filters and reverse osmosis for reuse in non-production activities at the brewery. The settled sludge was returned into the AS basin via a return sludge pump. Settled sludge from the clarifier was disposed of into the municipal sewage works, and this process was called "sludge wasting" (Mclean *pers. comm.* 2017). This was done to maintain the desired concentration of micro-organisms in the aeration basin of 350-400 ml/l volume of settleables (Spellman 2000, Mclean *pers. comm.* 2017). Sludge wasting was performed once or twice a week depending on the volume of settleables in the aeration basin, and was done when the volume of settleables increased

above 450 ml/l and was stopped when it decreased to 300-350 ml/l. The AS was managed by an independent commercial company (Proxa Pty Ltd, South Africa).

Figure 2.3: Activated sludge aeration basin (left) and clarifier (right) used to treat brewery effluent.

2.3.2 Data collection

The monitoring of water quality parameters and biomass production from both systems were carried out for six months, between October 2016 and March 2017.

The pH, temperature (^{o}C) and electrical conductivity (EC, µS/cm^{2}) of the incoming and outgoing effluent were recorded every second day using an electronic probe (Hanna, HI 991300, United Kingdom). Chemical oxygen demand (COD, mg/l, potassium dichromate method), total organic carbon (TOC, mg/l, persulfate method), total nitrogen (TN, mg/l, persulfate digestion method), total ammonia nitrogen (TAN, mg/l, salicylate method), nitrite (mg/l, ferrous sulfate method) and nitrate (mg/l, cadmium method) and phosphate (mg/l, molybdovanadate method) of the incoming and outgoing effluent were recorded every week, using a spectrophotometer (Merck Spectroquant Pharo 100 spectrophotometer,

product number 100706, Darmstadt, Germany) and commercially available test kits, according to standard methods (Merck Pty Ltd, Darmstadt, Germany). The COD, TOC and TN analyses were performed on unfiltered and filtered water samples, whereas TAN, nitrite, nitrate and phosphate analysis where done on filtered water samples. Soluble COD, TOC and TN were represented by filtered samples while their particulate fractions were calculated by subtracting filtered values from unfiltered values. Water samples were filtered through 8.0 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125, Whatman plc, Maidstone, United Kingdom).

Waste activated sludge samples and algal biomass samples were collected every two weeks for elemental analysis of C, H, N, and S (Agilent 7900 ICP-MS Agilent technologies, Santa Clara, United States of America, EPA 2000) which was carried out at a commercial laboratory (Central Analytical Facilities, Stellenbosch University, South Africa).

Total suspended solids were recorded using standard methods, where 50 ml of wastewater was filtered through 0.2 µm filter paper (APHA 2005, EPA 2007). The filter paper was previously placed in an oven set at 105 ^{o}C and dried for an hour and weighed (initial weight) before being used to filter a sample. The filter paper was then placed in the oven and allowed to dry for at least 24 h, until a constant weight was achieved, before being reweighed (final weight). Total suspended solids content of the sample was then calculated using Equation 2.1 (APHA 2005, EPA 2007):

$$\text{Total suspended solids g/l} = (\text{final weight (g)} - \text{initial weight (g)}) \times 20 \quad [2.1]$$

Total solids (TS) and volatile solids (VS) of the feed, clarified effluent and settled biomass were recorded (0.001 g/l) once a week. Ten millilitres of sample was placed in a pre-weighed crucible, and placed in a drying oven at 105 ^{o}C for a minimum of 24 h, until a

constant mass was achieved (APHA 2005, EPA 2007). Crucibles were allowed to cool down in a desiccator for at least ten minutes, and then were weighed and placed in a muffle furnace (Neytech Vulcan benchtop muffle furnace, RK-33855-25, Illinois, United States of America) set at 550 ^{o}C for two hours. Samples were then allowed to cool in a desiccator for an hour and reweighed. Total and VS were then calculated using Equations 2.2 and 2.3 (APHA 2005, EPA 2007):

$$\text{Total solids g/l} = (W_2 - W_1) / 10 \times 1000 \quad [2.2]$$

$$\text{Volatile solids g/l} = (W_2 - W_3) / 10 \times 1000 \quad [2.3]$$

where:

W_1 = weight of the crucible in g;

W_2 = weight of crucible and oven dried sample in g; and

W_3 = weight of crucible and ash in g.

The biomass produced per volume of effluent treated from the HRAP system was calculated once a week using Equation 2.4:

$$\text{Dry weight algae produced g/m}^3 = [(TS_2 \times (V_1+V_2)) - (TS_1 \times V_1)] / V_2 \quad [2.4]$$

where:

TS_1 = Total solids of sample from HRAP collected at day 1 in g/m^3;

TS_2 = Total solids of sample from HRAP collected at day 2 in g/m^3;

V_1 = Volume of HRAP system in m^3; and

V_2 = Volume of effluent treated m^3.

Biomass production in the AS system was conducted every week over 24 h. No sludge wasting was done over the period when biomass production was being calculated. Since the AS system and clarifier had been running at a stable state (stable sludge blanket in clarifier) for over five years, it was assumed that the sludge exiting the aeration basin and entering the aeration basin by the return AS line were equal (Speece *et al.* 1983, Snyder and Wyant 2005, Jenkins and Wanner 2014). Biomass production per volume of effluent treated from the AS system was calculated using Equation 2.5 (Speece *et al.* 1983, Snyder and Wyant 2005):

$$\text{Dry weight sludge produced g/m}^3 = ((TS_2 - TS_1) \times V_1)/V_2 \quad [2.5]$$

where:

TS_1 = Total solids of sample from AS basin on day 1 in g/m^3;

TS_2 = Total solids of sample from AS basin on day 2 in g/m^3;

V_1 = Volume of AS basin in m^3; and

V_2 = Volume of effluent treated m^3.

Activated sludge organic carbon balance was calculated using Equation 2.6 and used to estimate the carbon released from respiration during the AS process (Tchobanoglous *et al.* 2003, Puig *et al.* 2008):

$$C_{in} = C_{out} + C_{biomass} + C_{respiration} \quad [2.6]$$

where:

C_{in} = Unfiltered total organic carbon in influent (kg/m^3) x inflow (m^3);

$C_{biomass}$ = Carbon concentration of settled biomass (g/kg) x biomass produced (kg/m^3); and

C_{out} = Unfiltered total organic carbon in clarified effluent (kg/m^3) x outflow (m^3).

The net carbon dioxide ($C\text{-}CO_2$) assimilated by HRAP was estimated using Equation 2.7 (Alcantara *et al.* 2013, Yuan *et al.* 2015).

$$C\text{-}CO_2 = C_{out} + C_{biomass} - C_{in} \quad [2.7]$$

The nitrogen gassed out during HRAP and AS processes was estimated via a mass balance using Equation 2.8 (Barker and Dold 1995, Puig *et al.* 2008):

$$N_{in} = N_{out} + N_{biomass} + N_{gas\ out} \quad [2.8]$$

where

N_{in} = Unfiltered total nitrogen in influent (kg/m^3) x inflow (m^3);

N_{out} = Unfiltered total nitrogen in clarified effluent (kg/m^3) x inflow (m^3); and

$N_{biomass}$ = Nitrogen concentration of settled biomass (g/kg) x biomass produced (kg).

Unidentified dissolved nitrogen (UDN) entering and exiting HRAP and AS treatment systems was calculated using Equation 2.9 (Barker and Dold 1995, Mekinia *et al.* 2009).

$$UDN = TN_{\ filtered\ sample} - (NH_4\text{-}N + NO_2\text{-}N + NO_3\text{-}N) \quad [2.9]$$

2.3.3 Statistical analysis

There were no replicates for both the HRAP and AS systems which limited the statistical analysis to a descriptive assessment. Data collected over six months are presented as mean ± standard error and average percent change. A regression analysis comparing data collected over time (d) was conducted to determine if the data from the HRAP and AS

system remained constant over the six month monitoring period. A linear regression analysis for each water quality parameter against HRT was carried out for both systems.

2.4 Results

The AS system at Ibhayi Brewery treated on average 842 m^3 of effluent per day with a HRT of 0.45 days (Table 2.1). The experimental HRAP systems treated on average 3.08 m^3 of effluent per day and operated at an average hydraulic retention time (HRT) of 3.08 days (Table 2.1). The electrical energy consumption of the HRAP system per cubic metre of effluent treated is less than half of the energy consumption of the AS system (Table 2.1).

Table 2.1: Mean (± standard error) volumes treated, energy consumption and dry weight biomass gained by the high rate algal ponding (HRAP) and activated sludge (AS) systems over six months.

	AS	HRAP	n
Volume treated (m^3/day)	842.06±32.35	3.08±0.02	60
Hydraulic retention time (days)	0.45± 0.04	3.80±0.03	60
Energy consumption (kW/m^3)	0.29± 0.01	0.11±0.01	60
Biomass production (g/m^3)	83.12±12.93	317.18±5.56	26

There was no significant change in biomass production over time for HRAP and AS treatment systems (Figure 2.4). The HRAP system produced an average of 317.18±27.76 g/m^3 of algal biomass, 3.8 times more than that produced from the AS system (83.12±64.91 g/m^3; Figure 2.4).

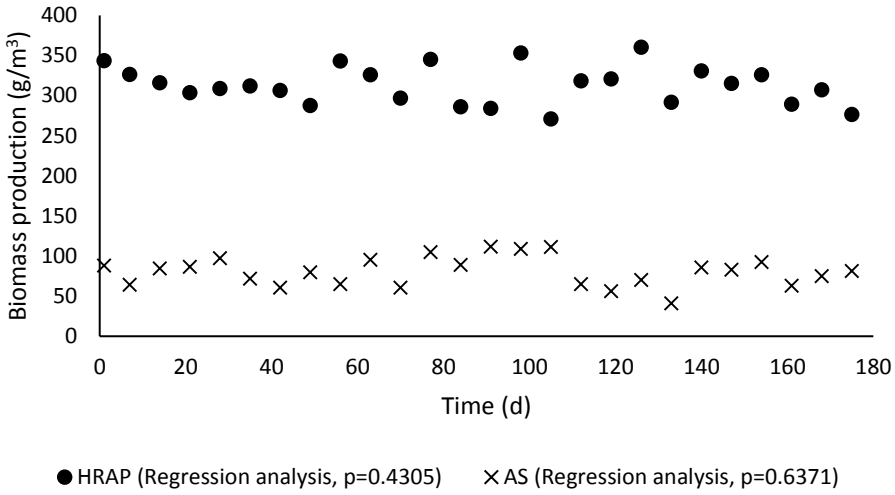

Figure 2.4: Biomass production from an activated sludge and high rate algal pond system used to treat brewery effluent.

Similarly, there was no significant changes in pH or DO concentration over time for HRAP and AS treatment systems (Figures 2.5 and 2.6). The pH of effluent entering the AS and HRAP systems was near neutral, with an increase during treatment in both systems (Figure 2.5). High rate algal ponds increased effluent pH to a mean of 9.95±0.30 while the AS system increased the pH to 8.41±0.20 (Table 2.2). The DO concentration of post-AD brewery effluent was low (0.17±0.12 mg/l) and also increased during HRAP and AS treatment (Figure 2.6). Dissolved oxygen concentrations in HRAP effluent were supersaturated during the day (9.20±0.96 mg/l) while operation procedures ensured that the DO concentration in the AS was maintained between 0.8-1.2 mg/l (Figure 2.6).

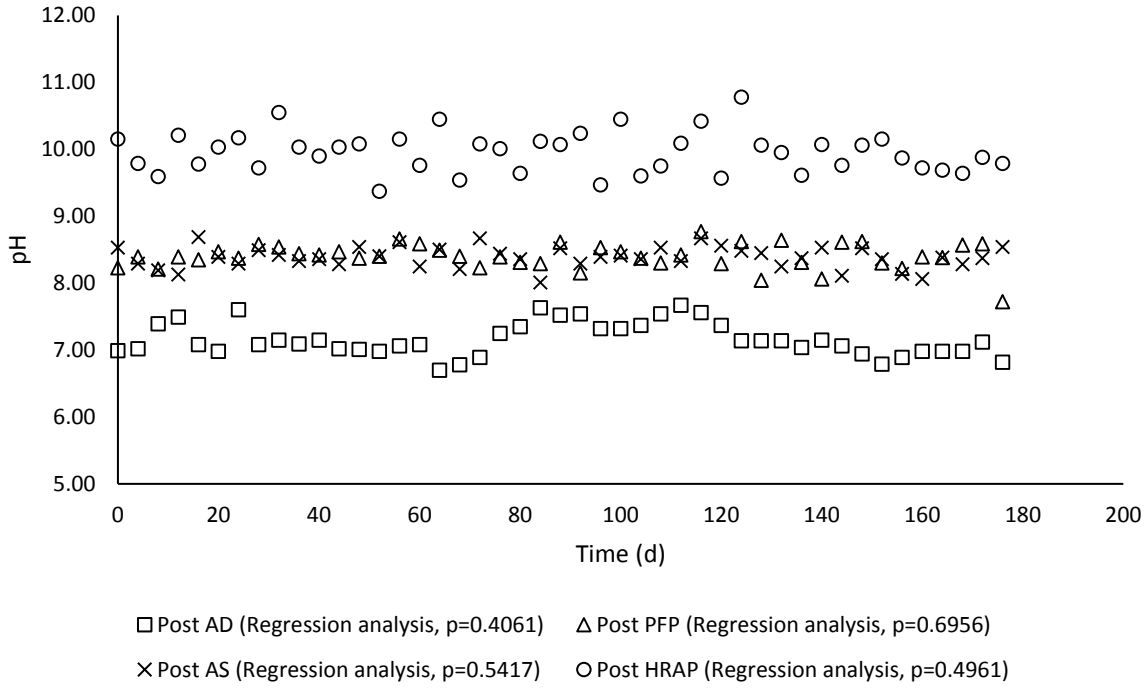

Figure 2.5: The pH of post-anaerobically digested brewery effluent entering and exiting the activated sludge and high rate algal pond treatment systems.

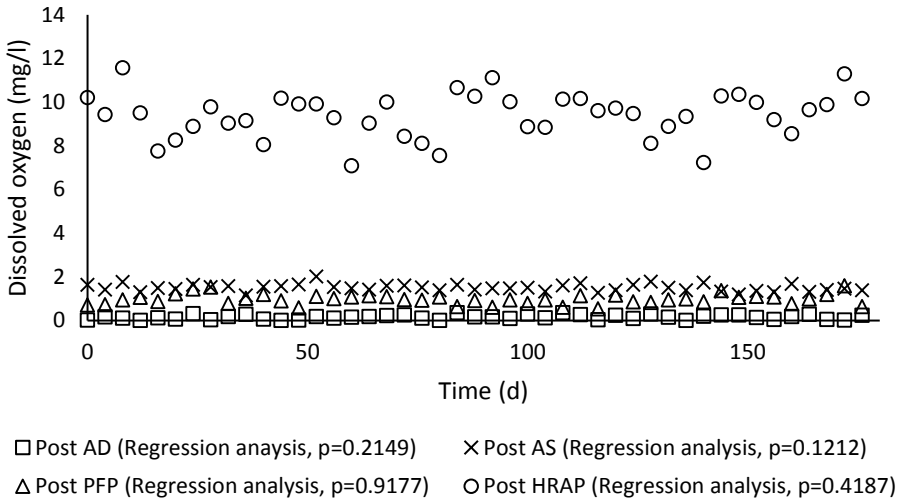

Figure 2.6: The dissolved oxygen of post-anaerobically digested brewery effluent entering and exiting the activated sludge and high rate algal pond treatment systems.

The average temperature in the aeration basin of the AS system was about two degrees higher than the temperature of the algal ponds (Table 2.2). Electrical conductivity increased

more in the HRAP (793.30±21.69 µS/cm^2) compared with that in the AS system (456.54±25.97 µS/cm^2; Table 2.2). Both, AS and HRAP treatment systems decreased the COD and TOC of post-AD brewery effluent to about 100 mg/l and 40 mg/l, respectively (Table 2.2). The TAN, nitrite and nitrate, concentrations of effluent leaving both the HRAP and AS systems were both well within the limits for discharge into a natural water resource (Table 2.2). Total ammonia concentrations in AS effluent (0.28±0.38 mg/l) were consistently lower than HRAP effluent (1.25±0.34 mg/l; Table 2.2). High rate algal ponds and AS treatment systems lowered the TN concentration of post-AD brewery effluent from 47.76±13.91 mg/l to an average of 12.21±4.31 mg/l (Table 2.2). The phosphate concentration of post-AD effluent (25.72± 1.46 mg/l) was decreased to (16.28± 1.35 mg/l) and (14.56± 1.46 mg/l) by the HRAP and AS systems respectively.

Table 2.2: Mean (± standard error) water quality parameters of effluent treated in high rate algal ponds (HRAP) and activated sludge (AS) over six months, n=45.

Parameter	Post-AD	Post-PFP	Post-AS	Post-HRAP	Discharge limits*
pH	7.12± 0.04	8.38± 0.02	8.41± 0.03	9.95± 0.04	5.5-9.5
Temperature (◦C)	31.78± 0.21	25.27± 0.22	26.38± 0.29	24.37± 0.36	
DO (mg/l)	0.17± 0.02	1.49± 0.03	1.07± 0.03	9.40± 0.15	
Conductivity (µS/cm^2)	3133.46±35.97	3256.91±53.28	3590.53±41.34	3929.76±28.63	1500
COD (mg/l)	361.69±15.89	204.02± 4.66	91.73± 0.80	97.67± 2.73	75
Total organic carbon (mg/l)	121.16± 5.08	57.76± 2.35	32.78± 0.42	46.62± 2.45	
PO_4-P	25.72± 1.46	21.46± 1.31	14.56± 1.46	16.28± 1.35	
TAN (mg/l)	32.47± 1.73	19.93± 0.91	0.28± 0.06	1.25± 0.05	3
NO_2-N (mg/l)	0.09± 0.01	0.83± 0.01	0.08± 0.01	0.27± 0.02	
NO_3-N (mg/l)	2.35± 0.10	8.71± 0.30	9.00± 0.57	6.62± 0.51	15
Total nitrogen (mg/l)	47.76± 2.08	34.93± 1.39	10.90± 0.67	13.51± 0.62	

Dissolved oxygen (DO), Chemical oxygen demand (COD), Anaerobic digestion (AD), Primary facultative pond (PFP), Total ammonia nitrogen (TAN). * Discharge limits into a natural water resource, National Water Act, Government Gazette No. 20526, 8 October 1999

The TAN and TN concentration of post-AD brewery effluent decreased significantly with an increase in HRT in both AS and HRAP treatment systems (Multiple regression $p<0.005$; Figures 2.7 and 2.8). The relationship between TAN and HRT can be described as $y=-3.99x + 33.60$ ($R^2=0.73$, $F_{(1,88)}= 365.29$, $p<0.0001$; Figure 2.7) in the HRAP and as $y=-71.52x + 32.47$ ($R^2=0.79$, $F_{(1,88)}= 347.63$, $p<0.0001$; Figure 2.7) in the AS. Total nitrogen and HRT is represented by the relationship $y=-3.96x + 45.88$ ($R^2=0.62$, $F_{(1,88)}=217.08$, $p<0.0001$; Figure 2.8) in the HRAP and $y=-74.61x + 44.47$ ($R^2=0.73$, $F_{(1,88)}=239.95$, $p<0.0001$; Figure 2.8) in the AS.

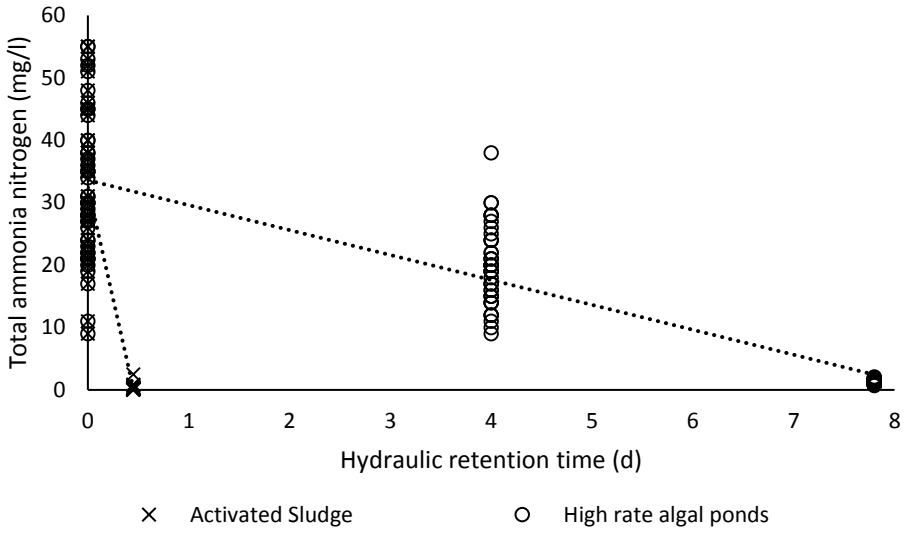

Figure 2.7: Brewery effluent total ammonia nitrogen concentration and related hydraulic retention time for activated sludge (y=-71.52x + 32.47; R^2=0.79, $F_{(1,88)}$= 347.63, p<0.0001) and high rate algal pond (y=-3.99x + 33.60; R^2=0.73, $F_{(1,88)}$= 365.29, p<0.0001) effluent treatment systems.

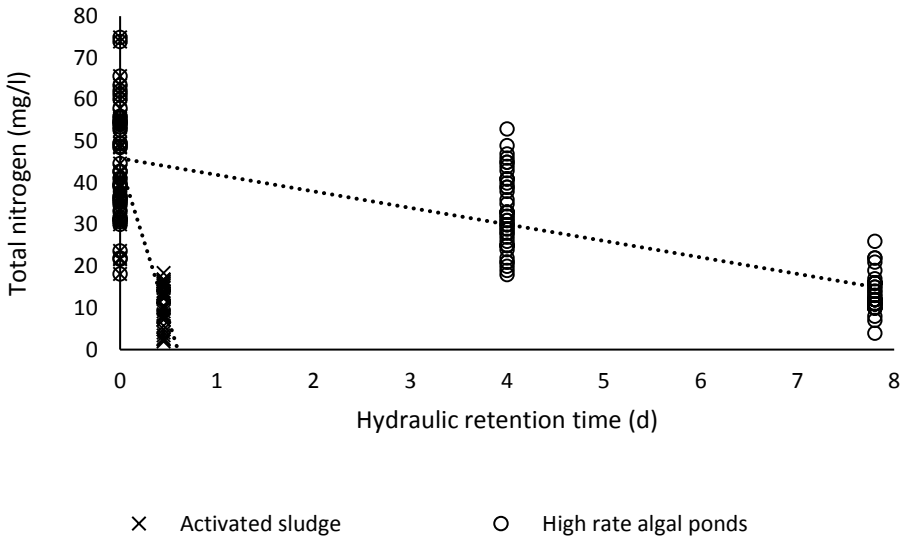

Figure 2.8: Brewery effluent total nitrogen concentration and related hydraulic retention time for activated sludge (y=-74.61x + 44.47; R^2=0.73, $F_{(1,88)}$=239.95, p<0.0001) and high rate algal pond (y=-3.96x + 45.88; R^2=0.62, $F_{(1,88)}$=217.08, p<0.0001) effluent treatment systems.

Seventy five percent of the total COD in the AD treated effluent entering the AS and PFP consisted of dissolved material in the treated effluent (AD outflow; Figure 2.9). The PFP further decreased the effluent COD and 90 % of the total COD entering the algal ponds from the PFP was in the form of soluble organic matter (PFP outflow; Figure 2.9). After treatment in the AS or HRAP systems the majority (i.e. >65 %) of the COD leaving the treatment systems was stabilised and in the form of particulates (AS outflow and HRAP outflow; Figure 2.9).

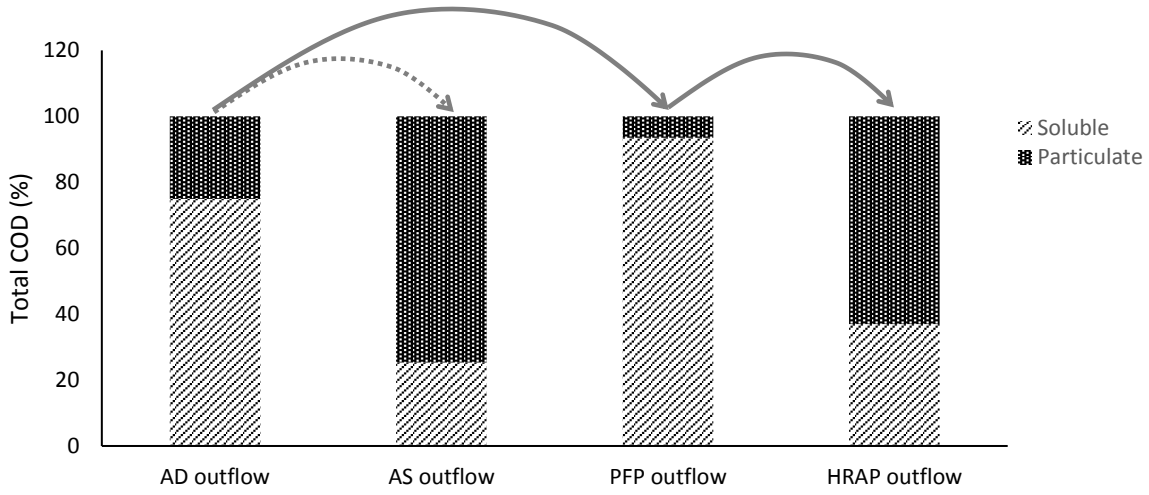

Figure 2.9: Mean particulate and soluble chemical oxygen demand (COD) entering and exiting the high rate algal pond (HRAP) and activated sludge (AS) effluent treatment systems. The outflow after anaerobic digestion (AD) forms the inflow for AS (dotted arrow) and it forms the inflow for the primary facultative pond (PFP) which in turn flows into the HRAP (solid arrows; n=25 per sample point per parameter).

The majority of the organic carbon entering both the AS and HRAP treatment systems, from the AD was in dissolved form (AD outflow; Figure 2.10). During the AS treatment process, about 50 % of the incoming organic carbon was released as carbon dioxide while about 20-25 % was incorporated into biomass and the remaining 25 % left the system in a dissolved form. The HRAP had a net consumption of carbon, where organic carbon leaving the ponds

was 1.8 times higher than that entering the ponds (Figure 2.10). However, 80 % of the organic carbon leaving the HRAP was stabilised into biomass. Dissolved organic carbon leaving AS and HRAP treatment process, was similar, and accounted for 20-30 % of the total incoming organic carbon.

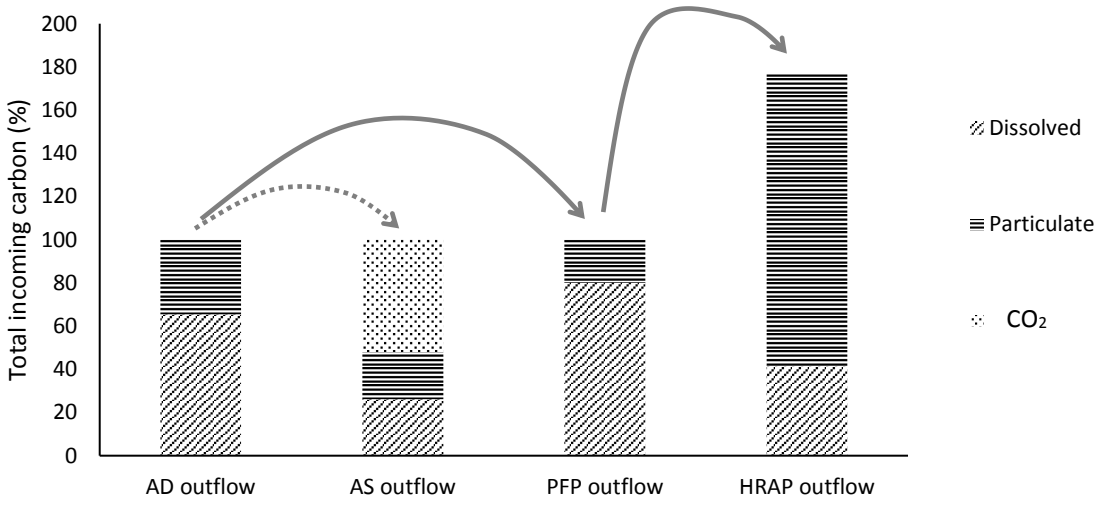

Figure 2.10: Mean particulate and soluble total organic carbon (TOC) entering and exiting the high rate algal pond (HRAP) and activated sludge (AS) treatment systems. The outflow after anaerobic digestion (AD) forms the inflow for AS (dotted arrow) and it forms the inflow for the primary facultative pond (PFP) which in turn flows into the HRAP (solid arrows; n=25 per sample point per parameter).

Total ammonia made up the majority (70 %) of the TN entering the aeration basin and PFP (47.76±13.91 mg/l; Figure 2.11). The remaining 30 % of incoming nitrogen was made up of particulate nitrogen (5-10 %), nitrite and nitrate (0-5 %) and UDN (10-15 %; Figure 2.11). The PFP decreased the portion of TN represented by TAN to 50-60 % and increased the portion of nitrate nitrogen to 20-25 % (Figure 2.11). Although these transformations occurred, the TN in the incoming and outgoing effluent of the PFP remained similar (Table 2.2).

Sixty-six percent of the total incoming nitrogen outgassed in the AS (Figure 2.11). Only 8.8 % of total incoming nitrogen was incorporated into biomass. The remaining 20 % left as nitrate in post-AS effluent and about five percent as unidentified dissolved nitrogen. The HRAP transformed 50 % of the total incoming nitrogen into biomass while only 25 % was released into the atmosphere (Figure 2.11). The remaining 25 % was made up of nitrate leaving in the effluent (20 %), and unidentified dissolved nitrogen accounted for five percent (Figure 2.11).

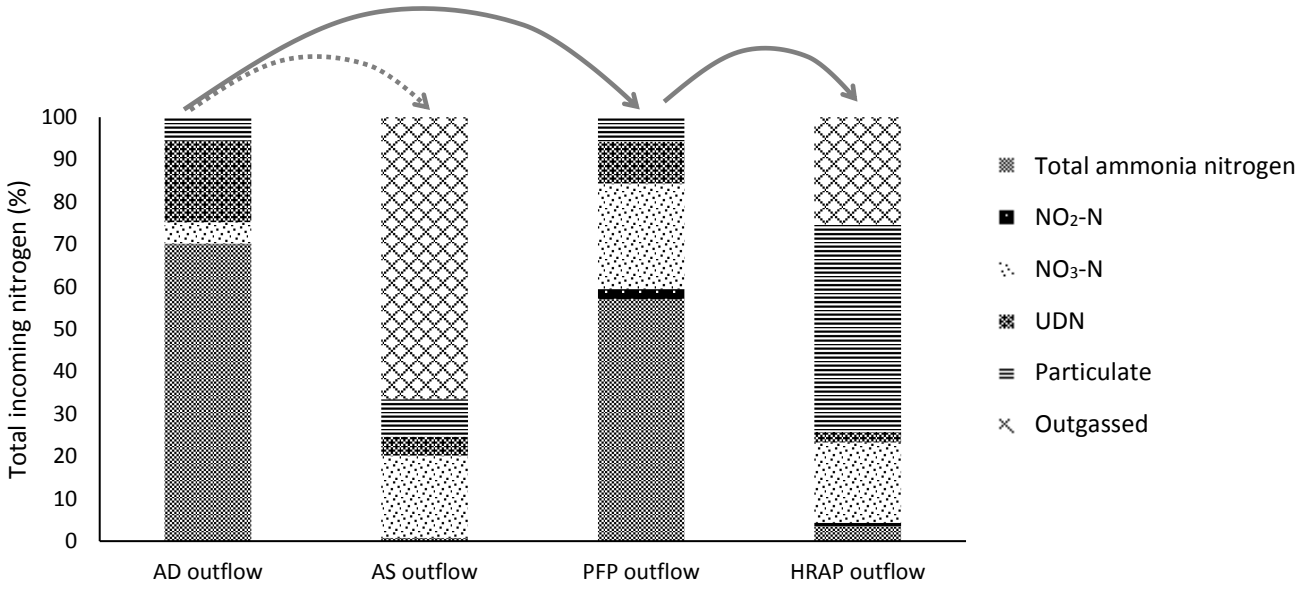

Figure 2.11: The form of nitrogen entering and exiting the high rate algal pond (HRAP) and activated sludge (AS) treatment systems, over six months. The outflow after anaerobic digestion (AD) forms the inflow for AS (dotted arrow) and it forms the inflow for the primary facultative pond (PFP) which in turn flows into the HRAP (solid arrows; n=25 per sample point per parameter). Unidentified dissolved nitrogen (UDN).

On average AS produced 60 g of C-CO_2 while HRAP consumed 84 g of C-CO_2 per cubic metre of effluent treated. The AS process produced 0.38 kg of biomass per kg COD removed while HRAP produced 3.6 times more biomass per kg COD removed (Table 2.3). The same trend was observed when looking at the biomass produced per kg of TOC fed or removed by the respective treatment systems (Table 2.3).

Table 2.3: Mean (± standard error) carbon dioxide emissions and biomass production from high rate algal ponds (HRAP) and activated sludge (AS) over six months, n=45.

	AS	HRAP
Net C-CO_2 production (g /m^3)	+60.17±5.82	-84.09±2.96
Biomass production (kg/kg COD fed)	0.27±0.03	0.95±0.04
Biomass production (kg/kg COD removed)	0.38±0.05	1.38±0.08
Biomass production (kg/kg TOC fed)	0.83±0.10	2.81±0.11
Biomass production (kg/kg TOC removed)	1.18±0.17	3.66±0.20

Chemical oxygen demand (COD), Total organic carbon (TOC)

The nitrogen and carbon concentrations of AS biomass were 50.04±6.03 g/kg and 321.67±28.26 g/kg respectively, similarly HRAP biomass had a nitrogen concentration of 53.90±9.01 g/kg and a carbon concentration of 373.00±36.50 g/kg. The mean ± the standard error of both the carbon and nitrogen concentrations of HRAP and AS biomass overlapped (Table 2.4). Settled biomass from the HRAP had a hydrogen concentration of 60.96±1.42 g/kg while waste sludge had a mean concentration of 51.24±6.06 g/kg (Table 2.4).

Table 2.4: Mean (± standard error) carbon, hydrogen, nitrogen and sulphur concentration of sludge produced in the primary facultative pond (PFP), high rate algal ponds (HRAP) and activated sludge (AS) treatment systems, n=25..

	AS	PFP	HRAP
Carbon (g/kg)	321.67±5.65	150.93±3.45	373.00±7.30
Hydrogen (g/kg)	51.24±1.21	20.13±0.56	60.96±0.28
Nitrogen (g/kg)	50.04±1.21	14.67±0.33	53.90±1.80
Sulphur (g/kg)	7.39±0.12	7.59±0.77	10.14±0.36

A HRAP system needed to treat the full volume of effluent currently treated by the AS (i.e. 850 m^3/day) at Ibhayi Brewery would consume 2.62 times less electrical energy than an AS system but would produce 3.8 times the amount of biomass (Table 2.5). The volume and surface area of a HRAP system required to treat all effluent were 10 and 100 times those needed by an AS system, respectively. However, the metabolic activity of the HRAP system

would consume 71 kg of C-CO_2 while the AS system would produce 51 kg of C-CO_2 per day (Table 2.5).

Table 2.5: The Impact of activated sludge (AS) and high rate algal pond (HRAP) systems needed to treat full volume effluent (850 m^3/d) at Ibhayi Brewery.

	AS	HRAP
Energy consumption (KW/day)	247	93
Reactor volume (m^3)	320	3199
Surface area required (m^2)	107	10665
Biomass produced (kg/day)	70	267
Carbon emissions (kg/day)	+51	-71

2.5 Discussion

The metabolic activity of micro-organisms in the effluent treatment process can influence the pH of the effluent. High rate algal ponds increased the neutral pH of post-AD effluent to near ten whereas AS increased the pH to eight. Carbon dioxide is generated during the AD of organic effluent and dissolves in the liquor, generating carbonic acid and carbonate alkalinity (Batstone *et al.* 2002, Van Rensburg *et al.* 2003). This causes an increase in both the alkalinity and acidity of the liquor (Van Rensburg *et al.* 2003). When AD effluent is aerated or exposed to the atmosphere, the volatile carbon dioxide expressed as carbonic acid is gassed off whereas the carbonate alkalinity is more stable and remains in the water (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). This results in a decrease in acidity and an increase in pH when post-AD brewery effluent is exposed to the atmosphere (Musvoto *et al.* 2000, Van Rensburg *et al.* 2003). In HRAP, photosynthesis causes the uptake of carbon dioxide and the release of O_2. The uptake of carbon dioxide from the water causes bicarbonate to dissociate, resulting in hydroxyl production, which increases the pH of the water (Tadesse *et al.* 2004). In both treatment systems, the pH of the effluent increased due to the outgassing of carbon dioxide when exposed to the atmosphere. The pH of HRAP

treated effluent was further increased due to the consumption of carbon dioxide by algae during photosynthesis.

Biological effluent treatment systems generally do not decrease the conductivity and salinity of effluents. On average HRAP increased brewery effluent conductivity by 25 % whereas AS increased the conductivity by 14 %. In biological effluent treatment systems, conductivity increase is mainly caused by evaporation and evapotranspiration (Tchobanoglous *et al.* 2003, Vymazal 2007). The rate of evaporation is determined by the temperature, air moisture content and surface area of water body (Klots 1991). Since the HRAP had a greater surface area than AS per volume unit of effluent treated, relatively more evaporation occurred in HRAP, and this is the most likely reason for the greater increase in effluent conductivity in that system.

One of the main functions of AS and HRAP is to lower the COD and remove unstable TOC from effluents and convert it into a more stable compound. Both treatment systems had similar TOC removal capacities, and both successfully lowered the COD to water reuse standards (<100 mg/l COD, Mclean *pers. comm.* 2017) but not to discharge limits into a natural water resource (<75 mg/l COD, DWAF 1996). The removal of organics from wastewater by biological systems such as HRAP or AS is achieved by the oxidization and mineralization of the organic matter in wastewater into biomass and more stable products such as carbon dioxide (Snyder and Wyant 2005, Garcia *et al.* 2006, Jenkins and Wanner 2014). Activated sludge and HRAP successfully oxidised unstable carbon compounds and inorganics into more stable forms (carbon dioxide and biomass) which aided in lowering the COD of treated effluent to reuse standards.

Nitrogen needs to be removed from effluent as it can pose an environmental and public health threat. Overall TN removal was similar for both systems, TAN, nitrite and nitrate concentrations being within the limits for discharge into a natural water resource (DWAF 1998). However, the form in which nitrogen was removed from each system differed. The form of nitrogen is influenced by many factors such as DO, pH, HRT and species of micro-organisms used in biological treatment process (Garcia *et al.* 2000, Tchobanoglous *et al.* 2003, Park and Craggs 2011). Both the AS and HRAP treatment systems where effective at removing inorganic nitrogen compounds (ammonia, nitrite and nitrate) from the effluent stream, resulting in its disposal posing minimal to no threat when released into a natural water resource.

When optimising the recovery of nutrients from brewery effluent the treatment system incorporates more nitrogen into biomass is more favourable as it can be used in downstream activities such as fertiliser production. The AS process released 66 % of incoming nitrogen into the atmosphere and only retained 8.8 % in its biomass, whereas the HRAP only released 25 % in a gaseous form and retained 50 % in its biomass. In both systems the remaining 20 % left as nitrate and about 5% as unidentified dissolved nitrogen. The assimilation of nitrogen into biomass in HRAP varies between 14-74 % and is influenced by a number of factors such as HRT, dissolved oxygen, pH, C/N ratio of effluent and form of nitrogen present in the effluent (Garcia *et al.* 2000, Park and Craggs 2011, Alcantara *et al.* 2015). In an experimental HRAP operated under similar conditions, nitrogen assimilation into biomass accounted for 47 % of total incoming nitrogen and 26 % was lost into the atmosphere: four day HRT, TN input of 50 mg/l, TN inflow made up of 84 % ammonia; (Park and Craggs 2011). Nitrogen lost to the atmosphere is mainly lost through; ammonia volatilization when the pH of water is above 9.0, and denitrification of nitrate to nitrogen

gas under anoxic conditions (Garcia *et al.* 2000, Park and Craggs 2011). Previous research on the same algal ponds at the research site at Ibhayi Brewery found that DO concentrations did not reach anoxic levels at night, mainly because the paddle wheels remained operational ensuring that the effluent remained well mixed (Jones *et al.* 2016). Hence the majority of nitrogen lost to the atmosphere was probably though ammonia volatilization since the average pH of HRAP effluent was above nine (Rose *et al.* 1996, Jones *et al.* 2016, Mogane 2016). The majority of nitrogen in HRAP systems was recovered in biomass with only 25 % being lost to the atmosphere.

The activated sludge system at Ibhayi Brewery automatically maintained its DO levels between 0.5-1.2 mg/l. These low DO concentrations allow an oxygen concentration gradient within the flocs (Munch *et al.* 1996, Law *et al.* 2012). Nitrifying bacteria occur in the outer layer, where aerobic conditions are present (Law *et al.* 2012). The denitrifiers reside in the middle anoxic zone of the flocs (Munch *et al.* 1996, Law *et al.* 2012). This allows nitrification and denitrification to occur simultaneously, which is what the AS process is designed and operated to do (Eckenfelder and Grau 1992, Mekinia *et al.* 2009). The removal of total incoming nitrogen using simultaneous nitrification/denitrification systems, varies from 40-95 % depending on the HRT, pH, DO, sludge age, temperature and C/N ratio (Brindle *et al.* 1998, Grunditz and Dalhammar 2001, Matsumoto *et al.* 2007).

During biological effluent treatment processes, waste products in the water are metabolised and incorporated into biomass which eventually need to be disposed of. The HRAP treatment system produced 3.8 times more biomass per volume of effluent treated or kilogram of COD removed when compared to AS. The majority of the micro-organisms present in AS are heterotrophic and utilise organic carbon in the wastewater to produce

biomass (Tchobanoglous *et al.* 2003, Waltz 2009). High rate algal ponds contain both heterotrophic and autotrophic micro-organisms (Evans *et al.* 1997, Tchobanoglous *et al.* 2003, Park *et al.* 2011). The autotrophic algae are able to utilise inorganic carbon and sunlight to produce biomass (El Ouarghi *et al.* 2003, Freeman 2005, Park *et al.* 2011) and this results in HRAP producing three times the biomass when compared to AS systems used to treat the same effluent.

Carbon emissions from effluent treatment plants have become a topic of concern due to global warming and climate change (Koop and van Leeuwen 2017). On average, the AS system produced 60 g of C-CO_2 while HRAP consumed 84 g of C-CO_2 per cubic meter of effluent treated. Heterotrophic bacteria in the AS oxidise organic carbon to produce energy, biomass, water and carbon dioxide (Tchobanoglous *et al.* 2003, Jenkins and Wanner 2014). Algae in the HRAP are able to utilise carbon dioxide to produce glucose during photosynthesis (Freeman 2005). A major benefit of the HRAP is that they have a net consumption of carbon dioxide while AS systems have a net production of carbon dioxide. As such, AS contributes to the anthropomorphic impact on global warming, whereas HRAP reduces this impact.

High rate algal ponds contain phototrophic micro-organisms which play a vital role during the effluent treatment process. Phototrophic micro-organisms require light as an energy source and therefore the treatment efficiency, surface area and HRT required is dependent on light intensity and photoperiod (Green *et al.* 1996, Craggs *et al.* 2014, Jones *et al.* 2016). This study was conducted over the summer months (late spring to early autumn) and an increase in HRT will be needed in winter to ensure similar nutrient removal efficiencies in the HRAP. The HRT of HRAP needs to be increased by 1-6 days during the winter months to

maintain sufficient effluent treatment efficiencies, with higher increases in HRT required as one moves further away from the equator (Green *et al.* 1996, Craggs *et al.* 2014, Jones *et al.* 2016). The HRT of the HRAP at the Ibhayi Brewery research site had to be increased from 3.8 to 6.0 d during winter to ensure that effluent was treated to reuse standards (Jones *et al.* 2016). High rate algal ponds are more suitable for: (a) equatorial climates, for example, where there is not much variation in light intensity and photoperiod throughout the year; or (b) where space is not limited and where HRT can be increased in winter without compromising the total volume of effluent treated (Guieysse *et al.* 2013, Craggs *et al.* 2014).

A comparison was conducted on the inputs and outputs of a HRAP or AS system needed to treat the full volume of effluent at Ibhayi Brewery. On the one hand, HRAP consume 71 kg of C-CO_2 and require 2.62 time less electrical energy than AS systems. On the other hand, AS systems require 10 times less volume and 100 times less surface area than HRAP. The AS system also produces about 3.8 time less biomass when compared to HRAP systems. Garfi *et al.* (2017) conducted a life cycle analysis comparing HRAP to AS and found that HRAP produced 2.5 times the biomass and a quarter of the C-CO_2 emissions. High rate algal ponds require 50 times more surface area and half the electrical consumption when compared to AS (Park *et al.* 2011, Craggs *et al.* 2011). The HRAP uses less energy and releases less carbon into the atmosphere but it requires substantially more land and produces more waste biomass. The decision as to which system is better to treat effluent is not straight forward and site-specific factors need to be taken into consideration.

Conclusion

Both HRAP and AS treatment systems are capable of lowering effluent COD and removing TN, ammonia and nitrate to reuse standards in non-production activities at Ibhayi Brewery. Each treatment system, however, has its advantages and disadvantages. The advantages of HRAP include a 50 % reduction in electricity consumption, recovering 50 % of incoming TN into biomass and sequestering atmospheric carbon dioxide. The major disadvantages of HRAP are their large surface area requirement and increasing effluent pH (>9.0) and conductivity. Activated sludge systems require small areas of land and produce less waste biomass than HRAP, however they consume more electrical energy and release carbon dioxide into the atmosphere. The main nitrogen removal from the AS system was denitrification with only 8.8 % of incoming nitrogen being recovered into sludge biomass. Activated sludge systems are ideal for industrial areas where space is limited and waste biomass production has to be minimalised. High rate algal ponds are suitable technologies for effluent treatment where space is not a constraint and where there are viable options to utilise the nitrogen recovered in the waste biomass. Both systems are effective at secondary effluent treatment; however, they have different nitrogen air emissions, nutrient removal mechanisms, biomass production and resource consumption rates. The suitability of the waste biomass produced for integration into other processes; e.g. such as the generation of energy or recovery of nutrient for agricultural fertiliser production, for example, needs to be determined in order to better understand which technology will be more appropriate for a zero-waste brewery effluent treatment process.

Chapter 3: Anaerobic digestion of the biomass produced by high rate algal ponds and activated sludge

3.1 Introduction

Biological effluent treatment technologies produce waste biomass that needs to be disposed of and this disposal can account for up to 50 % of the operational cost of the treatment facility (Perez-Elvira *et al.* 2006, Mo and Zhang 2013). At Ibhayi Brewery (SAB Ltd, South Africa), high rate algal ponds (HRAP) and activated sludge (AS) treatment technologies are effective at treating post-anaerobically digested (post-AD) brewery effluent to a standard suitable for reuse in aquaculture of *oreochromis mossambicus* and non-production activities (COD < 100 mg/l and turbidity < 7 NTU) such as bottle washing (Jones *et al.* 2016; Chapter 2). Both treatment technologies produce a biomass that poses an environmental threat if not disposed correctly. Sustainable and economically viable uses of waste sludge and algal biomass need to be identified to reduce the negative impact caused by its disposal, with the potential of generating a financial income.

The biomass generated in both AS and HRAP treatment systems is made up of micro-organisms that metabolise molecules in the wastewater for cell maintenance, reproduction and growth (Tchobanoglous *et al.* 2003). Their chemical composition is mainly carbon (30-50 %), nitrogen (4-10 %), hydrogen (5-10 %), oxygen (20-30 %), phosphorus (0.1-3%) and other trace elements (Ekama *et al.* 2007, Park and Li 2012, Ward *et al.* 2014, Tormo 2015). Waste sludge and algal biomass from brewery effluent treatment systems are not contaminated with heavy metals; with the main pollutants being composed primarily of carbon, nitrogen and phosphorus (Braeken *et al.* 2004, Kumar *et al.* 2010, Simate *et al.* 2011).

Open pond mixed culture algal biomass has the potential to be used as a biofuel, fertiliser or animal feed; for example, brewery effluent grown algae has been found suitable as a soya meal replacement in tilapia feeds (Santelices 2007, Jones *et al.* 2014, Alcantara *et al.* 2015). However, the dynamic microbial and algae species composition present in HRAP limit their commercial use in the food and pharmaceutical industries; and research is needed to identify their commercial value (Lee 2001). The methane potential of micro-algae falls within the reported yield for second generation biofuels, which has led to the interest in using algae as a feedstock for anaerobic digestion (AD; Chandra *et al.* 2012, Ward *et al.* 2014).

There exists an opportunity to anaerobically digest the sludge or algal biomass generated from the AS and HRAP effluent treatment processes to produce methane. The methane produced can be used to run motors, heat facilities and provide revenue for the treatment plant or industry generating the wastewater (Ward *et al.* 2008).

Anaerobic digestion is an energy efficient and environmentally sustainable method of removing organics from waste streams as it allows for the recovery of energy from organic waste (Angelidaki *et al.* 2003, Tchobanoglous *et al.* 2003). It is a biological process that converts organic carbon into a combustible gaseous mixture that is principally composed of methane and carbon dioxide, with hydrogen, H_2S and other trace gases as minor products (Lyberatos and Skiadas 1999, Tchobanoglous *et al.* 2003).

The maximum theoretical methane yield of algal biomass can be calculated from the protein, carbohydrate and lipid content of the algae (Sialve *et al.* 2009). These vary greatly between micro-algae species and culture conditions but normally algae have a protein content of 40-60 %, a lipid content of 10-20 % and a carbohydrate content of 10-30 %;

resulting in a theoretical methane yield of 600 ml/gVS fed (Richmond 2004, Ward *et al.* 2014, Uggetti *et al.* 2017).

Waste activated sludge generally has a low methane potential due to its low C/N ratio, with reported ranges between 5-10 (Heo *et al.* 2004, Ekama *et al.* 2007, Wan *et al.* 2011). The specific gas production from AS biomass is in the range of 180-500 ml/gVS fed (Bixio *et al.* 1999, Wook *et al.* 2000, Bolzonella 2005). The large variation in reported results is due to differences in substrate quality and digester operational procedures. Kaluza *et al.* (2014) reintroduced WAS into an upstream anaerobic digester and found that it did not decrease its performance and increased biogas production by 16 %. The low C/N ratio of WAS has been found to limit its feeding rate into anaerobic digesters, with total solids (TS) feeding rates above 4.8 $g/l_{reactor}/d$ causing ammonia toxicity and decreased biogas yield (Tchobanoglous *et al.* 2003, Bolzonella *et al.* 2005). The recommended TS feeding rate of WAS into anaerobic digesters is 1-2 $g/l_{reactor}/d$ (Tchobanoglous *et al.* 2003, Bolzonella *et al.* 2005).

Algal and WAS biomass generally have low biodegradability and a large number of studies have been conducted on using pre-treatment technologies to increase the specific gas production (SGP) of these biomasses (Navaneethan 2007, Mussgnug *et al.* 2010, Chen and Alzate *et al.* 2012, Gonzalez-Fernandez *et al.* 2012, Schwede *et al.* 2013). Heat treatment, chemical hydrolysis, high pressure, ultrasound and various other pre-treatment steps have been shown to increase the SGP of both algal and WAS biomasses by up to 170 % (Chen and Oswald 1998, Carrere *et al.* 2010, Mussgnug *et al.* 2010, Alzate *et al.* 2012, Gonzalez-Fernandez *et al.* 2012). Pre-treatment methods can increase the methane yield of algal and WAS biomasses, but the energy cost of pre-treatment normally exceeds the energy gained

from the extra methane production (Yen and Brune 2007, Lee *et al.* 2012, Lee *et al.* 2013, Lu *et al.* 2013). Therefore, no pre-treatment steps will be conducted during this study.

Research conducted on the AD of algae or waste activated sludge (WAS) is mainly limited to biochemical methane potential (BMP) and specific methane production (SMP) assays (Lin *et al.* 1997, Angelidaki *et al.* 2009, Seng *et al.* 2010, Ge *et al.* 2016), with limited literature on the continuous digestion of micro-algae (Bolzonella *et al.* 2005, Appels *et al.* 2008, Seng *et al.* 2010). To add to this, no research report/publication is available on the AD of brewery effluent grown algae or sludge. Furthermore, no literature has directly compared the biogas production from WAS and algal biomass from wastewater treatment systems.

3.2 Aims and objectives

The aim of this experiment was to evaluate the use of HRAP and AS biomasses for methane production during AD, to document its efficiency, and to describe the digestate (waste) that was produced from the AD process. This was done by feeding 2.5 l continuously stirred tank reactors (CSTR) for 90 days. Biogas production, biogas quality and various water quality parameters were recorded to determine the performance and dynamics of the AD process. The objectives of this experiment were to:

- evaluate the digestibility of HRAP and WAS biomass;
- assess the methane production potential from HRAP and WAS biomass in an anaerobic reactor; and
- assess the water quality parameters of the digestate from the two treatments.

3.3 Materials and methods

3.3.1 Experimental treatments

The two experimental treatments included settled biomass from: (1) HRAP and (2) AS effluent treatment systems. Each treatment was replicated three times, with a replicate consisting of a single anaerobic reactor.

Waste activated sludge from the commercial-scale AS system (Chapter 2, Section 2.3.1) and algal biomass from the pilot-scale HRAP system (Chapter 2, Section 2.3.1) used to treat brewery effluent at Ibhayi Brewery were collected every day. These slurries were settled for 12 h, to concentrate the sludge, and achieve a total solids content of 25 g/l. Gravitational settling of the waste biomass was chosen because this is practiced at Ibhayi brewery and a number wastewater treatment plants (WWTPs) use this method to separate sludge from the treated effluent (Bolzonella *et al.* 2005). Algal and WAS slurries that were collected and settled on one day was used to feed anaerobic reactors the following day. This sludge and algae formed the two feed sources for AD that were compared in this trial.

3.3.2 Experimental system

Design of the anaerobic reactors

The experiment was conducted in an insulated, temperature controlled room (37 ^{o}C). Digesters were made out of polyvinylchloride (PVC) chambers (150 mm diameter, 3.0 l volume) and were operated with a 2.5 l working volume and a 0.5 l head space (Figure 3.1). A PVC pipe connected the head space of the reactors to a water displacement flask (Figure 3.1). Each reactor was placed on a shaker plate (Labcon platform shaker, model SPO 15-MP,

California, United states of America) set at 120 rpm to ensure thorough mixing of the anaerobic sludge and feed. Experimental digesters were placed 0.5 m under water to check for any gas leaks, prior to starting the experiment.

Inoculation, conditioning and operation

The experimental anaerobic reactors were each inoculated with: (a) 1.0 l of granular sludge, obtained from the commercial-scale, methane producing up-flow anaerobic sludge blanket reactor at Ibhayi Brewery, and (b) 1.5 l of digestate from the same commercial-scale anaerobic digester (i.e. post-AD effluent). A sample was taken from each reactor to determine the VS and TS introduced into these digesters at the start of the experiment. Before reactors were fed the experimental treatments, they were conditioned with screened brewery effluent (untreated effluent that had passed through the drum filter; Chapter 2, Section 2.3.1) for 31 d at a feeding rate of 1.5 g VS/l.

The anaerobic reactors were then fed their respective feedstocks for a further 60 d, until a constant daily biogas production was reached in all digesters, before data were collected for a further 30 consecutive days. They were fed 150 ml/d of either sludge or algae which resulted in them having a hydraulic retention time (HRT) of 16.67 days and receiving between 1.5-2.0 g of TS/l/d of feed.

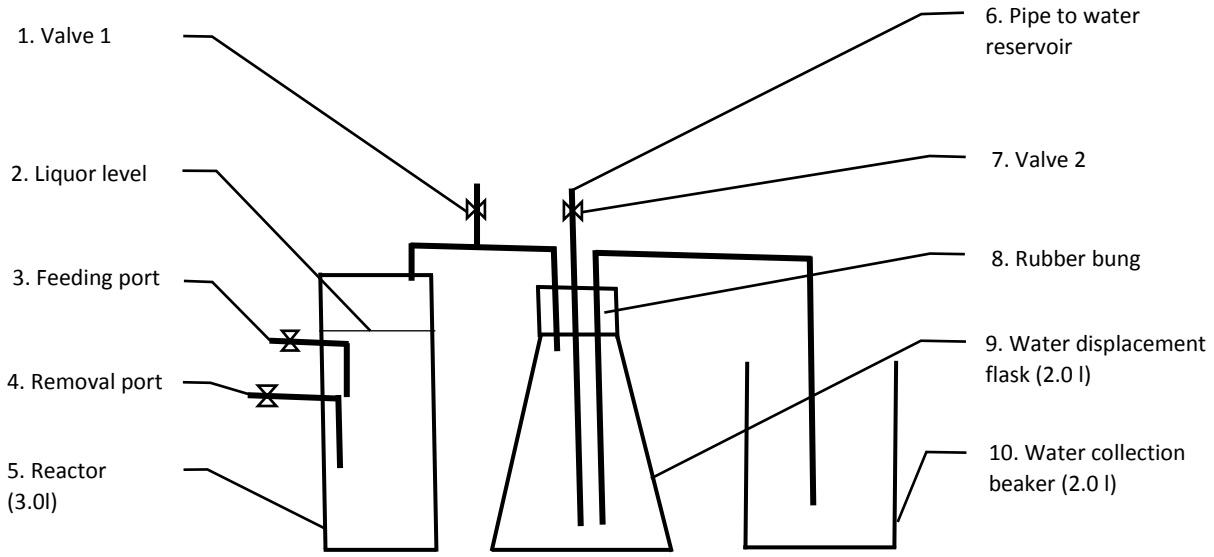

Figure 3.1: Schematic of the design of the anaerobic reactors.

The daily operational procedure included the following sequential steps:

1. Recording the volume of biogas produced;
2. Biogas analysis;
3. Removal of digestate;

   One hundred and fifty millimetres of digestate was removed through a removal portal using a syringe (Figure 3.1, point 4). This pipe extended inside each digester, to half way down the reactor (Figure 3.1, point 4).
4. Feeding the reactors;

   Reactors were fed fresh feed, following the removal of the same quantity of digestate. New feed was introduced into the digesters using a syringe via a feeding port situated on the side of the digesters (Figure 3.1, point 3).
5. Refilling the water displacement flask.

The water displacement flask was refilled every second day, and this was done by opening valve 2 (Figure 3.1, point 7) followed by opening valve 1 (Figure 3.1, point 1). The pipe from valve 2 was connected to a reservoir (Figure 3.1, point 6) which contained water adjusted to pH 2 with hydrochloric acid to stop the dissolution of carbon dioxide into the water (Carroll and Mather 1992, Ikumi *et al.* 2014).

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3.3.3 Data collection

The volume of biogas produced was recorded using a water displacement system, consisting of a 2.0 l conical water displacement flask (Figure 3.1, point 9) and a 2.0 l water collection beaker (Figure 1, point 10). The water in the beaker had a layer of vegetable oil on its surface to minimise evaporation. The volume of biogas produced was determined by subtracting the present day's beaker weight (0.1 g) from the previous day's beaker weight.

The methane and carbon dioxide percentage of the biogas were determined daily using the hand-held biogas analyser (Technovation Analytical Instruments, Series 2012 portable gas monitor: SR2012V2.0) connected to valve 1 (Figure 3.1, point 1). Readings were only taken when the instrument output values remained constant.

Water quality measurements from of the digestate where taken immediately after removal, with pH and alkalinity measurements carried out first. The pH, temperature (^{o}C) and conductivity ($\mu S/cm^2$) of the feed and digestate were recorded daily using an electronic probe (Hanna, HI 991300, United Kingdom). Chemical oxygen demand (COD, mg/l), total organic carbon (TOC, mg/l), total nitrogen (TN, mg/l), volatile fatty acids (VFA, mg COD/l), total ammonia nitrogen (TAN; mg/l), nitrite (mg/l), phosphate (mg/l) and chloride (mg/l) of the digestate and feed were recorded every third day using a spectrophotometer (Merck

Spectroquant Pharo 100 spectrophotometer, product number 100706, Darmstadt, Germany) and commercially available test kits, and standard methods (Merck Pty Ltd, Darmstadt, Germany). The COD, TOC and TN analyses were performed on unfiltered and filtered samples whereas the analyses of VFA, TAN, nitrite, phosphate and chloride were done on samples filtered through to 8.0 µm filter paper (Whatman 40 Ashless Circles, 125 mm diameter, Cat no. 1440 125, Whatman plc, Maidstone, United Kingdom).

The alkalinity of the digestate was determined by titration with 0.1 mol/l sulphuric acid (Ripley *et al.* 1986). Total alkalinity was calculated by the amount of acid titrated to obtain a pH of four, using Equation 3.1 (Ripley *et al.* 1986); where A is the amount of acid titrant in mg/l, N is the normality of the sulphuric acid solution and V is the volume of the sample (l) :

$$\text{Alkalinity (mg } CaCO_3\text{/l)} = (A \times N \times 50000)/V \quad [3.1]$$

Total solids and VS of the feed and digestate were recorded (0.001 g/l) twice a week according to the methods described previously (Chapter 2, Section 2.3.2).

Fortnightly feed and digestate samples were analysed for carbon, hydrogen, nitrogen, oxygen, sulphur, calcium, magnesium, potassium, sodium, zinc, copper, manganese, iron, phosphorus and aluminium (inductively coupled plasma mass spectrometry (EPA 2000) and X-ray fluorescence (EPA 2007a), Central analytical facilities, Stellenbosch University, South Africa).

A mass balance approach was used to estimate the mass of dissolved organic nitrogen leaving in the digestate using Equation 3.2; where total nitrogen entering the system equals the total nitrogen exiting the system (Puig *et al.* 2008, Alcantara *et al.* 2015):

$$N_{biomass\ in} + N_{dissolved\ in} = N_{biomass\ out} + N_{dissolved\ out} \quad [3.2]$$

where:

$N_{dissolved}$ = total ammonia nitrogen + NO_2-N + NO_3-N+ dissolved organic nitrogen.

3.3.4 Statistical analysis

Treatment means were compared using a Student t-test $p<0.05$. Data collected over the course of the trial were compared using a one-way repeated measures ANOVA ($p<0.05$). All data were checked for equality of variance and for the normal distribution of the residuals using Levene's test and a Shapiro-Wilk plot of the residuals, respectively. If the assumptions were not met, then the data were log or square-root transformed and checked for equal variance and normal distribution of residuals. If the assumptions were still not met, a non-parametric Mann-Whitney test was used to compare the data between treatments while a Mauchly's sphericity test was used to compare data collected over the course of the trial (Salkind *et al.* 2010). All analyses were performed using a statistical software package (Statistica, Version 10, StatSoft Inc, Tulsa, USA). Statistical analysis on pH data was done using hydrogen ion concentration.

3.4 Results

The carbon concentration and C/N ratio of algae biomass were significantly higher than those of the waste sludge (Student t-test, $p<0.05$; Table 3.1). Hydrogen concentration of algae was also significantly higher than sludge (Student t-test, $t=6.87$ $p=0.0023$; Table 3.1). Waste sludge had an average oxygen concentration of 248.46 g/kg which was significantly higher than the oxygen concentration of algae (Student t-test, $t=5.99$ $p=0.0039$; Table 3.1). Algal biomass had a sulphur concentration 0.99 g/kg higher than sludge biomass (Table 3.1).

The nitrogen concentrations of WAS and algae were similar (Student t-test, t=-0.28 p=0.7933; Table 3.1).

Table 3.1: Mean (± standard error) chemical composition of sludge and algae n=3 (Student t-test, p<0.05).

Element	Sludge	Algae	t	P
Carbon (g/kg)	328.14±4.94	349.08±2.71	3.71	0.0206#
Hydrogen (g/kg)	51.02±0.46	56.97±0.74	6.87	0.0023#
Nitrogen (g/kg)	52.52±0.76	51.25±0.57	-0.28	0.7933
Sulphur (g/kg)	10.01±0.57	9.02±0.34	1.47	0.2137
Oxygen (g/kg)	248.46±5.16	208.43±4.24	5.99	0.0039#
C/N ratio	6.39±0.16	6.96±0.11	2.84	0.0464#

# indicates significant differences between treatment means.

The mean pH of AS sludge was 8.35±0.03 which was significantly lower than the pH of algae slurry 8.70±0.03 (Student t-test, t=12.76 p=0.0002; Table 3.2). Algae and AS slurry had a similar alkalinity with a combined mean of 1280.23±16.15 mg $CaCO_3$/l (Table 3.2). Waste sludge had a significantly lower conductivity than algal slurry (Student t-test, t=5.55 p=0.0052; Table 3.2). The average TS and VS concentration of settled sludge and algal biomass was similar with combined means of 24.64±0.40 and 16.78±0.31 g/l respectively (Student t-test, p=0.0867; Table 3.2). The TOC of algal slurry was significantly higher than that of waste sludge with means of 8557.42±48.77 and 8209.27±41.67 mg/l respectively (Student t-test, t=8.26 p=0.0012; Table 3.2). The TN and nitrate concentrations of sludge and algae slurry fed to anaerobic reactors were similar (Student t-test, p>0.4045; Table 3.2). The mean TAN and nitrite (0.84±0.04 and 0.20±0.10 mg/l) concentrations of algal slurry were significantly higher than those of waste sludge (0.40±0.02 and 0.08±0.01 mg/l; Table 3.2).

Table 3.2: Mean (± standard error) characteristics of waste sludge and algae fed into anaerobic digesters n=3 (Student t-test, p<0.05).

Parameter	Sludge	Algae	t	P
pH	8.35 ± 0.01	8.70± 0.03	12.76	0.0002#
TA (mg $CaCO_3$/l)	1301.62±17.20	1258.84±15.87	-1.83	0.1416
Conductivity (µS/cm^2)	3279.29± 7.92	3452.88±30.26	5.55	0.0052#
TS (g/l)	25.24± 0.42	24.04± 0.50	-1.83	0.1413
VS (g/l)	17.29± 0.26	16.26± 0.37	-2.26	0.0867#
TOC (mg/l)	8209.27±30.44	8557.42±29.17	8.26	0.0012#
TN (mg/l)	1257.63±11.14	1243.34±10.56	-0.93	0.4045
TAN (mg/l)	0.40± 0.04	0.84± 0.06	5.82	0.0043#
NO_2-N (mg/l)	0.08± 0.01	0.20 ± 0.02	6.87	0.0024#
NO_3-N (mg/l)	8.89± 0.28	8.67 ± 0.13	-0.73	0.5077

Total alkalinity (TA), Total solids (TS), Volatile solids (VS), Chemical oxygen demand (COD), Total organic carbon (TOC), Total Nitrogen (TN), Total ammonia nitrogen (TAN), # indicates significant differences between treatment means.

The VS feeding rate for sludge and algae fed digesters were similar, with a combined mean of 1.01±0.02 g/$l_{reactor}$/d (Student t-test, p>0.0867; Table 3.3). The temperature of the liquor in sludge and algae fed anaerobic reactors was similar with a combined mean of 35.18±0.05 ^oC (Student t-test, t=0.11, p=0.92; Table 3.3).

Table 3.3: Mean (± standard error) operational conditions for anaerobic digesters used to digest waste sludge and algae n=3 (Student t-test, p<0.05).

Parameter	Sludge	Algae	t	P
Temperature (^oC)	35.18±0.05	35.17±0.05	0.11	0.9210
HRT (d)	16.67±0.00	16.67±0.00		
Feeding (gTS/$l_{reactor}$/d)	1.51±0.03	1.44±0.03	-1.83	0.1413
Feeding (gVS/$l_{reactor}$/d)	1.04±0.02	0.98±0.02	-2.26	0.0867

Total solids (TS), Volatile solids (VS), Hydraulic retention time (HRT).

The pH of digestate from algae and sludge fed digesters was similar (Student t-test, t=2.69 p=0.0545; Table 3.4). Total alkalinity of the liquor of sludge and algae fed digesters was similar with a combined mean of 1884.19±24.68 mg $CaCO_3$/l (Table 3.4). The VFA concentration of algae and sludge fed digesters remained below 100 mg COD/l throughout the experiment (Table 3.4). Digestate from algae fed digesters had a significantly higher EC

when compared to sludge fed digesters (Student t-test, t=5.18 p=0.0066; Table 3.4). The average TOC concentration of algae digestate was significantly higher than that of sludge digestate (Student t-test, t=12.70 p=0.0002; Table 3.4). Digestate from sludge and algae fed digesters had similar TN and TAN concentrations, with combined means of 1246.14±6.55 and 361.78±3.67 mg/l respectively (Student t-test, p>0.4066; Table 3.4). Nitrate and nitrite nitrogen concentrations of digestate from all digesters remained below 1.00 and 0.07 mg/l respectively (Table 3.4).

Table 3.4: Mean (± standard error) characteristics of digestate from waste sludge and algae fed anaerobic digesters n=3 (Student t-test, p<0.05).

Parameter	Sludge	Algae	t	P
pH	7.32 ± 0.08	7.20± 0.09	2.69	0.0545
VFA (mg COD/l)	< 100.00	< 100.00		
TA (mg $CaCO_3$/l)	1896.61± 29.00	1871.76± 20.84	-0.70	0.5248
Conductivity (µS/cm^2)	3481.90 ± 6.07	3656.14± 33.11	5.18	0.0066#
TS (g/l)	17.71 ± 0.46	15.92± 0.31	-2.99	0.0405#
VS (g/l)	11.24 ± 0.34	10.66± 0.21	-1.46	0.2189
TOC (mg/l)	5507.03± 7.08	5837.03± 25.01	12.70	0.0002#
TN (mg/l)	1247.10± 13.32	1245.19± 3.41	0.72	0.5121
TAN (mg/l)	336.22± 7.93	327.34± 5.36	-0.93	0.4066
NO_2-N (mg/l)	< 0.07	< 0.07		
NO_3-N (mg/l)	< 1.00	< 1.00		

Volatile fatty acids (VFA), Total alkalinity (TA), Total solids (TS), Volatile solids (VS), Chemical oxygen demand (COD), Total organic carbon (TOC), Total Nitrogen (TN), Total ammonia nitrogen (TAN), # indicates significant differences between treatment means.

The TS and VS destruction was similar between sludge and algae fed digesters with combined means 30.21±0.69 and 34.30±0.89 % respectively (Student t-test, >0.1554; Table 3.5). Biogas production per TS fed to algae and sludge digesters was similar (Student t-test, t=1.78 p=0.1505; Table 3.5). The average biogas production per VS fed was similar between algae and sludge fed digesters (Student t-test, t=2.64 p=0.0578; Table 3.5) with a combined average of 780.68±31.13 ml/gVS removed (Table 3.5). Biogas produced from the AD of algae had a significantly higher methane concentration when compared to gas produced from the

AD of sludge (Student t-test, p<0.0050; Table 3.5). Algal biomass produced significantly less carbon dioxide per VS when compared to waste sludge (Student t-test, p<0.0380, p<0.011; Table 3.5).

Table 3.5: Mean (± standard error) biogas, methane and carbon dioxide production from anaerobic digesters fed with waste sludge or algae n=3 (Student t-test, p<0.05).

Parameter	Sludge	Algae	t	P
Total solids destruction (%)	29.47± 2.00	30.95± 0.75	1.75	0.1554
Volatile solids destruction (%)	34.84± 1.54	33.76± 0.74	-0.63	0.5627
Biogas production (ml/gTS fed)	160.00± 3.12	168.87± 3.90	1.78	0.1505
Biogas production (ml/gVS fed)	233.53± 4.29	250.28± 4.68	2.64	0.0578
Biogas production (ml/gVS removed)	726.68±39.63	822.68±52.63	1.46	0.2188
CH_4 concentration of biogas*(%)	60.08± 0.18	64.73± 0.81	5.59	0.0050#
CO_2 concentration of biogas* (%)	27.37± 0.43	22.94± 0.24	-8.97	0.0009#
CH_4 production (ml/VS fed)	140.25± 3.13	161.72± 1.51	6.17	0.0035#
CO_2 production (ml/gVS fed)	64.61± 1.38	53.98± 3.19	-3.05	0.0380#

Volatile solids (VS), * Percentage expressed per volume, # indicates significant differences between treatment means.

Particulate bound nitrogen accounted for more than 98 % of the total nitrogen entering both algae and sludge fed anaerobic digesters (Figure 3.2). Sludge and algae fed digesters had similar nitrogen conversion percentages, converting an average of 26.90±0.36 % of incoming nitrogen into ammonia, while 71.25±0.62 % remained in particulate form and a mean of 2.22±0.13 % left in a dissolved form (Student t-test, Mann-Whitney U, p>0.30; Figure 3.2).

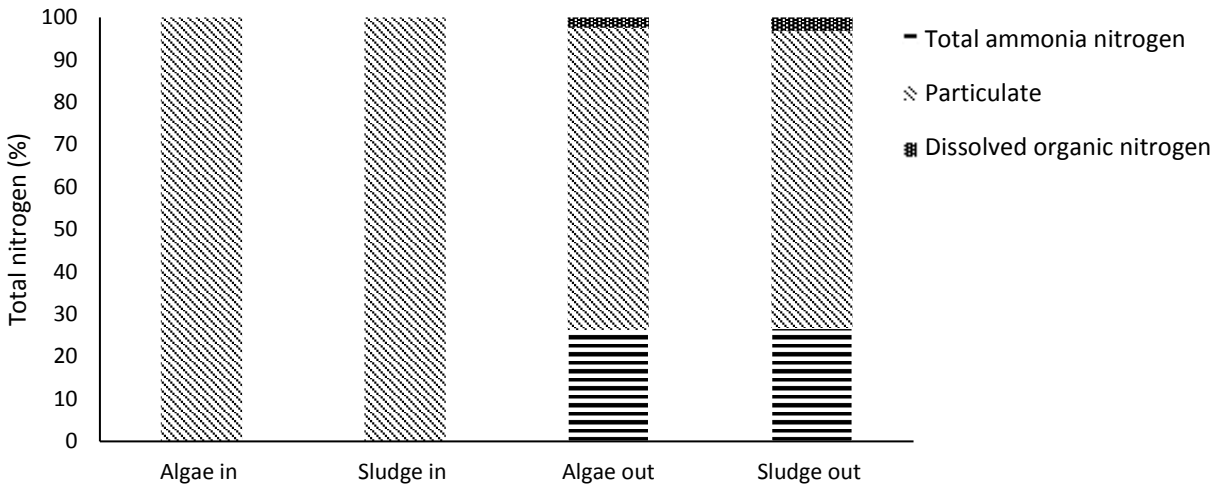

Figure 3.2: The form of nitrogen entering (algae in, sludge in) and exiting (algae out, sludge out) anaerobic digesters fed with waste sludge or algae.

3.5 Discussion

Algae and waste sludge biomass generally have a low biogas yield due to their low concentration of digestible substrate and C/N ratio. Algae and AS sludge had similar gas production rates per TS and VS fed. The average gas production from algae was 250 ml/gVS fed while sludge fed digesters produced 233ml/gVS fed. Literature reports a specific gas production (SGP) of 180-500 ml/gVS and 160-400 ml/gVS for semi-continuous and continuously fed anaerobic digesters receiving micro-algal or sludge substrates respectively (Samson and Leduy 1986, Bixio *et al.* 1999, Wook *et al.* 2000, Bolzonella 2005). Higher SGP rates have been recorded but these are from BMP assays which do not accurately represent the performance of a commercial anaerobic digester. The AD of algal and WAS sludge biomass involves the degradation of whole cells and hydrolysis has been identified as a rate limiting step (Gavala *et al.* 2003, Tchobanoglous *et al.* 2003, Appels *et al.* 2008). Pre-treatment disrupt the contents of cells to anaerobic bacteria which can increase methane yield and digestibility of substrates; however, they also increase processing costs and

operational energy consumption (Wook *et al.* 2000, Elliott and Mahmood 2007, Khanal *et al.* 2007, Lin *et al.* 2009). When the energy consumption of the pre-treatment step is taken into account the energy gained from increased methane production is lower (Tchobanoglous *et al.* 2003, Yen and Brune 2007, Lee *et al.* 2013, Lu *et al.* 2013). The low digestibility and C/N ratio of algal and WAS biomass limits digester TS feeding rates with most literature recommending a feeding rate of 1-2 $g/l_{reactor}/d$ (Tchobanoglous *et al.* 2003, Bolzonella *et al.* 2005). The results from this study are consistent with other studies that model the AD of algae and WAS slurries operated under mesophilic temperatures with no pre-treatment.

The TS and VS destruction is a vital parameter evaluating the suitability of a substrate for AD. Digesters fed algae or sludge had similar TS and VS destruction rates. Gas production per VS destruction was similar between WAS and algae fed digesters with a combined mean gas production of 774 ml/gVS removed. Under mesophilic conditions (34-38 ^{o}C) reported gas production for WAS and algae fed CSTR range between 600-900 ml/gVS, with the majority of the literature reporting 700-800 ml/gVS (Samson and Leduy 1986, Bixio *et al.* 1999, Wook *et al.* 2000, Bolzonella 2005). The VS reduction rate observed in this study was similar to the literature with most studies reporting a 25-50 % VS reduction in CSTR fed algae or sludge at 1-2 $gVS/l_{reactor}/d$ and operating at a HRT of 10-40 days (Bixio *et al.* 1999, Bolzonella 2005, Karlsson 2010, Ikumi *et al.* 2014). A VS reduction rate of 30-40 % and a gas production rate of 600-900 ml/gVS can be used when modelling the AD of brewery effluent grown algae or sludge.

Particulate nitrogen is transformed into soluble nitrogen during AD and high concentrations may inhibit the process. Over 98 % of nitrogen entering sludge and algae fed anaerobic digesters was in a particulate form. During the AD process about 30 % of this incoming

nitrogen was transformed into TAN resulting in digestates having an average TAN concentration of 361.5±5.19 mg/l. Ammonium toxicity during AD has been reported at concentrations above 1100 mg/l and is dependent on pH (Hansen *et al.* 1998, Tchobanoglous *et al.* 2003). Up to 50 % of the particulate nitrogen was converted to ammonium during BMP digestion trials of mix cultured micro-algae (Alcantara *et al.* 2015). Thirty five percent of particulate bound nitrogen was converted into TAN during continuous digestion trials of untreated WAS (Tormo 2015). The ammonia concentrations recorded during this study are well below reported concentrations that inhibit the AD process (Hansen *et al.* 1998, Tchobanoglous *et al.* 2003); as such digesters fed algae or WAS should not experience ammonia toxicity when operated under similar conditions to that of this trial.

The pH and alkalinity of substrates can influence the performance and stability of the anaerobic digester. The average pH of algae and sludge fed to the digesters was 8.70 and 8.35 while the average pH of the liquor in the respective digesters was 7.20 and 7.32. The optimal pH range for the liquor in anaerobic reactors is 6.5-8.0 (Tchobanoglous *et al.* 2003, Chernicharo 2007). Even though the pH of feed was relatively high, compared to standard operating procedures, for anaerobic digesters, the pH within the digesters remained stable and there was no need for pH adjustment (Tchobanoglous *et al.* 2003). The pH in anaerobic digesters is influenced by the acidity generated from hydrolysis and bicarbonate alkalinity generated from carbon dioxide and ammonia/ammonium production (Gallert *et al.* 1998, Hafner and Bisogni 2009). The production of hydrogen ions during AD normally exceeds the production of bicarbonate alkalinity, resulting in a decrease in the pH of the liquor (Rajeshwari *et al.* 2000). Therefore, most commercial anaerobic digesters require the addition of sodium hydroxide to maintain a stable pH of liquor and AD process. Waste

activated sludge and algae have a sufficient nitrogen content to allow the production of ammonia generated alkalinity which can maintain digester pH. These substrates are suitable for continuously fed anaerobic digesters as the acidity generated from hydrolysis and alkalinity produced during AD was able to maintain a stable pH in the reactors when fed 1.5 gTS/l/d.

Volatile fatty acid concentration can be used as an indication of AD stability. The VFA concentration of digestate in all reactors remained below 125 mg/l, with the majority of the readings being below 50 mg/l. It is important to keep a balance between the rate of VFA production and the rate of methanogenesis, as methanogenic archaea are more susceptible to the accumulation of VFA and corresponding pH drop than the other micro-organisms involved in AD (Jiang 2012). As such, pH and VFA concentration are good indicators of reactor stability (Rajeshwari *et al.* 2000, Buyukkamaci and Filibeli 2004). High concentrations of VFA and digester acidification are normally caused by overfeeding or unstable environmental conditions in the digester (Tchobanoglous *et al.* 2003). Normal operational standards for anaerobic digesters require that VFA concentrations remain below 100 mg/l (Tchobanoglous *et al.* 2003, Bolzonella *et al.* 2006, Mclean *pers. comm.* 2017). The low VFA concentrations recorded during the experiment represents a stable AD process, indicating that algae and WAS are suitable substrates for AD with a recommended feeding rate of 1.5 $kgTS/m^3/d$ for commercial continuously fed reactors (Bolzonella *et al.* 2006).

The methane and carbon dioxide composition of biogas varies between different substrates and digester operating conditions. Biogas from algae fed digesters had a significantly higher methane content and lower carbon dioxide content when compared to WAS fed digesters. The carbon dioxide to methane ratio of gas depends on the oxygen state of the carbon in

the substrate, with a positive relationship existing between the reduced carbon content of the substrate and methane production (Angelidaki and Sanders 2004). The carbon, hydrogen and oxygen concentration of organic matter can be used to estimate the theoretical methane and carbon dioxide yield of a substrate, with an increasing oxygen concentration decreasing the methane yield ($C_nH_aO_b + (n - a/4 - b/2)H_20 \rightarrow (n/2 + a/8 - b/4)CH_4 + (n/2 - a/8 + b/4)CO_2$; Symons and Buswell 1933, Angelidaki and Sanders 2004). According to this formula the respective theoretical methane yield of algal and sludge biomasses was 18.41 and 16.06 moles of methane per Kg of biomass digested. Algal biomass had a higher hydrogen concentration and lower oxygen concentration (less oxidised) when compared to AS sludge, resulting in a higher methane concentration from algae fed digesters. Reported ranges for the hydrogen and oxygen content of sludge and algae overlap but algal biomasses generally seem to have a higher hydrogen concentration and lower oxygen concentration (Ekama *et al.* 2007, Park and Li 2012, Ward *et al.* 2014). This is primarily because algae have a higher lipid concentration when compared to WAS, resulting in a higher hydrogen concentration (Wang and Park 2015). Algae have a higher hydrogen concentration and lower oxygen concentration than WAS that is grown on the same effluent which will result in the algal biomass producing a gas with a higher methane content when compared to that produced using WAS.

Anaerobic digestion can be used as a stabilisation and pre-treatment step when sludge biomasses are destined for agricultural land application. Digestate from algae and WAS fed digesters had similar TAN concentrations, with a combined mean of 331.78±5.19 mg/l. Inorganic nitrogen fertilisers used in agriculture contain ammonia in concentrations that vary from 2-46 % (Singh and Agrawal 2008, Dobhal and Singhal 2017). Anaerobically digested sludge from WWTPs has been found to contain sufficient nitrogen and ammonia to

be used as an inorganic fertiliser replacement (Lyberatos *et al.* 2004, Quilbe *et al.* 2005, Singh and Agrawal 2008). Antolin *et al.* (2005) compared the use of anaerobically digested WAS to a commercial fertilizer on barley fields for four years and found a higher yield in sludge application treatment; however, an increase in heavy metal concentration of the grain was observed. Anaerobically digested algae or WAS has the potential to be used as a fertiliser replacement, however chemical analysis of the digestate and field trials need to be conducted to ensure that the digestate does not cause toxic effects to the receiving environment.

Conclusion

Brewery effluent grown algal and WAS biomasses are suitable feed stocks for continuously stirred anaerobic digesters and this trial suggests that this process can be exploited commercially. It is recommended to operate WAS and algae fed anaerobic digesters at a TS feeding rate of 1-2 $g/l_{reactor}/d$ and a HRT between 15 and 35 days. Under these conditions a low VFA concentration and a VS reduction rate of 30-35 % can be expected. The low C/N ratio allowed the production of ammonia generated alkalinity which was able to maintain a stable bulk liquor pH. There was no difference in the biogas production from anaerobic digesters fed WAS or algae obtained from a brewery effluent treatment system. Algae and WAS fed anaerobic digesters had an average gas production of 241 ml/gVS fed. Algal biomass treating the same effluent (post-AD brewery effluent) as conventional AS, had a higher hydrogen and lower oxygen concentration (less oxidised) than WAS. This resulted in the biogas from algae fed anaerobic digesters having a higher methane and lower carbon dioxide content than WAS fed digesters. The water quality parameters of the digestate from

both WAS and algae fed digesters was similar and the digestate has the potential to be used as a fertiliser in agriculture.

Chapter 4: The suitability of waste biomass from activated sludge and high rate algal ponds as a fertiliser in agriculture

4.1 Introduction

Organic and inorganic pollutants are converted into biomass during the treatment of wastewater in biological systems such as constructed wetlands, high rate algal ponds (HRAP) and activated sludge (AS; Spellman 2000, Tchobanoglous *et al.* 2003, Kadlec and Wallace 2009). Practices and technologies need to be developed that allow the sustainable disposal of biomass, especially where the recovery or reuse of resources trapped in the biomass are considered (Singh and Agrawal 2008, Gianico *et al.* 2015). Wastewater biosolids are nutrient rich organic solids which have the potential to be used for fertiliser, soil amendment or energy production (Tchobanoglous *et al.* 2003, Guo 2012, Dobhal and Singhal 2017).

Inorganic fertilisers are used in agriculture to increase the production of agricultural lands, which are needed to meet the ever-growing needs of the human population (Dobhal and Singhal 2017). The production and use of these fertilisers cause have a major environmental impact by causing waterway pollution, air pollution, a decrease in soil fertility and contributing to global warming (Singh and Agrawal 2008, Hospido *et al.* 2010). The world's fertiliser needs are expected to increase two- to three-fold by 2025 (Dobhal and Singhal 2017). However, the production of inorganic fertilisers is an energy expensive process which produces waste products and causes environmental degradation (Singh and Agrawal 2008, Hospido *et al.* 2010). Therefore, plant nutrients that have been assimilated into biomass through biological wastewater treatment needs to be exploited as a fertiliser in crop productions systems (Martinez *et al.* 2002, Tchobanoglous *et al.* 2003). This will aid in both

reducing the environmental impacts of biosolid disposal and will decrease the reliance of agriculture on inorganic fertilisers (Sommers 1977, Martinez *et al.* 2002).

The composition and quality of biosolids varies greatly and are mainly dependent on the type of wastewater treated and the technologies used during treatment (EPA 1999, Lyberatos *et al.* 2004, Quilbe *et al.* 2005). Their characteristics determine the choice of disposal, for example those containing heavy metals are normally incinerated to produce energy, but those that meet regulatory requirements for the disposal of heavy metals and pathogens can be used for land application (DWAF 1997, EPA 1999, Mo and Zhang 2013).

Biosolids may contain heavy metals and/or pathogens that could contaminate the receiving environment or pose health risks when used as a fertiliser (Kolpin *et al.* 2002, Bright and Healey 2003). This waste can be classified into different categories, depending on its stabilisation treatment, pathogen count and heavy metal concentration (DWAF 1997, EPA 1999). The stabilisation of biomass destined for land disposal incorporates processes that decrease pathogen count, odour levels and VS concentration (EPA 1999, Tchobanoglous *et al.* 2003, Karanja 2011). The most common stabilisation methods include alkali stabilisation, anaerobic digestion (AD), aerobic digestion, composting and/or heat drying (EPA 1999, Tchobanoglous *et al.* 2003, Karanja 2011).

Before it can be applied to land, waste biomasses from wastewater treatment plants (WWTPs) must meet certain requirements or regulations, which are based on pathogen counts and heavy metal concentration (DWAF 1997, EPA 1999). These requirements serve to regulate the acceptable soil application rates and whether the biomasses can be used for agricultural purposes or non-agricultural purposes, such as land reclamation from mining sites (DWAF 1997, EPA 1999). Since brewery effluent is an organic effluent and free of

enteric pathogens, the waste biomass from AS and HRAP treatment systems falls within the limits for use on agricultural land (DWAF 1997).

Biosolids can supplement or replace commercial fertilisers as they contain nitrogen, phosphorus and various micro-nutrients such as copper, zinc, molybdenum, boron, calcium, iron, magnesium, and manganese (EPA 1999, Lyberatos *et al.* 2004, Quilbe *et al.* 2005, Singh and Agrawal 2008). The organic matter content can be used to increase the cation exchange capacity (CEC) of the soil and can aid in amending soils that are carbon depleted (Ramulu 2002, Lyberatos *et al.* 2004, Quilbe *et al.* 2005), and thus can potentially improve crop production. For example, the yield of maize, amaranthus, cowpea and crossandra fertilised with sewage sludge was the same as, or higher than, that of plants fertilised with a commercial inorganic fertiliser (Chitdeshwari *et al.* 2002). The yield of barley fertilised with waste activated sludge (WAS) applied to the soils at 15 t/ha dry weight was significantly higher than barley fertilised with a commercial inorganic fertilizer (Antolin *et al.* 2005). After four years of annual application of WAS from a sewage treatment facility, the soil's CEC, total organic carbon and available nitrogen increased significantly; however, so did the heavy metal concentration in the harvested grain (Antolin *et al.* 2005).

The majority of studies have reported an improvement in physical properties when sludge is applied to soils (Epstein 1975, Tsadilas *et al.* 1995, Nielson *et al.* 1998). The application of sludge to soils increases its organic matter content, which aids in stabilising the soil structure by increasing inter-particulate cohesion within aggregates and enhancing their hydrophobicity (Diacono and Montemurro 2010). Municipal solid waste applied to the soil, every two years, increased soil aggregate stability by 29 %, thus increasing its resistance to erosion (Annabi *et al.* 2007). The application of sewage sludge to the soil has been shown to

improve its water holding capacity, porosity and bulk density (Epstein 1975, Ramulu 2002, Ojeda *et al.* 2003).

The availability of macro-nutrients in biosolids varies greatly and is dependent on sludge composition, soil type and pH, climate and stabilisation step (Sommer 1977, Warman and Termeer 2005, Singh and Agrawal 2008). The availability of phosphorus in sludge varies from 3 % up to 80 % (Carliell and Wheatley 1997, Singh and Agrawal 2008), while that of nitrogen varies from 45-85 % (Coker *et al.* 1987, Warman and Termeer 2005, Singh and Agrawal 2008). Coker *et al.* (1987) found that initially 25 % of the nitrogen in anaerobically digested sewage sludge was available to plants, in the form of ammonia, and the subsequent availability of nitrogen to plants was due to the decomposition of the sludge in the soil.

Anaerobic digestion is a recommended stabilisation step prior to the land disposal of waste sludge as it allows the recovery of carbon into an energy source (biogas) and can increase the availability of nutrients to plants (Tchobanoglous *et al.* 2003). This process results in the conversion of protein bound nitrogen to ammonia which can be utilised by plants and can increase the nutrient availability of biosolids. Anaerobically digested WAS had higher phosphorus availability than heat dried WAS (Lyberatos *et al.* 2004). To date, there is only one publication which compares the use of AD to increase the nutrient availability of WAS to plants (Warman and Termeer 2005). Sludge pre-treated using AD resulted in a higher yield of *Zea mays* when compared to sludge that was composted (Warman and Termeer 2005). No other current literature has compared the use of AD to possibly increase the fertiliser value of effluent grown algae and to document the subsequent effect on soil fertility.

High rate algal ponds and AS are effective brewery effluent treatment technologies which both produce a biomass than can be used in agriculture. The suitability of these biomasses

as a fertiliser needs to be assessed and can aid in deciding which technology is favourable in a particular situation. Algae have resilient cell walls which can decrease their decomposition rate and thus reduce the availability of nutrients to plants when compared to WAS (Markou *et al.* 2012). This is the first study which compares the use of algae and sludge as a fertiliser replacement, where both the algae and the sludge are produced from effluent treatment systems that have been used to treat the same effluent.

4.2 Aims and objectives

The aim of this study was to determine the suitability of algae and WAS produced from a brewery effluent treatment system as a fertiliser in agriculture. This was done by comparing the change in soil characteristics and the growth of a crop fertilised with algae or sludge to a conventional inorganic fertiliser. This study determined what effect an AD pre-treatment step may have on the fertiliser quality and the fertility of the receiving soil.

The objectives of this study were to:

- compare plant growth and soil characteristics of plots fertilised with WAS, algae or inorganic fertiliser;
- compare plant growth and soil characteristics between soils fertilised with anaerobically digested and non-anaerobically digested algae or WAS; and
- determine the suitability of anaerobically digested and non-anaerobically digested WAS or algal biomass as an inorganic fertiliser replacement.

4.3 Materials and methods

4.3.1 Experimental species, system and irrigation

Three week old swiss chard (*Beta vulgaris* cv. Fordhoek giant) seedlings were purchased from a commercial nursery (Moorland Seedlings Pty Ltd, Humansdorp, South Africa). They were planted at a density of 16 plants per square metre, in raised beds (1.0 x 1.0 m, with a soil depth of 0.7 m; Figure 4.1), that were filled with the amended soils (Section 4.3.1). Each soil amendment treatment was randomly assigned to three raised beds, such that the treatments were replicated three times with a replicate consisting of a single raised bed.

They were irrigated once a day, except during rain (Smith and Heinrich 2011, Laboski and Peters 2012), and received a total of 377 mm of water over the thirteen-week growth trial (275 mm irrigation and 102 mm of rain).

Figure 4.1: Experimental raised beds showing them after seedlings were planted (left) and with mature Swiss chard in the beds (right).

4.3.2 Treatments

The five treatments were prepared by adding the following soil amendments to a sandy loam top-soil (10 % silt, 20 % clay, 70 % sand; Macvicar *et. al* 1977): (1) un-digested algae (algae); (2) anaerobically digested algae (AD-algae); (3) un-digested sludge (sludge); (4) anaerobically digested sludge (AD-sludge); and (5) a commercial inorganic fertiliser (inorganic-fertiliser) that served as a reference control (Hygrotech Pty Ltd, South Africa; Registration number K5709; Act 36 of 1947). These fertilisers were applied to the soil at a nitrogen application rate equivalent to 80 kg/ha (Smith and Heinrich 2011, Laboski and Peters 2012), two days before the soil was planted and they were mixed into the top five centimetres of the soil.

Two semi-continuously fed anaerobic digesters were used to digest the settled sludge and algal biomass. Both had a total volume of 220 l, with a head space of 60 l and an operating volume of 160 l. They were stirred with a submersible mixer (Sobo, WP 400M, South Africa) for five minutes every half an hour, and were situated in a temperature controlled room (37 ˚C). The digesters were seeded with sludge obtained from a biogas producing up-flow anaerobic sludge blanket reactor at Ibhayi Brewery (Chapter 2, Section 2.3.1). They were fed once a day following the removal of the equivalent volume of digestate. The settled algae and sludge fed to the digester had a total solids content of 25 g/l (Chapter 2, Section 2.3.1). Each digester was fed 9.5 l of either algae or sludge per day resulting in a feeding rate 1.5-2.0 g of TS/$l_{reactor}$/d. These two digesters where operated for 60 days, before the digestate was collected and applied to the soil as a fertiliser.

Undigested sludge and algae were obtained from the AS and HRAP system described in Chapter 2 (Section 2.3.1) while anaerobically digested sludge and algae were obtained from the experimental digesters fed with algae and sludge.

4.3.3 Data collection

A sample of the un-digested and anaerobically digested sludge and algae that were applied to the soil were subject to elemental analysis [inductively coupled plasma mass-spectrometry (EPA 2000) and X-ray fluorescence, (EPA 2007a)] at an independent laboratory (Central Analytical Facilities, Stellenbosch University, South Africa).

At the beginning of the trial, ten plants were randomly taken from the population of seedlings that were used for the experiment, and were weighed to determine the mean starting mass (0.1 g). At this time, the chlorophyll concentration index (CCI) of each plant was also recorded using a chlorophyll concentration meter (CCM-200 Plus Chlorophyll Concentration Meter, Opti-Sciences Inc., USA) on the uppermost fully expanded leaf of each plant, and this was repeated every four weeks. After five weeks, the plants were ready for harvesting, when all the large fully expanded leaves from each plant were removed and weighed (0.1 g). This was repeated every two weeks until the experiment was terminated, after 13 weeks. At the end of the trial, all the above ground biomass was harvested and weighed (0.1 g).

At the beginning of the trial three plants were randomly chosen and used for leaf chemical analysis. The biggest, uppermost fully expanded leaves from these plants were used for chemical analysis. These plants were not used in the experiment due to the destructive nature of the sampling. At the end of the trial leaves from each plot were collected and used for chemical analysis. All samples were analysed for aluminium, calcium, copper, iron, manganese, magnesium, nitrogen, phosphorus, potassium, sodium and zinc concentrations at an analytical laboratory (Inductively coupled plasma mass spectrometry; Eckard *et al.*

1998, de Figueiredo and Thurtell 1998; Cedara, Department of Agriculture, Kwa-Zulu Natal, South Africa).

Daily temperature and rainfall data were recorded using a rainfall gauge and a thermometer (Hanna, HI 991300, United Kingdom) situated next to the experimental area.

Air filled porosity (AFP), infiltration rate, bulk density and moisture concentration of the soil were measured, in each replicate at the beginning and end of the trial (Ramulu 2002, ISO 2017). Infiltration rates were determined using a 30 cm diameter ring infiltrometer placed 15 cm into the soil with 12 cm protruding above the surface. Five litres of water were poured into the ring infiltrometer and the time taken for the water to drain into the soil was recorded (Ramulu 2002, ISO 2017). Infiltration rate was then calculated (Equation 4.1, Ramulu 2002, ISO 2017):

$$\text{Infiltration rate (cm/min)} = (\text{volume of water added/surface area})/\text{time} \quad [4.1]$$

Air filled porosity (AFP), bulk density and water holding capacity were measured in each replicate at the beginning and end of the trial (Ramulu 2002, ISO 2017). The apparatus used was a 110 mm plastic pipe with an end cap that had four 3.0 mm holes drilled into it. The pipe was bored into the soil to get an undisturbed soil sample. A gauze was placed over the top of the vessel and submerged in water to just above the surface of the soil, for 60 min. The holes in the bottom were then sealed and the vessel was moved into a tray, where the holes were unblocked. The vessel was left to drain for 30 min and the amount of water collected was measured. Air filled porosity was calculated using Equation 4.2 (Ramulu 2002, ISO 2017). Directly after the AFP test the vessel was placed in a drying oven at 105 ^{o}C and allowed to dry for a minimum of 24 h, until a constant mass was achieved. Water holding

capacity was calculated using Equation 4.3 (Ramulu 2002, ISO 2017), and bulk density was then calculated using Equation 4.4 (Ramulu 2002, ISO 2017).

$$\text{Air filled porosity (\%)} = (\text{volume drained/volume of soil}) \times 100 \quad [4.2]$$

$$\text{Water holding capacity (\%)} = ((\text{wet weight} - \text{dry weight})/\text{volume}) \times 100 \quad [4.3]$$

$$\text{Bulk density (g/cm}^3\text{)} = \text{dry weight/volume} \quad [4.4]$$

Soil aggregate stability was measured, in each replicate, at the beginning and end of the experiment using five grams of 2-5 mm aggregates (Le Bissonnais 1996). Samples were placed in distilled water and allowed to stand for ten minutes. The distilled water was then removed with a pipette and the aggregates transferred onto a 0.05 mm sieve which was immersed in ethanol and shaken five times with a gentle regular helical rotation movement. The >0.05 mm aggregates on the sieve were collected, dried at 40 °C, and then gently sieved using a column of six sieves: 2.00, 1.00, 0.50, 0.20, 0.10, and 0.05 mm (Le Bissonnais 1996). The aggregate stability was represented by the mean weight diameter (MWD) of aggregates and was calculated using Equation 4.5 (Le Bissonnais 1996):

$$\text{Mean weight diameter} = \sum (d \times m) / 100 \quad [4.5]$$

where d was the mean diameter between the two sieves (mm) and m was the weight fraction of aggregates remaining on the sieve (%).

The chemical analysis of the soil was determined at the start of the trial (n=5). At the end of the trial, a sample was taken from the bed of each replicate for soil chemical analysis. Soil samples were sent to a commercial analytical laboratory and analysed for pH, cation exchange capacity (CEC), carbon, calcium, copper, potassium, phosphorus, sodium, magnesium, manganese, nitrogen and zinc (Ambic-2-extractable and KCl-extractable;

Hunter 1975, Farina 1981; Cedara, Department of Agriculture, Kwa-Zulu Natal South Africa). The sodium adsorption ratio (SAR) of the soil was calculated using Equation 4.6, where sodium, calcium, and magnesium are expressed in milliequivalents per litre, (meq/l) obtained from a saturated paste soil extract (Qadir and Schubert 2002).

$$\text{Sodium adsorption ratio} = \text{Na} \div \sqrt{\frac{\text{Ca} + \text{Mg}}{2}} \qquad [4.6]$$

Samples of anaerobically digested and un-digested sludge or algae applied to the soil were tested for *Escherichia coli.* Similar analyses were repeated on soil and leaf samples taken from each replicate every four weeks at the Ibhayi Brewery laboratories (IS 17994 method; ISO 2014).

4.3.4 Statistical analysis

The experimental design allowed for: (1) a multi-factor analysis of variance (ANOVA) where the treatments included two soil-amendments (algae and sludge) both of which were either subject to either AD or were left un-digested; and (2) a one-way ANOVA that included a comparison the four treatments described above and a fifth inorganic-fertilizer treatment that acted as the reference-control. If no significant interactions were observed between soil-amendment factor and/or AD pre-treatment factor (Multi-factor ANOVA), then the statistical analysis generated from the one-way ANOVA/Kruskal Wallis ANOVA were used. All analyses were carried out at $p<0.05$ and, when differences were found, a Tukey's multiple range analysis was used at $p<0.05$. Data collected over the course of the trial were compared using multi-factor repeated measures or one-way ANOVA or a non-parametric Mauchly's sphericity test, if ANOVA assumptions were not met ($p<0.05$; Salkind *et al.* 2010). All data were checked for equality of variance and for the normal distribution of the

residuals using Levene's test and a Shapiro-Wilk plot of the residuals, respectively. If the assumptions were not met, then the data were log or square-root transformed and checked for equal variance and normal distribution of residuals. If the assumptions were still not met, a non-parametric Mann-Whitney U test or a Kruskal-Wallis ANOVA was used to compare the data between treatments. All analyses were performed using a statistical software package (Statistica Version 10, StatSoft Inc, Tulsa, USA). Statistical analysis on pH data was done using hydrogen ion concentration.

4.4 Results

Sludge and algae biomass applied to the soil were within the heavy metal limit for application to agricultural land (Table 4.1; DWAF 1997). Sludge biomass had a chromium concentration between 243.5-223.3 mg/kg, which was below the land application limit. Algal biomass had a four times higher chromium concentration (1043.7-1057.1 mg/kg) than sludge biomass (Table 4.1). Mercury concentrations in algal and sludge biomasses where below 3.5 mg/l. All results came back negative for the presence of *E. coli* in algae, sludge soil and on the plant leaves.

Table 4.1: Heavy metal composition of sludge and algae applied to soil and limits for disposal of sludge on agricultural land n=3 (DWAF 1997).

Parameter	Limit	Algae	AD-algae	Sludge	AD-sludge
Cadmium (mg/kg)	15.7	0.6	1.8	2.6	0.9
Cobalt (mg/kg)	100.0	6.5	4.0	2.0	2.5
Chromium (Cr^{3+}) (mg/kg)	1750.0	1043.7	1057.1	243.5	223.3
Copper (mg/kg)	50.5	38.1	41.6	36.3	48.8
Mercury (mg/kg)	10.0	<3.5	<3.5	<3.5	<3.5
Nickel (mg/kg)	200.0	59.2	63.1	45.4	55.3
Lead (mg/kg)	50.5	10.9	19.7	10.3	21.4
Arsenic (mg/kg)	15.0	2.0	8.6	3.2	7.6
Selenium (mg/kg)	15.0	0.3	1.3	0.8	1.4

Anaerobically digested algae (AD-algae), Anaerobically digested sludge (AD-sludge).

Harvested Swiss chard biomass was not influenced by an interaction between fertiliser treatment and time (Repeated measures ANOVA, $F_{(12,30)}$=0.85, p=0.6045) or by an interaction between biomass type (algae vs sludge) and AD (Repeated measures multi-factor ANOVA, $F_{(3,24)}$=1.54, p=0.2288). However, there was a significant difference in biomass harvested between fertiliser treatments, with a higher average biomass harvested from algae and sludge fertiliser treatments when compared to the inorganic-fertiliser treatment (Figure 4.2; Repeated measures ANOVA, $F_{(4,10)}$=48.62, p<0.0001). The mean biweekly yield from the inorganic-fertiliser treatment was 3.45±0.89 kg/m^2 while algae and sludge treatments had a similar fortnight yield of 5.08±0.73 kg/m^2 (Figure 4.2).

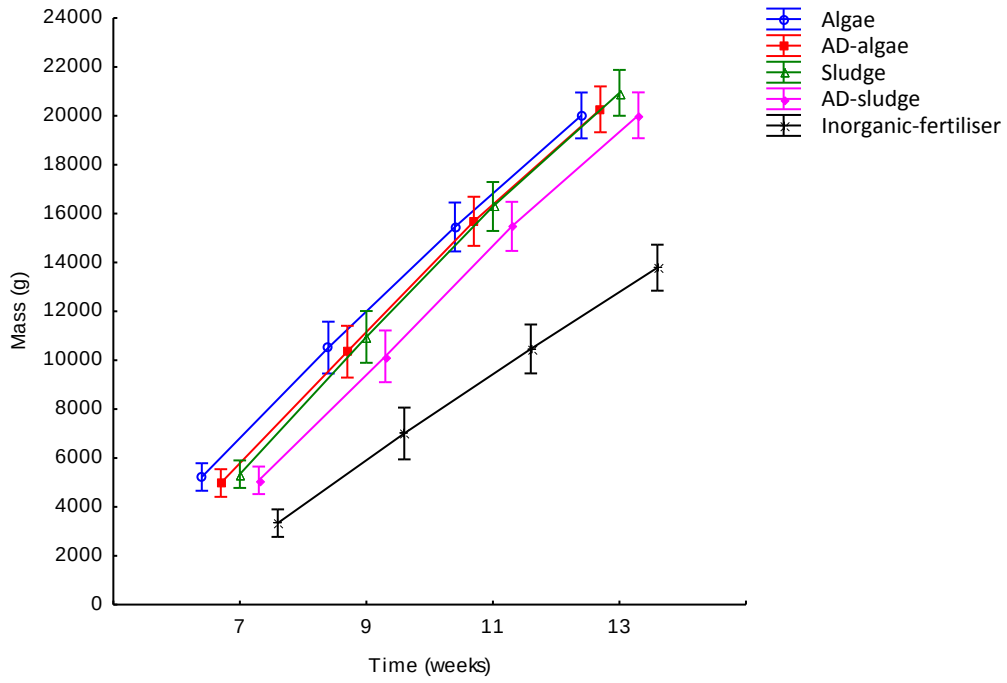

Figure 4.2: Mean (±95 % confidence interval) biomass harvested from Swiss chard plants subject to the experimental fertiliser treatments (Repeated measures ANOVA, $F_{(4,10)}$=48.62, p<0.0001). Anaerobically digested algae (AD-algae), Anaerobically digested sludge (AD-sludge).

Chlorophyll concentration index of Swiss chard leaves was not influenced by an interaction between time and fertiliser treatment (Repeated measures ANOVA, $F_{(12,30)}$=0.72, p=5.995).

There was no interaction between biomass type (sludge vs algae) and AD on CCI (Repeated measures multi-factor ANOVA, $F_{(3,24)}$=0.93, p=0.4412). However, the CCI of Swiss chard leaves increased significantly over the first eight weeks of the trial, after which it remained constant (Table 4.2); but there was no significant difference between the CCI of Swiss chard plants cultivated under all the fertiliser treatments (Table 4.2, Repeated measures ANOVA, $F_{(4,10)}$=0.85, p=0.6045).

Table 4.2: The mean (± standard error) chlorophyll concentration index of Swiss chard leaves subject to the experimental fertiliser treatments n=3 (Repeated measures ANOVA, $F_{(12,30)}$=0.85, p=0.6045). Anaerobically digested algae (AD-algae), Anaerobically digested sludge (AD-sludge).

Time (weeks)	Algae	AD-algae	Sludge	AD-sludge	Inorganic-fertiliser
0	16.10±2.45	23.20±2.54	19.33±3.14	12.47±3.26	15.07±1.05
4	34.07±4.75	27.06±4.87	31.67±4.24	34.23±5.21	26.43±1.44
8	50.33±4.03	50.50±5.78	42.17±5.34	46.23±6.30	38.00±5.16
13	42.33±5.40	36.53±9.87	45.30±5.61	41.17±2.73	43.77±3.64

The chemical concentration of plant leaves was not influenced by an interaction between factors such as biomass type (sludge vs algae) and AD pre-treatment (Multi-factor ANOVA, p>0.05). Similarly, no significant differences were observed in leaf elemental concentration between biomass type and AD pre-treatment (Multi-factor ANOVA, p>0.05). There was also no significant difference in the nitrogen concentration of Swiss chard leaves cultivated under the five fertiliser treatments (Table 4.3). The mean nitrogen concentration of leaves from the algae, sludge and inorganic-fertiliser treatments were 51.23±1.25 g/kg, 51.51±1.66 g/kg and 41.19±6.82 g/kg respectively (Table 4.3). The nitrogen concentration of leaves increased significantly from the beginning to the end of the trial in algae and sludge fertiliser

treatments (Table 4.3; Kruskal Wallis, p>0.65, $F_{(5,20)}$=11.80, p=0.0376). The potassium and phosphorus concentrations of Swiss chard plants were similar between all fertiliser treatments (Table 4.3; Kruskal Wallis, p>0.05). The iron concentration in leaves from the inorganic-fertiliser treatment was significantly lower than that of leaves from the algae and sludge treatments (Table 4.3; Kruskal Wallis, p>0.65, $F_{(5,20)}$=15.06, p=0.0101). There was no difference in the concentration of iron in the leaves of Swiss chard plants subject to the algae or sludge fertiliser treatments (Table 4.3). Except for iron, there was no significant difference in all the other nutrient (calcium, magnesium, sodium, zinc, copper, manganese and aluminium) concentrations in the leaves between all five fertiliser treatments (Table 4.3; One-way ANOVA/Kruskal Wallis, p>0.60). The final aluminium, zinc, copper and phosphorus concentration of the leaves was significantly lower than their starting concentrations, for all fertiliser treatments (Table 4.3; Kruskal Wallis, p<0.05).

Table 4.3: The mean (± standard error) leaf chemical concentration for Swiss chard plants grown using different fertilisers, after 13 weeks and at the start of the trial. Superscripts in the same row represent significantly different treatment means n=3 (One-way ANOVA/Kruskal Wallis, p<0.05).

Parameter	Start	Algae	AD-algae	Sludge	AD-sludge	Inorganic-fertiliser	F/H	P value
Nitrogen (g/kg)	39.24±0.55^{a}	50.84± 1.04^{b}	51.73±1.45^{b}	50.56±1.65^{b}	52.45±1.67^{b}	41.19±6.82^{ab}	H=11.80	0.0376$^{\#}$
Calcium (g/kg)	8.12±0.32	9.30± 0.77	8.29±0.28	8.25±0.31	8.41±0.38	8.70±0.58	F=0.91	0.5042
Magnesium (g/kg)	7.50±0.17	8.78± 0.43	8.64±0.16	8.78±0.19	8.77±0.42	8.44±0.39	H=9.27	0.0988
Potassium (g/kg)	50.83±0.90	59.65± 2.79	61.39±4.15	59.57±0.81	58.55±2.61	56.20±2.79	H=8.71	0.1212
Sodium (g/kg)	18.27±1.76	30.13± 2.00	30.55±1.55	30.47±1.44	30.93±1.47	31.10±1.74	H=10.87	0.0541
Zinc (mg/kg)	61.56±1.54^{a}	39.74± 5.44^{b}	34.00±0.89^{b}	37.79±2.78^{b}	36.89±2.38^{b}	42.10±4.55^{b}	H=12.10	0.0334$^{\#}$
Copper (mg/kg)	52.98±3.21^{a}	7.37± 0.91^{b}	6.34±0.64^{b}	6.27±0.52^{b}	6.13±0.60^{b}	6.98±0.97^{b}	H=11.89	0.0363$^{\#}$
Manganese (mg/kg)	88.29±2.20	99.42±12.85	85.48±3.35	88.18±5.21	86.88±6.34	93.52±5.39	H=1.875	0.8685
Iron (mg/kg)	0.11±0.10^{a}	0.31± 0.03^{b}	0.28±0.03^{b}	0.32±0.03^{b}	0.29±0.03^{b}	0.12±0.02^{a}	H=15.06	0.0101$^{\#}$

Phosphorus (g/kg)	8.39±0.18[a]	2.62± 0.17[b]	2.75±0.21[b]	2.98±0.09[b]	2.63±0.27[b]	2.91±0.23[b]	H=11.82	0.0374[#]
Aluminium (g/kg)	0.78±0.03[a]	0.28± 0.02[b]	0.26±0.03[b]	0.26±0.03[b]	0.27±0.03[b]	0.22±0.02[b]	H=12.43	0.0294[#]

Anaerobically digested algae (AD-algae), Anaerobically digested sludge (AD-sludge), [#] indicates significant different treatment means.

The physical properties of the soil were not influenced by an interaction between factors biomass source and AD pre-treatment (Multifactor ANOVA, $p>0.05$). The AD pre-treatment step had no significant effect on the soil's physical properties (Multifactor ANOVA, $p>0.05$). After 13 weeks, there was no significant difference in the bulk density, porosity, water holding capacity, infiltration rate and mean weight diameter of soils subject to the five fertiliser treatments (Table 4.5; One-way ANOVA/Kruskal Wallis, $p>0.05$). The combined mean porosity, water holding capacity and mean weight diameter of the soils were 33.90±0.71 %, 22.57±0.72 % and 1.57±0.27 mm respectively (Table 4.5).

All measured soil chemical concentrations where not influenced by an interaction between biomass type and AD pre-treatment (Multifactor ANOVA, $p>0.05$). However, the carbon and nitrogen concentrations of soils fertilised with AD sludge or algae were significantly lower than of soils fertilised with undigested sludge or algae (Multifactor ANOVA, $P<0.05$; Table 4.4). The zinc concentration of the soil was significantly higher in the algae fertilised soils

when compared to sludge fertilised soils (Table 4.6; Multifactor ANOVA, $F_{(1,8)}$=85.51, p=0.0001). No other significant difference was observed between soil chemical concentration and biomass source or AD pre-treatment step (Multifactor ANOVA, p>0.05).

Table 4.4: The mean (± standard error) carbon and nitrogen concentration of soils fertilised with the algae and sludge treatments. Superscripts in the same row represent significantly different treatment means n=3 (Multifactor ANOVA, p<0.05).

Parameter	Algae	AD-algae	Sludge	AD-sludge	$F_{(1,8)}$ value	P value
Carbon (g/kg)	58.70±1.04[a]	54.82±1.05[b]	57.03±0.81[a]	55.29±0.76[b]	9.221	0.0161[#]
Nitrogen (g/kg)	3.64±0.04[a]	3.47±0.06[b]	3.66±0.04[a]	3.53±0.06[b]	7.569	0.0250[#]

Anaerobically digested algae (AD-algae), Anaerobically digested sludge (AD-sludge), [#] indicates significant differences between treatment means.

Table 4.5: The mean (± standard error) starting and final physical characteristics of soils subject to the different fertiliser treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means n=3 (One-way ANOVA/Kruskal Wallis, p<0.05).

Parameter	Start	Algae	AD-algae	Sludge	AD-sludge	Inorganic-fertiliser	F/H value	P value
Bulk density (g/cm^3)	1.42±0.01	1.41±0.06	1.44±0.03	1.35±0.06	1.38±0.03	1.42±0.04	F=2.303	0.0764
Porosity (%)	36.17±0.67	33.48±1.57	34.08±2.19	36.61±1.62	33.75±0.88	31.55±0.72	F=1.020	0.4301
Water holding capacity (%)	22.43±0.68	23.01±0.98	20.49±1.35	22.79±1.65	24.99±2.49	21.58±0.99	F=0.947	0.4691
Infiltration rate (cm/min)	0.80±0.02	0.82±0.11	0.92±0.10	0.95±0.04	0.72±0.03	0.82±0.05	H=8.367	0.2127
Mean weight diameter (mm)	1.56±0.09	1.55±0.04	1.67±0.03	1.49±0.05	1.56±0.06	1.59±0.10	F=0.788	0.5782

Anaerobically digested algae (AD-algae), Anaerobically digested sludge (AD-sludge).

Table 4.6: The mean (± standard error) chemical characteristics of soils subject to the different fertiliser treatments. Values in the same row represented by a different superscript symbol represent significantly different treatment means n=3 (One-way ANOVA/Kruskal Wallis, p<0.05).

Parameter	Start	Algae	AD-algae	Sludge	AD-sludge	Inorganic-fertiliser	F/H value	P value
pH	7.60± 0.03	7.63± 0.05	7.53± 0.04	7.65± 0.03	7.64± 0.05	7.57± 0.05	F=0.67	0.6502
Exchangeable acidity (cmol(+)/kg)	0.09± 0.00	0.09± 0.01	0.09± 0.01	0.10± 0.01	0.09± 0.01	0.10± 0.00	H1.69	0.8900
CEC (cmol(+)/kg)	26.52± 0.34	27.97± 1.25	29.57± 3.89	25.90± 0.45	28.23± 0.79	24.22± 0.36	H=10.23	0.0691$^{\#}$
Phosphorus (mg/kg)	37.50± 1.21^{a}	55.93± 3.03^{b}	57.26± 3.07^{b}	58.37± 3.79^{b}	56.21± 2.36^{b}	79.79± 3.61^{c}	F=28.35	0.0000$^{\#}$
Potassium (mg/kg)	618.00±17.87^{a}	691.33±52.27^{a}	651.33±19.38^{a}	653.33±3.80^{a}	686.33±32.91^{a}	826.33±50.60^{b}	F=4.20	0.0156$^{\#}$
Calcium (g/kg)	4.49± 0.04	4.79± 0.08	4.64± 0.07	4.51± 0.12	4.75± 0.09	4.64± 0.15	F=1.78	0.1812
Copper (mg/kg)	1.16± 0.08	1.33± 0.12	1.17± 0.07	1.27± 0.12	1.43± 0.12	1.20± 0.06	F=1.22	0.3499
Magnesium (mg/kg)	518.80±17.06	544.00±16.46	516.67±22.59	506.67± 9.35	515.67±29.54	517.00±18.01	F=0.37	0.8632
Manganese (mg/kg)	30.80± 0.97^{a}	51.67± 2.91^{b}	47.33± 2.19^{b}	48.67± 1.45^{b}	46.33± 6.36^{b}	53.00± 3.51^{b}	H=12.28	0.0311$^{\#}$
Sodium (mg/kg)	774.80±13.66^{a}	1001.67±38.97^{b}	943.67±33.07^{b}	940.67±39.87^{b}	922.67±27.65^{b}	782.00±13.32^{a}	F=12.72	0.0001$^{\#}$
Sodium absorption ratio	2.91± 0.04^{a}	3.66± 0.13^{b}	3.51± 0.13^{b}	3.54± 0.12^{b}	3.40± 0.13^{b}	2.90± 0.08^{a}	F=11.31	0.0002$^{\#}$
Zinc (mg/kg)	4.16± 0.21^{a}	14.83± 1.41^{b}	12.53± 0.92^{b}	5.10± 0.50^{a}	5.53± 0.46^{a}	4.10± 0.40^{a}	H=15.27	0.0093$^{\#}$

Anaerobically digested algae (AD-algae), Anaerobically digested sludge (AD-sludge), Cation exchange capacity (CEC), $^{\#}$ indicates significant differences between treatment means.

At the end of the trial, the carbon (54.46±0.92 g/kg) and nitrogen concentration (3.42±0.05 g/kg) of soils fertilised with algae or sludge was significantly higher than soils fertilised with inorganic fertiliser (50.01±1.43 and 3.12±0.06 g/kg respectively; One-way ANOVA, $p<0.05$). The soil pH, exchangeable acidity and CEC were similar between all fertiliser treatments (Table 4.6; One-way ANOVA/ Kruskal Wallis, $p>0.05$). The soil phosphorus and potassium concentrations were higher in inorganic-fertilised soils compared to algae or sludge fertiliser soils (Table 4.6; One-way ANOVA, $F_{(5,14)}=28.35$, $p<0.0000$). After 13 weeks, the calcium, copper, magnesium and manganese concentration of the soil were similar between all five fertiliser treatments (Table 4.6; One-way ANOVA/Kruskal Wallis, $p>0.05$). Soil fertilised with inorganic-fertiliser had a higher potassium concentration than soils fertilised with algae or sludge (Table 4.6; One-way ANOVA, $F_{(5,14)}=4.20$, $p<0.0156$). The SAR and sodium concentration were significantly higher in algae and sludge fertiliser soils when compared to soils fertilised with inorganic fertiliser (Table 4.6; One-way ANOVA, $p<0.05$). Algae and sludge fertilised soils had a similar sodium concentration and SAR with a combined mean of 952.17±34.89 mg/kg and 3.53±0.13, respectively (Table 4.6). Inorganic fertiliser and sludge fertilised soils had a similar zinc concentration (Table 4.6; One-way ANOVA $p>0.05$).

4.5 Discussion

One of the limitations for applying biosolids to soils is their heavy metal content. Algae and sludge biomasses used in this study had heavy metal concentration below the limits for agricultural land application in South Africa (DWAF 1997). The copper concentration in both algae and sludge was 5-10 mg/kg below the limit. The heavy metal concentration of waste biomass from effluent treatment facilities is mainly influenced by the type of effluent

treated. For example, food processing effluent carries less metal than domestic or mine-drain effluent which is usually contaminated with heavy metals. It is also influenced by the chemical processes used in effluent treatment; for example chemicals used in flocculation (Tchobanoglous *et al.* 2003, Quan-ying *et al.* 2007). Generally, waste sludge from domestic sewage and non-food production industries contains high concentrations of heavy metals and is not suitable for agricultural land application (Alvarez *et al.* 2002, Quan-ying *et al.* 2007). Sludge originating from the treatment of organic effluents such as food processing industries normally have a heavy metal concentration suitable for application to soils (Stocks *et al.* 2002, Kanagachandran and Jayaratne 2006). The sludge and algal biomasses used in this study originated from a brewery effluent treatment process and were within the heavy metal limits for use on agricultural land.

Biosolids have the potential to supplement or replace commercial fertilisers as they contain nitrogen, phosphorous and various micro-nutrients needed to support plant growth (Quilbe *et al.* 2005, Singh and Agrawal 2008). In this study, the yield of Swiss chard plants cultivated in soil fertilised with sludge or algae was significantly higher than the yield from inorganic-fertiliser treatments. Similarly, the yields of maize, amaranthus, cowpea and crossandra fertilised with WAS were the same or higher than plants fertilised with a commercial fertiliser (Chitdeshwari *et al.* 2002, Warman and Termeer 2005, Singh and Argawal 2008). However, barley fertilised with sewage sludge had a lower yield when compared to inorganic fertiliser, but the addition of inorganic potassium to the sludge increased the yield of barley, indicating that the sludge was deficient in potassium (Miah *et al.* 1999). The present study shows that sludge and algae can be utilised as a inorganic fertiliser replacement when applied to the soil at the same nitrogen loading rate and can even increase crop yield.

Heavy metals can accumulate in the leaf tissue of plants and render them unsafe for consumption when sludge is used as a fertiliser. Of the heavy metals tested, iron was the only metal present at a higher concentration in Swiss chard leaves from sludge and algae fertilised treatments when compared to the inorganic-fertiliser treatment. A single application of waste activated sludge to the soil, at 112 t/ha dry weight, increased the copper, zinc, iron and chromium concentration of snap beans and flax plants (Dowdy *et al.* 1978, Tsakou *et al.* 2002). Land application of sludge has been shown to increase the foliar cadmium, chromium, copper, iron, manganese and zinc concentration of agricultural crops (Hernandez *et al.* 1991, Morera *et al.* 2002, Bozkurt and Yarilgac, 2003, Warman and Termeer 2005, Singh and Argawal 2008). The acidity of the soil influences the accumulation of heavy metals in crop tissue due to their increase in solubility as pH decreases; therefore it is advisable to only apply waste sludge to soils with a pH above 7.0 (Hernandez *et al.* 1991, DWAF 1997, Benitez *et al.* 2001). Itanna (2002) found Swiss chard and lettuce to have the highest metal foliar concentration (arsenic, chromium, iron, lead) of eight vegetable species grown in soils fertilised with sewage sludge. The accumulation of heavy metals in plant tissue varies between plant species, with Swiss chard assimilating more soil iron into its leaves than cabbage, kale, potato, red beet and cauliflower (Itana 1998, Itana 2002). The land application of algae and waste activated sludge from a brewery effluent treatment process increased the foliar iron concentration, however the concentration was still well within the limits for human consumption. The foliar heavy metal concentration of crops grown in sludge fertilised soils needs to be continuously monitored if practiced commercially as there is a possibility of heavy metal contamination, especially in acidic soils.

Biosolids have the ability to improve the soil's physical characteristics and are thus commonly used for soil reclamation. There was no difference in the physical properties of

the soil receiving algae, waste sludge or inorganic fertiliser. The literature reports an improvement in the soil's physical properties when sludge is applied (Tsadilas *et al.* 1995, Nielson *et al.* 1998, Singh and Argawal 2008, Diacono and Montemurro 2010). The organic matter added to the soil from WAS improved the bulk density, porosity and water holding capacity of the soil, after one year (Ramulu 2002, Ojeda *et al.* 2003, Diacono and Montemurro 2010). Organic matter concentration of the soil increases when sludge is applied to soil and this aids in stabilizing soil structure by increasing inter-particle cohesion within aggregates and increasing their hydrophobicity, thus increasing soil structural stability (Bronick *et al.* 2005, Diacono and Montemurro 2010). Organic matter is the parameter most closely related to soil structural stability (Albiach *et al.* 2001, Diacono and Montemurro 2010). The improvement in soil structural stability is normally only noticed one to two years after the application of sludge (Sodhi *et al.* 2009, Tejada *et al.* 2009). During this study no differences where noticed in the soil's physical characteristics and structure due to the short period of the trial (13 weeks). However, after prolonged application (>two years) an increase in the soil's physical structure would be expected (Ojeda *et al.* 2003, Diacono and Montemurro 2010).

When applying sludge to soils the possibility of contaminating them with heavy metals exists. Of the heavy metals analysed, zinc was the only element that was higher in algae fertilised soils when compared to soils fertilised with inorganic fertiliser. Various authors have reported an increase in soil iron, copper, zinc, manganese nickel, chromium, cadmium and lead concentrations when WAS is applied to soil (Hernandez *et al.* 1991, Lopez-Mosquera *et al.* 2000, Singh and Argawal 2008, Lu *et al.* 2012). Zerzghi *et al.* (2010) applied anaerobically digested WAS to desert soils for 20 years and reported no detrimental effect on the soil's heavy metal content or soil fertility. It is important to apply sludge from

WWTPs to soil below the permitted application rate of (8.0 t/ha/y) and to ensure that its heavy metal content is always within the limits for land application (DWAF 1997, EPA 1999). If this is carried out, in conjunction with regular soil monitoring procedures, the land application of sludge from WWTPs should not have any detrimental effect on the soil's chemical fertility (Zerzghi *et al.* 2010).

Cation exchange capacity is a measure of a soil's ability to hold exchangeable cations (Robertson *et al.* 1999). There was no difference in the CEC of soils receiving the different fertiliser treatments. It is mainly influenced by pH, organic matter and clay particles (Sollins *et al.* 1988, Robertson *et al.* 1999). The majority of the literature has reported an increase in soil CEC arising from the land application of sludge (Maiti *et al.* 1992, Montemurro *et al.* 2006, Kaur *et al.* 2008, Kukal *et al.* 2009, Diacono and Montemurro 2010). This increase was attributed to the higher organic matter content in the soil, which is negatively charged, and thus increase the soil's ability to retain positively charged nutrients, when the sludge was applied to the soil for periods of five to thirty years (Kaur *et al.* 2008, Kukal *et al.* 2009, Diacono and Montemurro 2010). During this study, algae and sludge fertilised soils had a higher carbon content than soils fertilised with inorganic fertiliser, however, this increase was not enough to significantly increase CEC in the 13 weeks of this trial. Prolonged application of WAS or algae is likely to increase the CEC, as reported elsewhere (Kaur *et al.* 2008, Kukal *et al.* 2009, Diacono and Montemurro 2010).

Sodium contamination of agricultural soils is the leading cause of rendering soils unsuitable for agriculture (Qadir *et al.* 2003, Muyen *et al.* 2011). In this study, the sodium concentration and SAR of soils fertilised with algae or sludge were significantly higher than those of the soils fertilised with inorganic fertiliser. Karami *et al.* (2012) applied various

organic wastes (animal manure, waste sludge and plant derived organic wastes) to soils and, after six months, they recorded a decrease in the soil's SAR. This was due to an increase in soil magnesium and calcium concentration and not a decrease in soil sodium concentration (Karami *et al.* 2012). The land application of animal manures to calcareous soils with a sodium concentration greater than 0.9 g/l resulted in an increase in soil sodium concentrations (Bernal *et al.* 1993). The application of sewage sludge to potting soil that was planted with olive trees resulted in an increase in soil sodium concentrations, SAR and decreased plant growth due to osmotic and sodium stress (Gasco and Lobo 2006). The application of brewery effluent grown algae and sludge to land increased the soil's SAR which could be accompanied by a deterioration in the soil's structural stability and crop yield.

In South Africa there are no sodium guidelines or limits for the disposal of waste sludge on agricultural land. In this study, the soil's sodium concentration increased when WAS or algae was applied to it. Future research should monitor the influence of WAS on the soil's SAR and sodium concentration, as prolonged application could lead to soil salinization, rendering it unsuitable for agriculture (Gasco and Lobo 2006, Muyen *et al.* 2011). Thus, regulations on the application rates of sludge to agricultural soils should also consider limit values for sodium (not only heavy metals) to avoid to accumulation of sodium in the soil and consequent deterioration in the soil's fertility.

Soil pH plays a major role in the availability of micro-nutrients to plants (Epstein and Bloom 2005). There was no difference in the pH of soils subject to the experimental fertiliser treatments. The influence that WWTP derived sludge has on soil pH varies considerably, and is dependent on the pH of the receiving soil, the pH of the biosolids and rate that sludge is

applied to the soil (Nielson *et al.* 1998, Garcia-gil *et al.* 2004, Meng *et al.* 2005, Butler and Muir 2006, Bastida *et al.* 2008). Changes in pH have been correlated with the calcium carbonate content of the sludge and acid production during decomposition in the soil (Sommers 1977, Tsadilas *et al.* 1995, Singh and Argawal 2008). If the pH of the soil is similar to that of the biosolids applied to it, there is unlikely to be a change in pH; however further decomposition of the biosolids can cause a decrease in soil pH (Singh and Argawal 2008). The application of sludge or algae to the soil at nitrogen rate of 80 kg/ha did not alter the soil pH; however prolonged disposal and further biological degradation my result in a change in pH.

The use of inorganic fertilizers in agriculture results in the leaching of nitrate and ammonia, which can lead to eutrophication of surrounding water bodies (Withers *et al.* 2014). After only 13 weeks, the nitrogen concentration of soils fertilised with algae or sludge was significantly higher than soil fertilised with inorganic fertiliser. At the beginning of the trial all fertilisers were applied at the same application rate. Nitrogen is removed from the soil by plant uptake, by leaching into surrounding water bodies or by transformation into a gaseous form (N_2, NH_3, NO, NO_2, N_2O; Carpenter *et al.* 1998, Withers and Lord 2002, Withers *et al.* 2014). More nitrogen was assimilated into plant biomass in the algae and sludge fertilised treatments as plants grown in these treatments had a greater biomass production and similar leaf nitrogen content when compared to inorganic fertilised plants. Therefore, a greater portion of nitrogen must have left the soil via leaching or outgassing to the atmosphere in inorganic-fertiliser treatments when compared to sludge or algae fertilised treatments.

The amount of nitrogen leached from soils is dependent on the form of nitrogen supplied, the crop utilisation rate and the irrigation regime, while the mass of nitrogen lost to the atmosphere is influenced by soil temperature, oxygen concentration, moisture and the amount of available organic carbon and nitrogen (Bremner 1997, Brentrup *et al.* 2000, Snyder *et al.* 2009, Signor *et al.* 2013). Nitrogen leaching is predominant in carbon deficient soils containing more inorganic nitrogen than crops can utilise (Bremner 1997, Signor *et al.* 2013). The nitrogen in the inorganic-fertiliser treatment was present in the form of ammonia and nitrate, whereas in the sludge and algae treatments it was present as ammonia and other organic compounds (Hygrotech Pty Ltd, South Africa; Chapter 3, Section 3.4). Since ammonia and nitrate are highly soluble in water (Rivetta *et al.* 2008, Hollister *et al.* 2012), more nitrogen was probably lost to leaching from the inorganic-fertiliser treatment when compared to the algae and sludge fertilised treatments where 50-60 % of the nitrogen applied to the soil was in an insoluble particulate form. Walsh *et al.* (2012) reported greater nitrate and ammonia leaching from a sandy clay loam fertilised with ammonium nitrate when compared to anaerobically digested sludge. However, Schroder *et al.* (2010) concluded that the extent of nitrogen leaching from grassland soils was not influenced by the nitrogen source (organic vs inorganic), the main factor being balancing nitrogen supply with crop demand. More nitrogen was retained in the sludge or algae fertilised soils than the inorganic-fertiliser soils because all nitrogen supplied by the inorganic fertiliser was in a soluble form (ammonia and nitrate) while 50-99 % of the nitrogen supplied by the sludge and algae was in an insoluble form.

Sludge derived organic fertiliser can be advantageous over inorganic-fertilisers as a portion of its nitrogen is in an insoluble form and only becomes available to plants after microbiological decay in the soil, thus resulting in less nitrogen being leached into

surrounding water bodies (Sanger *et al.* 2011, Walsh *et al.* 2012, Withers *et al.* 2014). Future studies should determine the effect wastewater derived sludge has on the metabolic community structure and activity of a soil and collect and analyse the leachate from the soil to determine the amount of nitrogen lost via leaching and to the atmosphere.

During AD insoluble particulate bound nitrogen is converted to ammonia, which can be utilised by plants (Angelidaki and Sanders 2004). There was no difference in the crop yield, CCI and chemical leaf concentration of Swiss chard plants grown in soils fertilised with anaerobically digested or non-anaerobically digested algal or sludge biomass. Heat dried WAS that was previously subject to AD had a higher concentration of plant available inorganic phosphorus than non-anaerobically digested heat dried WAS (Lyberatos *et al.* 2004). The yield of *Zea mays* from sludge fertiliser treatments was higher in soils fertilised with AD WAS when compared to soils fertilised with untreated WAS (Warman and Termeer 2005). The application of sewage sludge increased the soil's microbial activity, respiration and enzymes activities, indicating the soil's microbes are able to degrade the sludge into constituents that are available for plant uptake (Banerjee *et al.* 1997). The microbial community present in the soil was able to degrade insoluble particulate bound nutrients into soluble plant-available nutrients, resulting in no difference in crop yield between soils fertilised with anaerobically digested or non-anaerobically digested biomass (Morera *et al.* 2002, Antolin *et al.* 2005, Abbott and Murphy 2007).

Anaerobic digestion is able to recover a portion of the carbon in waste biomass into a fuel source (methane) before it is applied to the soil. The carbon concentration of soils fertilised with AD sludge or algae was significantly lower than soils fertilised with undigested sludge or algae. During AD the carbon content of organic matter is converted into methane and

carbon dioxide, thus decreasing its carbon concentration (Tchobanoglous *et al.* 2003, Angelidaki and Sanders 2004). Anaerobic digestion is a recommended pre-treatment step prior to the land disposal of waste sludge as the process can decrease its odour, pathogen count, as well as the greenhouse gas emissions from further decomposition in the soil (EPA 1999, Warman and Termeer 2005, Singh and Agrawal 2008). The AD stabilisation step is beneficial as it allows the recovery of energy from waste solids, do not have any negative effects on plant growth or soil fertility and decreases the amount of carbon applied to the soil.

Conclusion

The sludge and algal biomasses produced from a brewery effluent treatment process were within the heavy metal limits for use on agricultural land. Also, no *E.coli* were detecetd in the biomass, soil or on the Swiss chard leaves. These waste products can be utilised as an inorganic fertiliser replacement when applied to the soil at the same nitrogen loading rate and can even increase crop yield, as was recorded in this study. There was no difference in the fertiliser quality of algae and WAS originating from a brewery effluent system, since both biomasses supported good crop growth and had no negative effects on the soil's fertility.

The nitrogen applied to the soil from algae and sludge biomass appeared to leach out of the soil less than the nitrogen (ammonia and nitrate) supplied by inorganic fertilisers. This was because the majority of the nitrogen supplied by algae and sludge was in an insoluble form. These biomasses offered a good alternative to inorganic fertilisers as the slow nutrient release aids in reducing the nutrient contamination conventional inorganic fertiliser based

agriculture has on surrounding water bodies. No difference was observed in the soil's physical fertility when algae or sludge was applied to the soil; however, this trial was only run for three months and an understanding of the long-term impacts on the soil is needed.

Anaerobic digestion is a viable pre-treatment step for sludge and algal biomasses as it recovers a portion of the carbon as an energy source and does not decrease their quality as fertilisers. The application of sludge or algae generated from a brewery effluent treatment system increased the soil sodium concentration, so it is recommended that regulations on the application rate of sludge to agricultural land should consider the limit values for sodium to avoid to its accumulation in the soil and deterioration in soil fertility.

Chapter 5: Life cycle assessment of activated sludge and high rate algal ponds coupled with resource recovery

5.1 Introduction

Wastewater treatment plants (WWTPs) play a major role in reducing the environmental impacts (eutrophication, ecotoxicity and acidification) caused by the discharge of untreated effluent into receiving water bodies. However, they too have an environmental impact by consuming natural resources during construction and operation and via the production of waste products such as sludge, nitrogen oxides (NO_x) and carbon dioxide (Lopsik 2013, Mo and Zhang 2013, Garfi *et al.* 2017). Therefore, in addition to the stated functional objectives of employing WWTPs to address the problem of treating effluents, consideration also needs to be given, when designing and constructing WWTPs, to minimise the use of natural resources, water and energy, reducing waste generation and enabling the recovery of energy and plant nutrients (Lundin *et al.* 2000, Mo and Zhang 2013). Environmental criteria, and the technical and economic aspects of WWTPs all need to be taken into consideration when selecting the most appropriate technologies (Molinos Senante *et al.* 2014, Garfi *et al.* 2017). Studies on biological effluent treatment technologies conclude that their environmental impacts can be reduced by minimizing sludge production (increasing sludge retention time, upgrading from conventional activated sludge to membrane bio-reactors, cell lysis-cryptic growth and uncoupling metabolism) and through the recovery of nutrients, energy and water (Roeleveld *et al.* 1997, Lundin *et al.* 2004, Mo and Zhang 2013, Velho *et al.* 2016).

Life cycle analysis (LCA) can be described as the “cradle-to-grave” assessment of the environmental, social and economic aspects and potential impacts associated with a

product, process, or service (Lundie *et al.* 2004, ISO 2006, Mo and Zhang 2013). This analysis attempts to evaluate the environmental, social and economic impacts associated with construction, inputs, outputs and disposal of an activity (Lundie *et al.* 2004, Mo and Zhang 2013). Life cycle analysis has become standard in the study of environmental impacts associated with energy consumption, greenhouse gas emissions and criteria pollutants (Chester *et al.* 2010). It is one of the most all-round tools used to assess the potential impacts of a product or system which has a standardized international structure (ISO standards 1404; Lundin and Morrison 2002, Lundie *et al.* 2004, ISO 2006).

Life cycle analysis offers the ability to evaluate the impact of all aspects of wastewater treatment coupled with energy and nutrient recycling technologies (Lundin and Morrison 2002, Lundie *et al.* 2004, Mo and Zhang 2013). There are only a few studies that compare the environmental impact of conventional WWTP technologies, such as activated sludge (AS) for example, to low alternative nature based technologies, such as constructed wetlands (CW) and high rate algal ponds (HRAP; Garfi *et al.* 2017). These systems are more environmentally sustainable than AS systems due to their lower energy and chemical consumption during operation (Dixon *et al.* 2003, Machado *et al.* 2007, Fuchs *et al.* 2011, Yildirim and Topkaya, 2012, Garfi *et al.* 2017). The majority of LCA studies comparing nature-based systems to conventional AS systems conclude that they have a lower global warming potential, aquatic toxicity and resource consumption (Siracusa and La Rosa 2006, Zhou *et al.* 2009, Roux *et al.* 2010, Mo and Zhang 2013). These alternative systems, however, have a greater land utilisation requirement due to their higher hydraulic retention time (HRT) and surface area requirement (Siracusa and La Rosa 2006, Zhou *et al.* 2009, Roux *et al.* 2010).

Many LCA studies on biological wastewater treatment technologies fail to include the end use of the generated biomass or whether it is regarded as a resource or a waste product (Siracusa and La Rosa 2006, Zhou *et al.* 2009, Roux *et al.* 2010, Mo and Zhang 2013). The fate of the generated biomass from WWTPs must be included in a LCA study as its disposal has been shown to have an influence on environmental impacts (Lundin *et al.* 2004, Mo and Zhang 2013). Most studies view sludge as a waste to be disposed of, and do not consider the option of recycling nutrients from sludge (Lundin *et al.* 2004, Siracusa and La Rosa, 2006, Zhou *et al.* 2009, Roux *et al.* 2010). A comprehensive LCA should incorporate interactions with surrounding technical systems such as power generation, agriculture, fertiliser production and other related material flows, in addition to the operation and construction of the wastewater treatment systems.

The environmental load of alternative wastewater treatment systems varies greatly between published studies. This is mainly due to differences in functional units, impact categories, , systems boundaries, sludge handling options and allocation choices. Most studies report on the climate change, fossil fuel depletion, ecotoxicity, and eutrophication impacts caused by the energy consumption and emissions to air and water from biological WWTPs (Lundin *et al.* 2000, Houillon and Jolliet 2005, Brown *et al.* 2010, Mo and Zhang 2013). The systems boundaries of some LCA studies only include the operational phase (Hospido *et al.* 2005, Houillon and Jolliet 2005, Brown *et al.* 2010) while others include the construction and operational phases, (Peters and Lundie 2001, Hong *et al.* 2009, Peters and Rowley 2009).

System boundaries in a LCA should be selected according to the dynamics of the system and goal of the study (Lundin *et al.* 2000). For example, a LCA must consider the environmental

impact from the production and disposal of chemicals if they are used in the effluent treatment process; especially, if the process is being compared to one that does not use chemicals (Lundin *et al.* 2000). It is also critical that the environmental burden or waste biomass disposal is included when comparing different biological effluent treatment methods (Lundin *et al.* 2004, Hong *et al.* 2009).

Individual effluent treatment technologies have been evaluated and compared using LCA but there is very little literature in which combinations of these technologies are compared. There are only two published studies which compare the environmental burden of HRAP to an AS treatment system (Garfi *et al.* 2017, Arashiro *et al.* 2018). However, both studies compared HRAP to an AS system that were in different regions and treated different effluent types. The LCA conducted by Arashiro *et al.* (2018) had different destinations for the biomass produced from the HRAP and AS systems, which did not facilitate an equivalent comparison between the two systems. This study aims to address this gap by conducting a LCA comparing HRAP to an AS system used to treat the same effluent, in the same location, at the same time, and with the same biomass disposal options.

The quality of treated effluent discharged into aquatic systems and sludge disposal methods have a major influence on the environmental performance of WWTPs (Hospido *et al.* 2010, Foley *et al.* 2010, Fang *et al.* 2016). The only two published LCAs comparing HRAP to an AS system are vague in describing the quality of treated effluent leaving both systems and only describe the total nitrogen and phosphorous concentrations (Garfi *et al.* 2017, Arashiro *et al.* 2018). The form of nitrogen entering the environment in treated effluent may play a major role in the environmental impact of the effluent treatment technology. Nitrite is more toxic to aquatic organisms than nitrate, because the nitrogen in nitrite is in an unstable

oxidation state, with toxic concentrations of nitrate to fish 100 times lower than that of nitrite (Lucas and Southgate 2012). Therefore, any LCA of alternative wastewater treatment technologies needs to include a detailed inventory of emissions to water, air and land.

The inventory data used in the two LCA studies on HRAP were theoretical and were collected from various published papers (Garfi *et al.* 2017, Arashiro *et al.* 2018). This may have a significant influence on the results and it also reduces the robustness of the conclusions drawn from the studies. In order to increase the robustness of LCA studies on HRAP, it needs to be compared to conventional WWTPs that treat the same effluent using onsite data that includes treatment performance and emissions. Both LCA scenarios should also be coupled with various combinations of viable sludge handling technologies and utilise inventory and emission data recorded from real life scenarios or experiments.

There is a lack of literature that reviews the energy, nutrient and water recovery in HRAP treatment technologies, coupled with biogas and fertiliser production from the generated algal biomass (McCarty *et al.* 2011, Mo and Zhang 2013). Individual resource recovery technologies have been evaluated and compared (HRAP, AS and anaerobic digestion (AD)) using LCA but literature is lacking with regard to the utilisation of combinations of these technologies and hardly any studies evaluate the environmental impacts of these technologies (Mo and Zhang 2013). This study aims to address these issues by determining the best combination of effluent treatment technologies that allow maximum recovery of resources while minimising environmental impact.

5.2 Aims and objectives

The aim of this study was to conduct a thorough LCA comparing HRAP to conventional AS technologies, where resource recovery and sludge disposal are taken into account. The study objectives were to:

- document and describe the environmental burden of a HRAP and AS effluent treatment system coupled with energy and nutrient recovery options;
- identify the major processes which contribute to the environmental burden of both systems; and
- identify shortcomings and contribute to improving the methods used in the field of LCA on nature-based wastewater treatment technologies

Data collected from onsite monitoring of HRAP and AS systems (Chapter 2), as well as from the utilisation of the generated biomass for methane production through AD (Chapter 3) and/or used in land application for crop production (Chapter 4), were needed to perform a robust LCA study (current chapter). This is the first LCA study that uses empirical data to compare the environmental impacts of AS and HRAP effluent treatment technologies, collected from the same effluent stream, under the same temporal conditions, and coupled with energy and nutrient recovery options. This was made possible by drawing together the data collected in Chapters 2, 3 and 4 of this thesis and creating a synthesis in the form of the LCA presented here.

5.3 Materials and methods

5.3.1 System description

Untreated brewery effluent was first passed through a 500 µm drum screen (Autrex Industrial Screening, Serial no. A 140/02, Model no. R 015) and then AD in an up-flow anaerobic sludge blanket reactor (Chapter 2). Post-AD effluent was then further treated in either an AS or HRAP system. The design, size and operation of the commercial AS system modelled in this chapter are described earlier (Chapter 2, Section 2.3.1). Effluent from these systems was suitable for reuse in non-production activities in the brewery such as bottle washing, irrigation or discharge into a natural water resource.

Sludge and algal biomass handling

Waste activated sludge (WAS) is the material that needs to be disposed of from the AS system (generated from sludge wasting; Chapter 2, Section 2.3.1) to maintain the desired settleable volume in the aeration basin. The algal biomass settled out from the HRAP-treated effluent also needs to be disposed of. Experiments were carried out where the WAS and algae were anaerobically digested to produce biogas (Chapter 3), after which the digestate was utilised as a fertiliser in Swiss chard production (Chapter 4). The results (biogas production and Swiss chard yield) from these experiments were used in the LCA modelling scenario (Chapters 2, 3 and 4).

5.3.2 Life cycle assessment

The LCA was conducted according to the standards of the International Organiation for Standardization (ISO), with the following main steps: goal and scope definition; inventory analysis; impact assessment; and interpretation of results (ISO 2000, ISO 2006, ISO 2006a).

Goal and scope definition

The goal of this study was to compare the environmental impact of two brewery effluent treatment technologies (activated sludge and high rate algal ponds), coupled with energy and nutrient recovery options (Figure 5.1 and 5.2). The functional unit adopted here was one cubic metre of treated effluent because these systems' main purpose was effluent treatment.

The system boundary included input and output flows of material, energy and resources during construction and operation of the plant over twenty years (Yildirim and Topkaya 2012, Garfi *et al.* 2017; Figures 5.1 and 5.2). Transportation of the materials used for the construction phase was not included in the analysis since it was only used during construction and had a minor influence on the overall impact of the treatment system (Machado *et al.* 2007, Lopsik 2013). Destruction of the effluent treatment plant was also not included in the analysis because previous studies have shown its impact have marginal effects on the overall impact of the treatment system (Machado *et al.* 2007, Lopsik 2013). Transport of WAS and algae to agricultural lands was estimated to be 30 km based on the average distance of farmers around Ibhayi Brewery.

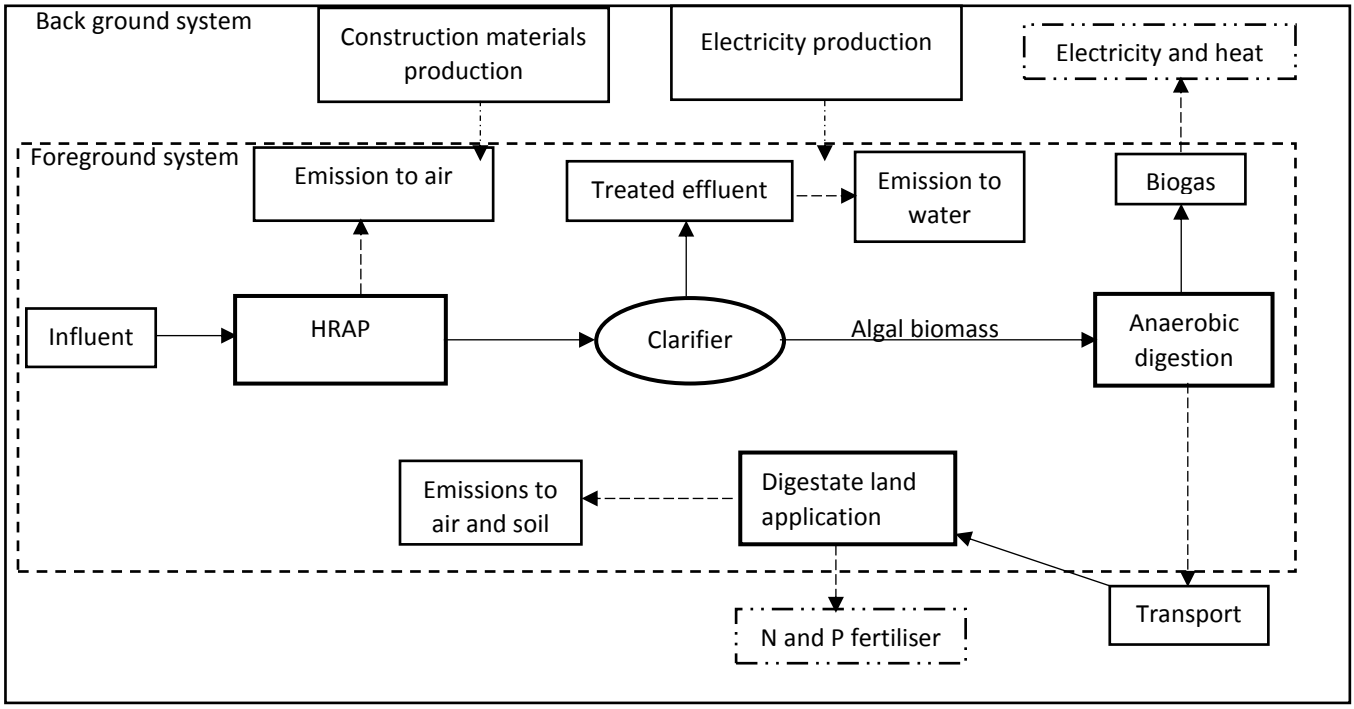

Figure 5.1: System boundary of the high rate algal pond scenario used to treat brewery effluent.

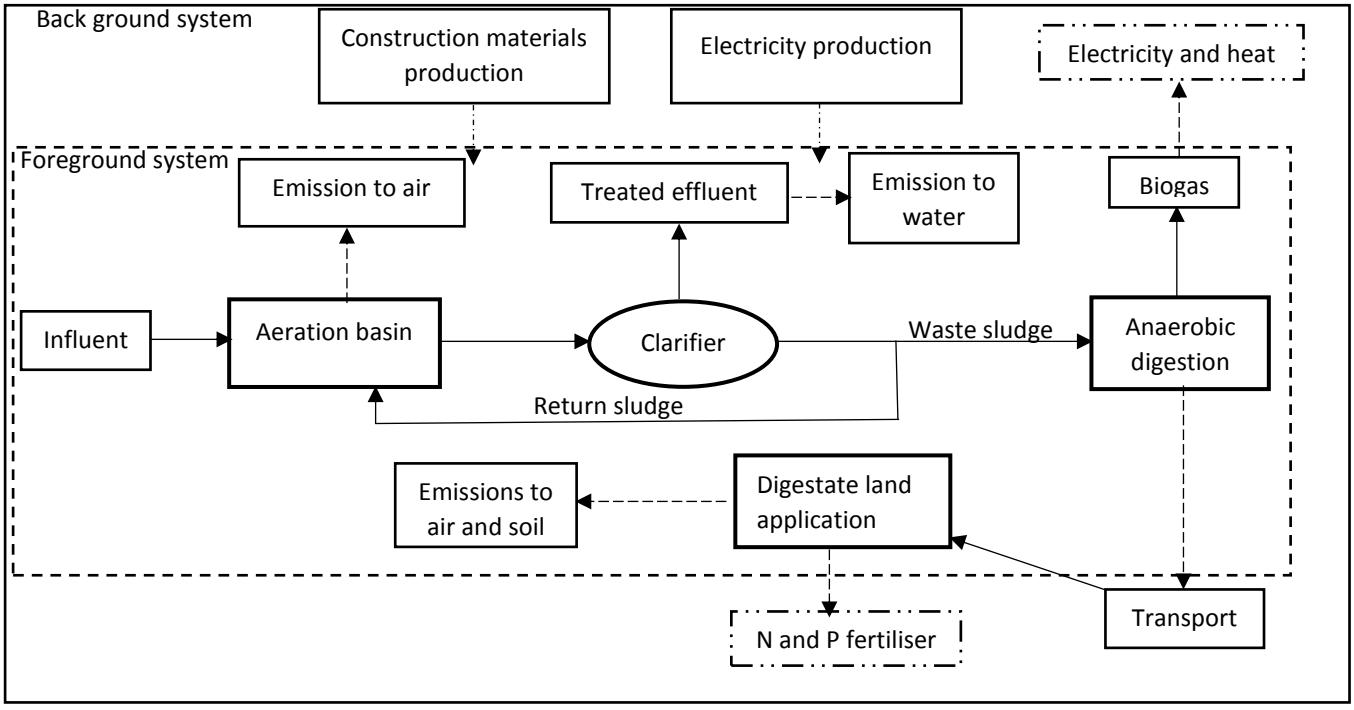

Figure 5.2: System boundary of the activated sludge scenario used to treat brewery effluent.

The system expansion method via avoided production was used to model the utilisation of generated by-products (biogas used for electricity generation, digested sludge and algae used as a fertiliser, Chapter 1, Section 1.2.7, ISO 2006, Garfi *et al.* 2017). The "avoided production" method is used when by-products indirectly decrease the impact that the main product or process has on the environment, because the production of the main product (and subsequent by-products) removes the need for a separate industry/process to manufacture the by-product that was produced (Guinee *et al.* 2002, ISO 2006). According to the "avoided production" method, the by-products result in the avoided production of conventional products (i.e. the products that would need to have been produced a separate process), and so the environmental impact related to the conventional product can be subtracted from the overall impact of the system (ISO 2006, Arashiro *et al.* 2018). In this study the digestate was considered as a substitute for chemical fertiliser while biogas was used to produce electricity and heat from a combined heat and power (CHP) unit. Previous trials (Chapter 4) found on a nitrogen and phosphorous basis, digestate from sludge and algae resulted in the same crop yield as chemical fertiliser. Inorganic fertiliser production was therefore avoided via digestate production on a nitrogen and phosphorous basis of 1 to 1 (Chapter 4, ISO 2006, Arashiro *et al.* 2018). The avoided production of electricity and heat generated from the combustion of biogas (60 % methane) from a CHP unit was 2 kWh/m^3 and 2.5 kWh/m^3 biogas respectively (ISO 2006, Collet *et al.* 2011, Sfez *et al.* 2015).

Inventory analysis

Inventory data used for the construction of the AS system were provided by the company (South African Breweries, Pty Ltd.) that built it (Table 5.1, Naiker *pers comm* 2018). For the construction of the HRAP system, inventory data were provided by the Institute for Environmental Biotechnology, Rhodes University (EBRU), who have an operational technical

scale HRAP system treating domestic wastewater (Table 5.1). Materials and energy used during operation of the systems were monitored over six months and used in this study (Table 5.1; Chapter 2). For both systems, carbon dioxide emissions/uptake from respiration/photosynthesis were estimated using a mass balance approach over the same period (Table 5.2; Chapter 2) Dinitrogen monoxide emissions from the AS were taken from previous studies conducting LCA analysis on AS systems (Table 5.2; Law *et al.* 2012, Garfi *et al.* 2017). Ammonia volatilisation from HRAP was estimated in a previous study conducting a mass balance of nitrogen of HRAP, also over six months (Table 5.2; Chapter 2). Background data (emissions from materials, chemicals, electricity and transport) were obtained from the Ecoinvent 3.1 database where the South African electricity mix was used for all electricity requirements (Moreno-Ruiz *et al.* 2014, Garfi *et al.* 2017).

Table 5.1: Inventory of inputs for the high rate algal pond (HRAP) and activated sludge (AS) brewery effluent treatment systems. Values are representative of the functional unit (1.0 m^3 of effluent treated).

Inputs	Unit	AS	HRAP
Construction materials			
Excavation	m^3/m^3	$1.32E^{-5}$	$3.18E^{-4}$
Concrete	kg/m^3	$9.61E^{-3}$	$2.70E^{-2}$
Water proof coating	kg/m^3	$9.04E^{-5}$	$1.79E^{-3}$
Metals	kg/m^3	$1.71E^{-3}$	$1.10E^{-2}$
Plastic (Polyvinyl chloride)	kg/m^3	$4.28E^{-5}$	$5.48E^{-3}$
Anaerobic digester			
Excavation	m^3/m^3	$9.83E^{-7}$	$3.74E^{-6}$
Concrete	kg/m^3	$1.02E^{-5}$	$3.86E^{-5}$
Water proof coating	kg/m^3	$5.11E^{-5}$	$1.96E^{-4}$
Metals	kg/m^3	$7.96E^{-4}$	$3.02E^{-3}$
Operation			
Electricity	kWh/m^3	$4.13E^{-1}$	$2.10E^{-1}$
Anaerobic digester			
Electricity	kWh/m^3	$2.54E^{-2}$	$9.65E^{-2}$

Biogas production data for the AD of WAS and algae were taken from laboratory scale continuously stirred tank reactor experiments conducted over six months (Chapter 3). The NH_3 and N_2O air emissions from the application of digestate on agricultural land were calculated using emission factors found in the literature (Lundin *et al.* 2000, Hospido *et al.* 2010, Arashiro *et al.* 2018). Methane emissions from agricultural land application of digestate were not considered as AD of liquid fertiliser does not occur in a predominantly dry climate and because the materials were stabilised via AD (Lundin *et al.* 2000, Arashiro *et al.* 2018). Heavy metal and nutrient emissions to the soil from the agricultural application of digestate were calculated from the complete elemental analysis of digestate obtained from previously conducted trials on the AD of WAS and algae and its suitability as a fertiliser (Chapters 3 and 4).

Table 5.2: Inventory of outputs for the high rate algal pond (HRAP) and activated sludge (AS) brewery effluent treatment scenarios. Values are representative of the functional unit (1.0 m^3 of effluent treated).

Outputs	Unit	AS	HRAP
Waste biomass production	g/m^3	8.31E 1	3.17E 2
Emission to water			
Chemical oxygen demand	g/m^3	9.17E 1	9.95E 1
NH_4	g/m^3	2.80E-1	1.25E 0
NO_2	g/m^3	1.10E-2	1.30E-2
NO_3	g/m^3	9.00E 0	6.62E 0
PO_4	g/m^3	3.41E 0	3.80E 0
Emission to air			
Operation of HRAP or AS			
CO_2	g/m^3	6.02E 1	-8.41E-1
N_2O	g/m^3	1.28E-1	
NH_3	g/m^3		8.75E 0
Digestate used as fertiliser			
N_2O	g/m^3	1.27E-1	4.96E-1
NH_3	g/m^3	1.59E 0	6.54E 0
Emission to soil			
Aluminium	g/m^3	1.52E 0	3.44E 0
Calcium	g/m^3	1.38E 0	5.46E 0
Iron	g/m^3	4.98E-1	1.89E 0
Potassium	g/m^3	3.84E-1	1.44E 0
Magnesium	g/m^3	3.19E-1	1.12E 0
Sodium	g/m^3	4.19E 0	1.59E 1
Phosphorus	g/m^3	1.70E 0	4.90E 0
Silicon	g/m^3	1.72E-1	1.27E 0
Zinc	g/m^3	4.91E-1	1.67E 0
Chromium	g/m^3	1.85E-2	7.40E-2
Manganese	g/m^3	2.07E-2	2.07E-2
Nickel	g/m^3	4.59E-3	1.84E-2
Copper	g/m^3	3.12E-2	1.10E-1
Strontium	g/m^3	1.37E-2	3.55E-1
Cadmium	g/m^3	7.59E-5	2.88E-4
Barium	g/m^3	6.37E-3	2.02E-2
Lead	g/m^3	1.78E-3	6.76E-3
Avoided products			
Electricity	kWh/m^3	2.66E-2	1.06E-1
Heat	kWh/m^3	3.32E-2	1.33E-1
N fertiliser	g/m^3	4.16E 0	1.59E 1
P fertiliser	g/m^3	1.78E 0	2.07E 0

Impact assessment

Environmental impacts were analysed using software (SimaPro 8 , The GreenHouse, Cape Town, South Africa) at the Recipe midpoint method (hierarchist approach) which is in accordance with the ISO 14040 standards (ISO 2000, Goedkoop *et al.* 2008, Pré

sustainability 2014, Garfi *et al.* 2017). The following impact categories were assessed: climate change; ozone depletion; terrestrial acidification; freshwater eutrophication; marine eutrophication; photochemical oxidant formation; particulate matter formation; metal depletion; fossil fuel depletion; human toxicity; terrestrial ecotoxicity; freshwater ecotoxicity; and marine ecotoxicity (ISO 2000, ISO 2006, Garfi *et al.* 2017). All impact categories were normalised at a global scale (ISO 2000, ISO 2006). Climate change and water footprint were normalised according to South African values. The normalisation values used for South Africa were 10 t CO_2 eq/capita/y for greenhouse gas (GHG) emissions and 100 m^3/capita/y for the blue water footprint (Pahlow *et al.* 2015, Climate transparency 2017).

5.3.3 Sensitivity and uncertainty analysis

Sensitivity analysis of the major assumptions (NH_3 emissions, N_2O emissions, transport of digestate) utilised in the inventory data were tested at ±10 % variation to identify how their variation might influence the results (Dixon *et al.* 2003). A sensitivity co-efficient was also calculated for the inclusion or exclusion of biogenic carbon dioxide to identify its influence on environmental categories. A sensitivity co-efficient for each environmental indicator was then calculated using Equation 5.1 (Dixon *et al.* 2003):

$$\text{Sensitivity} = \frac{\text{Output high-Output low}}{\text{Output default}} \div \frac{\text{Input high-Input low}}{\text{Input default}} \quad [5.1]$$

where

"Output" = environmental indicator (terrestrial acidification, freshwater eutrophication); and

"Input" = emission (N_2O, NH_3).

A Monte Carlo analysis was conducted to determine the robustness of the conclusions generated from the LCA (ISO 2006, Liu and Zhang 2012). A normal distribution and variation of 10 % was added to all output data when conducting the Monte Carlo analysis (ISO 2006, Liu and Zhang 2012). The analysis was run on 1000 simulations as recommended by the ISO standards (ISO 2006, Hung and Ma 2009).

5.4 Results

Out of the 13 environmental impact categories characterised, the potential impact from the HRAP system was lower for the following six of the categories: climate change; ozone depletion; photochemical oxidant formation; freshwater ecotoxicity; marine ecotoxicity; and fossil fuel depletion (Table 5.3). The HRAP system had half the climate change potential when compared to the AS system (Table 5.3). Ozone depletion and photochemical oxidant formation were 10 and 45 % lower in the HRAP when compared to the AS (Table 5.3). The HRAP scenario had double the human toxicity impact when compared to the AS system (Table 5.3). The terrestrial acidification, terrestrial ecotoxicity, marine eutrophication and freshwater eutrophication impact categories were lower for the AS (Table 5.3). However, marine and freshwater ecotoxicity impacts were lower for the HRAP system. The HRAP had a higher metal resource depletion while the AS had a higher fossil fuel depletion (Table 5.3).

Table 5.3: The environmental impacts of a high rate algal ponds (HRAP) and activated sludge (AS) brewery effluent treatment system, coupled with a biogas and fertiliser production scenario. Values are referred to one functional unit (1.0 m^3 brewery effluent treated).

Environmental impact	unit	HRAP	AS
Climate change	Kg/m^3 CO_2 eq	$4.16E^{-1}$	$8.08E^{-1}$
Ozone depletion	kg/m^3 CFC 11 eq	$4.11E^{-8}$	$4.64E^{-8}$
Terrestrial acidification	kg/m^3 SO_2 eq	$3.93E^{-2}$	$8.38E^{-3}$
Freshwater eutrophication	kg/m^3 P eq	$6.33E^{-3}$	$3.16E^{-3}$
Marine eutrophication	kg/m^3 N eq	$8.73E^{-3}$	$5.95E^{-3}$
Photochemical oxidant formation	kg/m^3 NMVOC	$1.34E^{-3}$	$2.55E^{-3}$
Particulate matter formation	kg/m^3 PM10 eq	$5.60E^{-3}$	$1.84E^{-3}$
Metal depletion	kg/m^3 Fe eq	$3.97E^{-2}$	$1.90E^{-2}$
Fossil fuel depletion	kg/m^3 oil eq	$1.32E^{-1}$	$1.97E^{-1}$
Human toxicity	kg/m^3 1,4-DB eq	$9.21E^{-1}$	$4.83E^{-1}$
Terrestrial ecotoxicity	kg/m^3 1,4-DB eq	$2.69E^{-3}$	$8.38E^{-4}$
Freshwater ecotoxicity	kg/m^3 1,4-DB eq	$4.27E^{-3}$	$7.05E^{-3}$
Marine ecotoxicity	kg/m^3 1,4-DB eq	$4.31E^{-3}$	$7.04E^{-3}$

Equivalents (eq), 1,4 dichlorobenzene (1,4 DB), Trichlorofluoromethane (CFC11), Non-methane volatile organic compounds (NMVOC), Particulate matter <10 microns in diameter (PM10).

Electrical energy consumption during the operation of HRAP and AS and digestate land application accounted for over 90 % of the climate change potential for the HRAP and AS treatment systems (Figure 5.3). For both HRAP and AS, digestate land application accounted for at least 80 % of the ozone depletion, terrestrial acidification and terrestrial ecotoxicity impact categories (Figure 5.3). Emissions to water were the main contribution to marine eutrophication for both the HRAP and the AS systems (Figure 5.3). Electrical energy consumption during the operation of HRAP and AS was the major contributor to photochemical oxidant formation, freshwater ecotoxicity and marine ecotoxicity for both treatment systems (Figure 5.3). Electrical energy consumption during the operation of HRAP and AS and transport of digestate, contributed to over 85 % of fossil resource scarcity for both treatment systems (Figure 5.3). Avoided fertiliser, electricity and heat production decreased climate change, ozone depletion, photochemical oxidant formation, particulate matter formation, freshwater ecotoxicity, marine ecotoxicity, metal depletion and fossil fuel scarcity by 10-40 % for HRAP and 5-10 % for AS systems respectively.

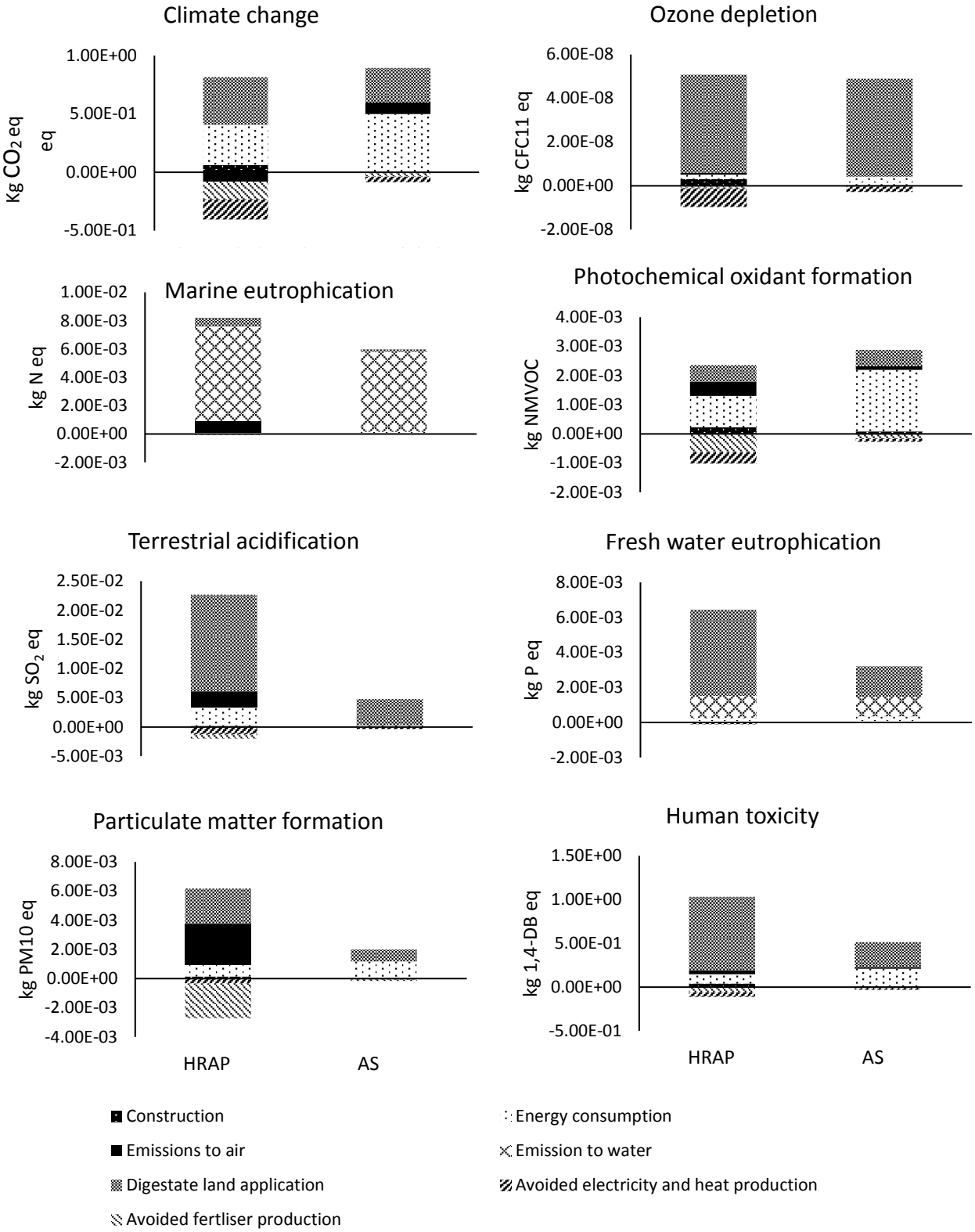

Figure 5.3: Potential environmental impacts of a high rate algal pond (HRAP) and activated sludge (AS) brewery effluent treatment system, coupled with a biogas and fertiliser production system. Values are referred to one functional unit (1.0 m^3 brewery effluent treated). Equivalents (eq), 1,4 dichlorobenzene (1,4 DB), Trichlorofluoromethane (CFC11), Non-methane volatile organic compounds (NMVOC), Particulate matter <10 microns in diameter (PM10).

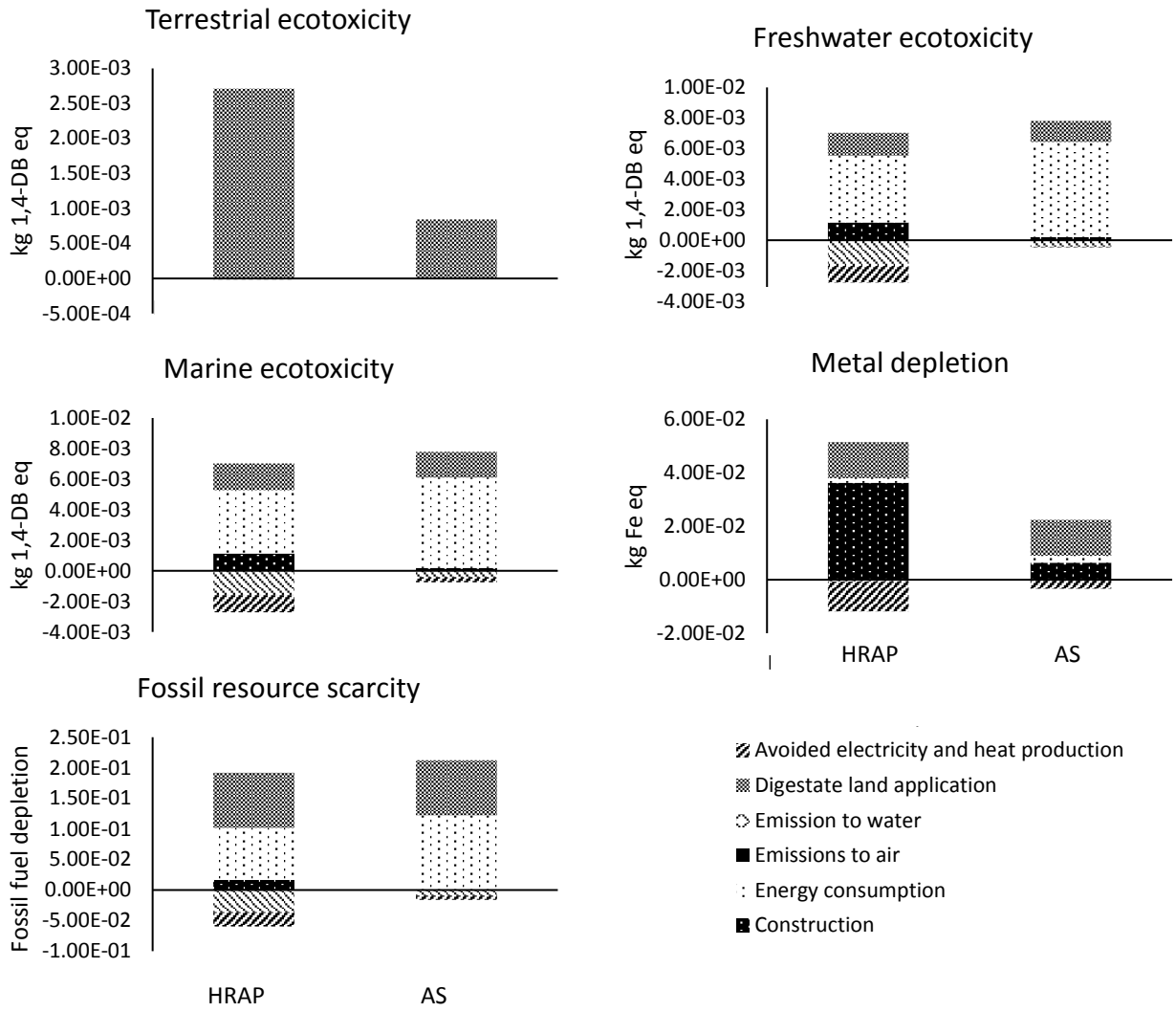

Figure 5.3 continued: Potential environmental impacts of a high rate algal pond (HRAP) and activated sludge (AS) brewery effluent treatment system, coupled with a biogas and fertiliser production system. Values are referred to one functional unit (1.0 m^3 brewery effluent treated). 1,4 dichlorobenzene equivalents (1,4 DB eq), Iron equivalents (Fe eq).

After normalisation, the five most significant categories, in descending order, were: freshwater eutrophication; marine eutrophication; human toxicity; freshwater ecotoxicity; and marine ecotoxicity (Figure 5.4). Climate change was sensitive to digestate transport and N_2O emissions for both HRAP and AS (Tables 5.4 and 5.5). A ten percent increase in these parameters would increase climate change potential by up to six percent (Tables 5.4 and 5.5).

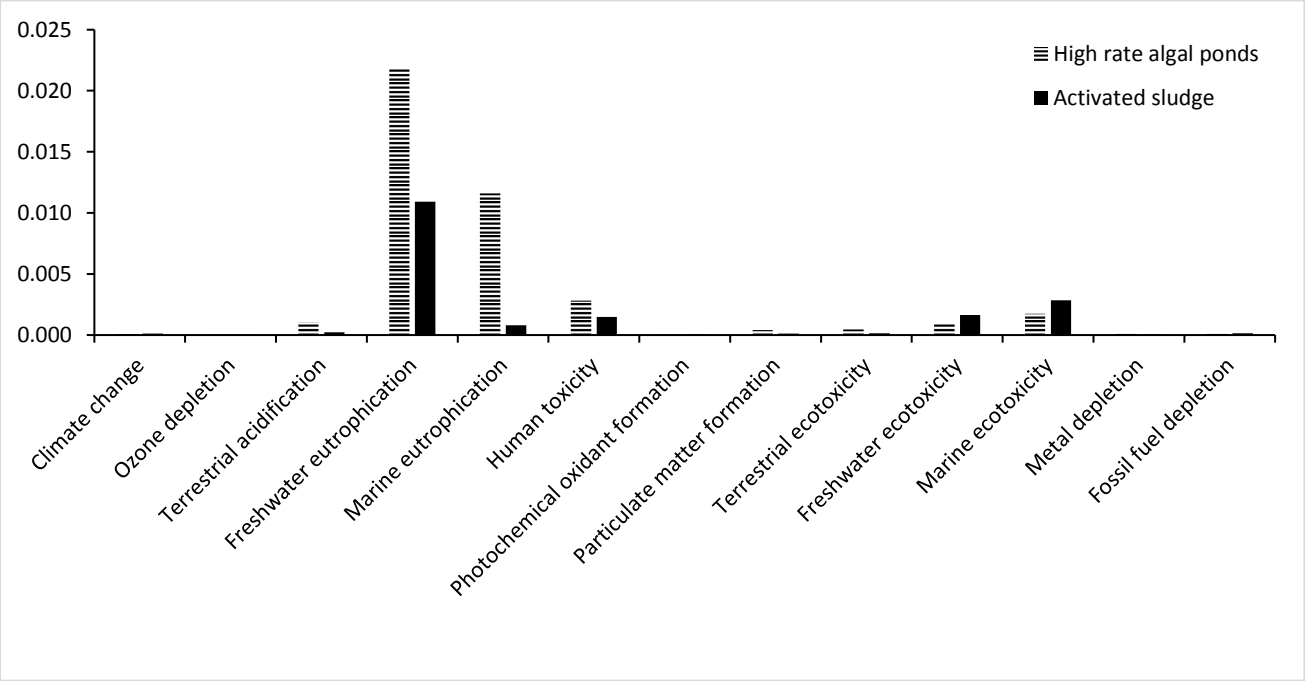

Figure 5.4: Normalised impact categories of a high rate algal pond and activated sludge system used to treat brewery effluent.

Digestate transport had the greatest influence on impact categories with a 10 % increase in digestate transport increasing ozone depletion by about 10 % for both systems (Tables 5.4 and 5.5). Nitrite emission into water from the treated effluent had an influence on marine eutrophication, with a ten percent increase resulting in marine eutrophication increasing by about five percent (Tables 5.4 and 5.5). A ten percent change in ammonia volatilisation in HRAP would change terrestrial acidification and particulate matter formation by five percent (Tables 5.4 and 5.5). Air emission of NH_3 and N_2O from the agricultural use of digestate influenced climate change, terrestrial acidification and particulate matter formation, however they were not significant at a five percent significance level (Tables 5.4 and 5.5). The inclusion or exclusion of biogenic carbon dioxide emissions affected the climate change potential of HRAP and AS scenarios by 16.3 and 8.0 % respectively (Tables 5.4 and 5.5).

Table 5.4: Inventory parameters for high rate algal ponds which influence environmental categories by at least one percent at a ten percent variation. Data here represent percent (%) change in impact category associated with a ten percent change in inventory parameters.

	Digestate transport	NO_2 water emissions	NH_3 volatilisation	NH_3 digestate	N_2O digestate	Biogenic CO_2
Climate change	±06.30	0	0	0	±03.76	±16.32
Ozone depletion	±10.89	0	0	0	0	0
Terrestrial acidification	±00.16	0	±05.45	±04.07	0	0
Photochemical oxidant formation	±04.20	0	0	0	0	0
Marine eutrophication	±00.03	±04.79	±00.99	±00.74	0	0
Particulate matter formation	±00.59	0	±05.00	±03.74	0	0
Metal depletion	±03.40	0	0	0	0	0
Human toxicity	±00.70	0	0	0	0	0
Terrestrial ecotoxicity	±00.27	0	0	0	0	0
Freshwater ecotoxicity	±03.10	0	0	0	0	0
Marine ecotoxicity	±03.85	0	0	0	0	0

Table 5.5: Inventory parameters for activated sludge which influence environmental categories by at least one percent at a ten percent variation. Data here represent percent (%) change in impact category associated with a ten percent change in inventory parameters.

	Digestate transport	NO_2 water emissions	N_20 emissions AS running	NH_3 digestate	N_20 digestate	Biogenic CO_2
Climate change	±03.19	0	±00.47	0	±00.47	±08.02
Ozone depletion	±09.64	0	0	0	0	0
Terrestrial acidification	±00.73	0	0	±04.65	0	0
Photochemical oxidant formation	±02.21	0	0	0	0	0
Marine eutrophication	±00.04	±05.55	0	±00.25	0	0
Particulate matter formation	±01.80	0	0	±02.76	0	0
Metal depletion	±07.09	0	0	0	0	0
Human toxicity	±01.34	0	0	0	0	0
Terrestrial ecotoxicity	±00.88	0	0	0	0	0
Freshwater ecotoxicity	±01.88	0	0	0	0	0
Marine ecotoxicity	±02.35	0	0	0	0	0

A ten percent variance on all output inventory data resulted in the HRAP having higher terrestrial acidification, freshwater eutrophication, marine eutrophication, human toxicity, particulate matter formation, terrestrial ecotoxicity and metal depletion impacts by 100 % of the generated models (Figure 5.5). The only impact category that was influenced by the ten percent variance added to all emissions was ozone depletion, where 91 % of the time AS systems had a higher impact than HRAP (Figure 5.5).

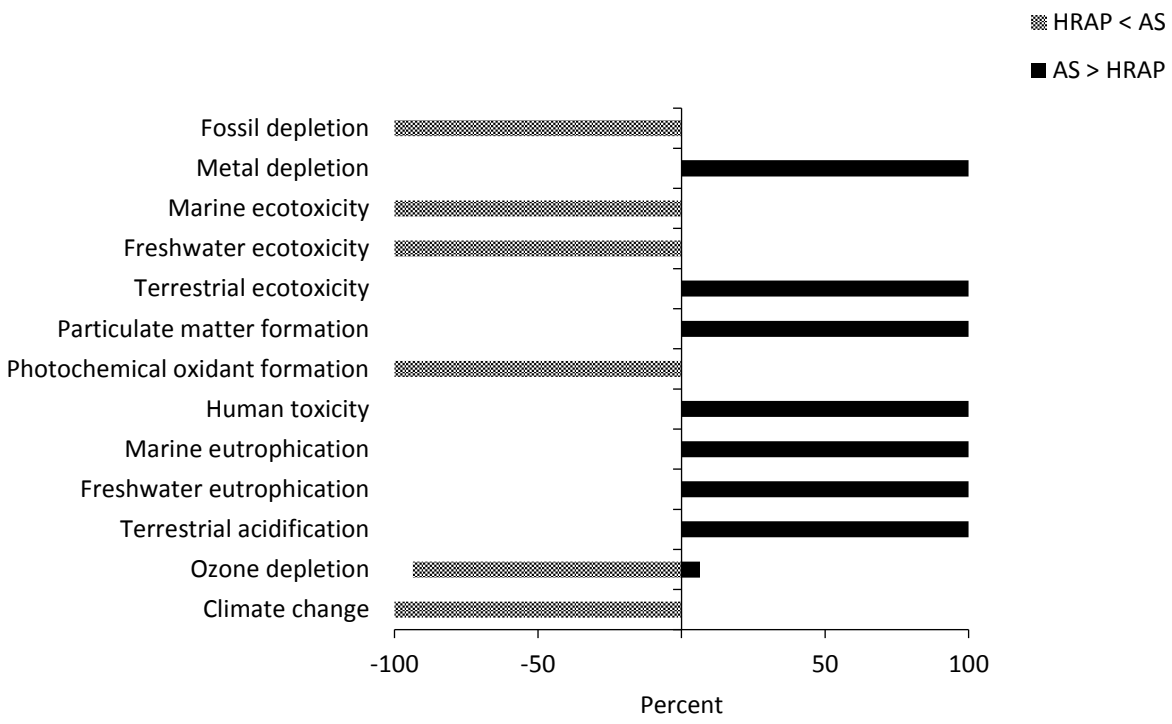

Figure 5.5: Results from Monte Carlo analysis showing percent of models where high rate algal pond (HRAP) scenario was higher or lower than the activated sludge (AS) scenario. Results were obtained from running 1000 models with ten percent variation added to all output and inventory data.

5.5 Discussion

Energy consumption during operation has a large influence on the environmental impact of wastewater treatment technologies (Mo and Zhang 2013). The electrical energy consumption was lower for the HRAP when compared to the AS, resulting in lower potential

impacts for categories which were mainly influenced by electrical energy consumption during operation. Previous LCA studies of WWTPs have concluded that technologies with lower operational energy use, have less of an impact on climate change, ozone depletion, freshwater and marine ecotoxicity, photochemical oxidant formation and fossil fuel depletion (Lundie *et al.* 2004, Yıldırım and Topkaya 2012, Garfi *et al.* 2017). Similarly, the HRAP scenario had less of an impact on climate change, photochemical oxidant formation, freshwater and marine ecotoxicity and fossil fuel depletion, and this was primarily due to lower operational energy consumption when compared to the AS.

Materials used for construction of WWTPs can influence the environmental impact over its lifetime. They contributed 30 % and 80 % to metal depletion for AS and HRAP systems respectively. However, construction materials contributed less than ten percent of all other impact categories. The HRAP treatment system had double the metal depletion impact when compared to the AS scenario. High rate algal ponds require more construction materials when compared to AS system due to the large area of land they occupy and the higher HRT they need to treat effluent to similar standards (Craggs *et al.* 2011, Arashiro *et al.* 2018). Materials used for construction are the major contributors to metal depletion for biological WWTPs and normally contribute to less than five percent of all other impact categories (Machado *et al.* 2007, Garfi *et al.* 2017, Arashiro *et al.* 2018). Since HRAP are depth limited, their efficiency is dependent on light penetration (Craggs *et al.* 2011, Byung-hyuk *et al.* 2018), besides, they have a higher HRT, they require more construction materials and have a higher metal depletion impact when compared to AS systems.

Ammonia volatilisation occurs in HRAP due to the high pH (>9.0) and this will have an influence on its environmental impact (Rose *et al.* 1996, Chen *et al.* 2003). The HRAP

scenario had a higher particulate matter formation impact which was mainly due to the higher air emissions from ammonia volatilisation and the heavy metal content of algae. A LCA comparing construction and operational impacts found similar results where ammonia volatilisation in HRAP caused them to have a greater particulate matter formation impact (Arashiro *et al.* 2018). The addition of carbon dioxide into HRAP reduced ammonia volatilisation by nine percent and increased nitrogen recovery (Park and Craggs 2011). Ammonia volatilisation from HRAP is an air emission concern and reducing this emission by decreasing pH through carbon dioxide injection is an option that needs to be assessed (Park and Craggs 2011, de Godos *et al.* 2013).

The normalisation of impact categories identifies which ones carry the highest environmental threat. After normalisation, the most significant impact categories were: freshwater eutrophication; marine eutrophication; human toxicity; freshwater ecotoxicity; and marine ecotoxicity. This result is consistent with the majority of LCA studies on biological wastewater treatment facilities (Hospido *et al.* 2005, Gallego *et al.* 2008, Lopsik 2013, Fang *et al.* 2016, Arashiro *et al.* 2018). Eutrophication and ecotoxicity impact categories pose the greatest environmental threat for biological wastewater treatment facilities.

Normalisation identified marine eutrophication as one of the important impact categories of both systems. Marine eutrophication is caused by nitrogen and phosphorus containing compounds entering the coastal environment and according to the ReCiPe method used in this study 7.3 % of nitrogen applied to land, 11 % of NH_3 emitted to the air and 100 % of nitrogen compounds released into freshwater enter the coastal environment (Goedkoop *et al.* 2008, Kitsiou and Karydis 2011). The fate of nitrogen (assimilation, nitrification,

denitrification) discharged into fresh water is not taken into account in the marine eutrophication method because 100 % of the nitrogen will enter the coastal environment; however, in reality the percent of nitrogen that will reach the coastal environment will be less than 100 % and will depend on many factors such as the distance to the coast, the season, the climate and the type of freshwater ecosystem it has to pass through (Clausen *et al.* 2009, Goedkoop *et al.* 2008). For standardisation amongst LCA work marine eutrophication is considered; however, nitrogen compounds released into the environment far from the coast (>200 km) are less likely to reach the marine environment compared to emissions released near the marine environment (Clausen *et al.* 2009, Goedkoop *et al.* 2008). This study compared a HRAP and an AS system situated in the same geographical location, situated close to the banks of an estuary, which was about 9.3 km away from the coastline and 1.7 km from the main channel of the estuary (which forms an integral part of the coastal environment), and therefore marine eutrophication impact was taken into account.

High rate algal ponds had a higher marine eutrophication than the AS. For both scenarios emission to water made up the majority (>75 %) of marine eutrophication, with nitrite and phosphate being the major contributors. Previous LCA studies have concluded that emission to water, and especially nitrite and phosphate are major contributors to marine eutrophication (Lundie *et al.* 2004, Corominas *et al.* 2013, Morelli *et al.* 2018). A LCA study comparing the construction and operational impact of HRAP and AS systems found HRAP had a lower marine eutrophication impact than an AS system (Arashiro *et al.* 2018). However, the authors only included total nitrogen emission to water and did not specify the form of nitrogen leaving in the treated effluent (Arashiro *et al.* 2018). Nitrite and phosphate concentrations in treated effluent from HRAP were higher than those in AS systems, which

resulted in HRAP having a higher marine eutrophication potential impact. It is important to specify the form of nitrogen being released into water when conducting a LCA. Nitrite and ammonia have a higher impact weight than total nitrogen, and only specifying total nitrogen will obscure the environmental impact of treated effluent released into the environment (Morelli *et al.* 2018). Specifying the form of nitrogen can influence the potential consideration of marine eutrophication impact.

The land application of sludge can result in heavy metal contamination of the soil and/or the pollution of surrounding water bodies and must be taken into consideration. Digestate land application accounted for over 65 % of terrestrial ecotoxicity, human toxicity, freshwater eutrophication, and terrestrial acidification for both AS and HRAP treatment systems, with HRAP having higher potential impacts in all above mentioned categories. Algal biomass from the HRAP had a higher heavy metal content when compared to WAS for the AS. These results are consistent with previous studies investigating the agricultural use of micro-algae and WAS (anaerobically digested or undigested), where terrestrial toxicity was higher when algae or WAS, were applied directly to the soil in comparison to incineration or composting (Fang *et al.* 2016, Sharma 2017, Arashiro *et al.* 2018). The increased impacts on terrestrial environments due to the recycling of nutrients and sludge in agriculture are unavoidable and it is important to ensure sufficient soil monitoring programmes are put in place when this practice is carried out (Tangsubkul *et al.* 2005). The HRAP scenario had higher impacts on terrestrial environments due to the higher heavy metal content of the algae and the greater biomass produced from the HRAP system. The HRAP system recovered more heavy metals in the algal biomass and when this biomass was applied to the soil it would have a concentrating effect over years of application, resulting in higher terrestrial ecotoxicity (Tangsubkul *et al.* 2005, Arashiro *et al.* 2018). In the AS scenario a greater portion of the

heavy metals remained in the effluent and were released into the environment (even though heavy metal concentrations were below discharge standards) where they were diluted in the receiving environment. This resulted in the AS system having a lower ecotoxicity scoring. If technologies were available to remove the heavy metals from the algal biomass prior to its application to agricultural land (Mo and Zhang 2013, Fang *et al.* 2016), this would reduce the ecotoxicity impacts of the HRAP.

High rate algal ponds require light for photosynthesis during the treatment process resulting in their treatment performance being influenced seasonally (Perez-Lopez *et al.* 2017, Arashiro *et al.* 2018). Seasonal variation was not included in the current study since the data collected to model the HRAP system were collected only over the summer. In another study, Lasissa *et al.* (2018) found that the electrical consumption and land footprint increased during the winter months, which increased the climate change, metal depletion, freshwater and marine ecotoxicity, photochemical oxidant formation and fossil fuel depletion of HRAP; however, these did not influence the overall conclusion of the LCA. In winter and summer HRAP had a higher impact for metal depletion and lower climate change, freshwater and marine ecotoxicity, photochemical oxidant formation and fossil fuel depletion impact categories than AS (Arashiro *et al.* 2018). High rate algal ponds are more suitable for climates near the equator where warm temperatures and high solar radiation are predominant throughout the year (Arashiro *et al.* 2018).

Freshwater and marine ecotoxicity were identified as important impact categories after normalisation. Electrical energy consumption during HRAP and AS operation was the major contributor (>65 %) to both these categories, with HRAP scoring a lower impact due to a lower operational energy consumption. The electrical energy inventory used in this study

was a South African energy mix, which is primarily (i.e. 77 %) comprised of coal generated electricity (Pré sustainability 2014, Jain and Jain 2017). The method of electricity generation has a major influence on the environmental impact of a product or service which uses electricity during its life cycle and it is important to select electricity inventory data that represent the scenario under study (ISO 2000, ISO 2006, Turconi *et al.* 2013). If these systems were built in a country with a large renewable energy contribution, they would have minimal freshwater and marine ecotoxicity. However, in South Africa, any electrical energy consumption during process operations results in greater impacts on the aforementioned impact categories due to the method of electricity production.

The sensitivity of environmental characteristics to inventory data is important in understanding their robustness (ISO 2006). A ten percent increase in digestate transport distance will increase climate change potential and photochemical oxidant formation of both HRAP and AS scenarios by up to seven percent and ozone depletion by ten percent. The transport distance of waste biomass from wastewater treatment facilities has a significant influence of climate change potential, photochemical oxidant formation and ozone depletion (Pasqualino *et al.* 2009, Fang *et al.* 2016, Arashiro *et al.* 2018). However, transport is not a fixed factor due to site specific needs, and biomass is normally applied close to wastewater treatment plant location (Pasqualino *et al.* 2009). Climate change potential, photochemical oxidant formation and ozone depletion were sensitive to digestate transport distance; however, transport distance is influenced by site specific needs and not by the type of treatment system used.

The results of the sensitivity analysis indicate how sensitive impact categories are to inventory assumptions. Climate change, terrestrial acidification and particulate matter

formation had a sensitivity co-efficient of less than five percent for NH_3 and N_2O air emissions from the land application of digestate. A ten percent increase in ammonia volatilisation will increase terrestrial acidification by 5.6 % for the HRAP scenario. A sensitivity co-efficient below five percent, indicates that the environmental categories are not significantly influenced (slightly sensitive) to inventory data variation (Clavreul *et al.* 2012, Fang *et al.* 2016). These results are consistent with the literature where climate change, terrestrial acidification and particulate matter formation were found to be slightly sensitive to NH_3 and N_2O air emissions from the land application of digestate (Rehl *et al.* 2012, Arashiro *et al.* 2018). The only study to report on the sensitivity of impact categories to air emissions from HRAP found terrestrial acidification to be slightly sensitive to ammonia volatilisation (Garfi *et al.* 2017). Environmental impact categories were only slightly sensitive to three assumptions considered in this study (digestate transport, NH_3 and N_2O air emissions), indicating that the main results are not strongly dependent on the assumptions considered.

The inclusion or exclusion of biogenic carbon dioxide will influence the climate change potential of an effluent treatment plant. Climate change potential will decrease by 16 % for the HRAP and increase by eight percent for the AS scenario if biogenic carbon dioxide is included in the LCA model. Previous authors found that the inclusion of biogenic carbon dioxide emissions from respiration during the AS treatment process increased climate change potential between 10-20 % (Cashman *et al.* 2014, Chai *et al.* 2015, Sharma 2017). To date no LCA studies have documented the influence of biogenic carbon dioxide inclusion in the LCA of HRAP. The inclusion or exclusion of biogenetic carbon dioxide will, however, not influence the overall result when comparing the climate change potential of HRAP and AS systems, with HRAP having a lower impact in both situations.

A Monte Carlo analysis (Figure 5.5) was used to understand the reliability of results in the LCA (ISO 2006, Bernard *et al.* 2014). Ozone depletion was higher in the AS scenario for 91 % of the models but only higher in the HRAP scenario seven percent of the time. For all other impact categories, all generated models with ten percent variation to inventory data had the same outcome as the current model. A confidence level of greater than 95 % indicates that the results of the LCA study are robust (ISO 2006, Bernard *et al.* 2014). The environmental impact comparisons remained the same when ten percent variation was added to all emission data.

Conclusion

The HRAP system had a higher metal depletion potential than the AS system because HRAP require more construction materials due to their larger surface. Normalisation identified freshwater eutrophication, marine eutrophication, human toxicity, freshwater ecotoxicity and marine ecotoxicity as the most significant impact categories for both treatment systems. Land application of digestate and operational energy consumption were the major contributors to the majority of the significant environmental impact categories identified after normalisation. This study found HRAP to have a lower climate change, freshwater and marine ecotoxicity when compared to the AS system, primarily due to its lower operation electrical energy consumption. The HRAP had higher impact for human toxicity due to the greater biomass applied to the land and higher heavy metal concentration in the algae. The higher eutrophication impact of HRAP was largely influenced by the higher nitrite and phosphate concentrations of the treated effluent. Future LCA models should describe the nitrogen species in effluent discharged into the environment as opposed only to stating

total nitrogen when analysing the eutrophication impact of nature based wastewater treatment technologies. It is important to understand how site specific factors will influence the marine eutrophication impact of wastewater treatment systems, especially if they discharge nitrogenous emission into the environment far away from the coastline.

Chapter 6: Discussion

6.1 Quality of treated effluent

High rate algal ponds (HRAP) and activated sludge (AS) technologies are effective at treating post-anaerobically digested (post-AD) brewery effluent to a quality suitable for reuse in non-production activities at the Ibhayi Brewery; e.g. cleaning in process water, boiler feed water and lawn irrigation. Both systems successfully lowered effluent chemical oxygen demand, total nitrogen (TN), total ammonia nitrogen (TAN), nitrite and nitrate concentrations; however, their waste biomass production, environmental footprint, nitrogen removal mechanism, effect on effluent pH and electrical conductivity (EC) differed and need to be taken into consideration when deciding which treatment technology is most suitable for specific situations.

High rate algal ponds increased the neutral pH of post-AD effluent to near ten whereas AS increased the pH to eight. During treatment, photosynthesis in the HRAP consumes carbon dioxide causing bicarbonate to dissociate, resulting in hydroxyl production and an increase in water pH (Tadesse *et al.* 2004). In both treatment systems, the pH of the post-AD effluent increased due to the outgassing of carbon dioxide when exposed to the atmosphere. The consumption of carbon dioxide during photosynthesis further increased effluent pH. The higher pH of HRAP treated effluent renders it less suitable for reuse in agriculture as certain micro-nutrients (iron, magnesium and manganese) become unavailable to plants at a pH above 8.5 (Lucas and Davis 1961, Tyson *et al.* 2007, Taylor *et al.* 2018).

High rate algal ponds increased brewery effluent conductivity by 25 % whereas AS increased the conductivity by 14 %. More evaporation occurred in the HRAP due to their larger surface

area and hydraulic retention time (HRT), which resulted in a greater increase in effluent conductivity, when compared to AS. The high EC (>3000 μS/cm^2) of treated effluents limits their use for the irrigation of agricultural crops due to the negative effect it has on soil salinity, structural fertility and crop growth (Aljaloud *et al.* 1993, Muyen *et al.* 2011, Dakoure *et al.* 2013, Taylor *et al.* 2018). Therefore, AS treated effluent is more suitable for crop irrigation than HRAP effluent due to its lower pH and EC. The As systems will are more suitable for the treatment of high EC wastewater where an increase in EC during treatment is unfavourable.

High rate algal ponds had a higher marine eutrophication impact when compared to the AS system. Emissions to water, from the treated effluent, accounted for over 75 % of this impact, with nitrite and phosphate being the major contributors. Nitrite and phosphate concentrations in treated effluent from HRAP were higher than in AS systems, which resulted in HRAP having a higher marine eutrophication impact. Arashiro *et al.* (2018) reported AS systems to have a higher marine eutrophication than HRAP due to the higher TN and phosphorus concentration in HRAP treated effluent. On the contrary, this study found that HRAP to had higher marine eutrophication impact due to higher concentrations of nitrite and phosphate in the treated effluent and ammonia emissions to the air via ammonia volatilisation. Furthermore, marine eutrophication was found to be the most sensitive to nitrite water emissions. Previous life cycle analysis (LCA) studies have concluded that emission to water, especially nitrite and phosphate, are major contributors to marine eutrophication (Lundie *et al.* 2004, Corominas *et al.* 2013, Morelli *et al.* 2018). It is important that future LCA models on wastewater treatment technologies specify (as detailed as possible) the form of nitrogen being released into water as nitrite and ammonia have a higher impact weight than total nitrogen. When comparing various water treatment

technologies using LCA models the water emissions for both systems must be reported at the same level of detail. Comparing two systems where emissions in one system are specified as total nitrogen while the other is described by ammonia, nitrite and nitrate lead to biased results because of their different impact weighting.

Marine eutrophication was one of the important impact categories that were identified after normalisation. However, the relative importance of this category needs to be understood. Nitrogen and phosphorus containing compounds entering the coastal environment contribute to marine eutrophication with a standard assumption that 7.3 % of nitrogen applied to land, 11 % of NH_3 emitted to the air and 100 % of nitrogen released into freshwater reach the coast (Goedkoop *et al.* 2008, ReCipe 2008, Kitsiou and Karydis 2011). Nitrogen compounds released into freshwater will be stabilised and assimilated by micro-organisms and plants in the freshwater environment and only a portion will enter the marine environment (Destouni *et al.* 2006, Goedkoop *et al.* 2008, Clausen *et al.* 2009). This portion is dependent on many factors, including the distance and time it has to travel, the concentration and volume released, the season, the climate and the type of freshwater ecosystem (Arheimer and Brandt 2000, Conley 2000, Destouni *et al.* 2006). If the treated effluent nitrogen and phosphorus concentrations are within the limits for discharge it can be assumed that they will have minimal impact on the receiving freshwater ecosystems and will be stabilised in the system, thus not reaching the coastal environment (DWAF 1998, Destouni *et al.* 2006). Marine eutrophication was included in this study because treated effluent that was not re-used was discharged into an estuarine environment. Precaution should be taken when literature compares the marine eutrophication impact of wastewater water technologies from different regions and sites/locations relative to the coast. For example, the discharge of treated effluent into bays or fjords (i.e. Saldanha Bay) are more

likely to suffer from eutrophication than exposed coastlines (i.e. east coast of South Africa) due to the sheltered nature of bays which allow nutrients to accumulate over time (Wolanski *et al.* 2019). Future LCA studies should take into account site specific circumstances and should incorporate a sensitivity co-efficient on the relative importance of the marine eutrophication impact category; especially if nitrogen and phosphorus are discharged into freshwater bodies far from the marine environment or within the discharge limits into a natural water resource.

6.2 Construction and operational impacts

Energy consumption during operation has a large influence on the environmental impact of wastewater treatment technologies (Mo and Zhang 2013). The electrical energy consumption was 40-55 % lower for HRAP when compared to the AS. Freshwater and marine ecotoxicity were identified as important impact categories after normalisation, with electrical energy consumption being the major contributor (>65 %) to both categories. Previous LCA studies of wastewater treatment plants (WWTPs) have concluded that technologies with lower operational energy use have less impact on climate change, ozone depletion, freshwater and marine ecotoxicity, photochemical oxidant formation and fossil fuel depletion (Lundie *et al.* 2004, Yıldırım and Topkaya 2012, Garfi *et al.* 2017). Similarly, the HRAP scenario had less of an impact on climate change, photochemical oxidant formation, freshwater and marine ecotoxicity and fossil fuel depletion, and this was primarily due to its lower operational energy consumption when compared to the AS. If these systems were situated where renewable energy was available, they would have minimal impact on freshwater and marine ecotoxicity. However, in South Africa higher

electrical energy consumption results in greater impacts on the aforementioned impact categories due to the method of electricity production (77 % coal generated electricity; Jain and Jain 2017). It is imperative to understand the method of electrical energy generation when conducting a LCA and deciding which technologies to use; as energy consumption will have minimal influence on climate change, photochemical oxidant formation, freshwater and marine ecotoxicity if electricity is generated from a renewable source in comparison to fossil fuel derived electricity.

Materials used for system construction contributed 30 % and 80 % to metal depletion impact for AS and HRAP systems respectively. However, construction materials contributed less than ten percent of all other impact categories. The HRAP treatment system had double the metal depletion impact when compared to the AS scenario. Since HRAP require more construction materials when compared to AS system due to the large area of land they occupy, as they are depth limited, they have a higher metal depletion impact when compared to AS systems (Craggs *et al.* 2011, Arashiro *et al.* 2018).

6.3 Nitrogen transformation, recovery and biomass production

The process by which nitrogen (total ammonia) was removed from brewery effluent differed between the HRAP and AS systems. The AS process released 66 % of incoming nitrogen into the atmosphere and only retained 8.8 % in its biomass, whereas the HRAP released 25 % in a gaseous form and retained 50 % in its biomass. Nitrogen lost to the atmosphere is mainly through; (1) ammonia volatilization when the pH of water is above 9.0 and (2) biological denitrification of nitrate to nitrogen gas under anoxic conditions (Garcia *et al.* 2000, Park and Craggs 2011). The bulk of the nitrogen lost to the atmosphere from the HRAP was via

ammonia volatilisation (due to the high pH >9.0 found in the HRAP), while it was via denitrification in the AS (due to a pH <8.5 and anoxic oxygen concentrations). Atmospheric ammonia aids in the formation fine particulate matters [$(NH_4)_2SO_4$, NH_4HSO_4, and NH_4NO_3] (Renard *et al.* 2004). These molecules are captured by surface waters, atmospheric moisture, and deposited on the soil, contributing to the marine eutrophication, terrestrial acidification and particulate matter formation impacts of HRAP (Renard *et al.* 2004, Pré sustainability 2014, Meng *et al.* 2018). The HRAP recovered a greater portion of nitrogen from effluent into biomass, making it more favourable for nitrogen recovery.

Waste biomass disposal from WWTPs remains one of the most difficult and expensive problems in wastewater treatment (Tchobanoglous *et al.* 2003). In this regard, AS systems are superior due to their lower biomass production. However, if technologies are available to utilise the biomass to produce energy and/or fertiliser then the HRAP system could be superior as the biomass can be used to produce biogas and a greater portion of nitrogen is recovered for reuse as a fertiliser.

6.4 Recovery of carbon in waste biomass

The HRAP treatment system produced 3.8 times more biomass per volume of effluent treated when compared to AS. The majority of the micro-organisms present in AS are heterotrophic bacteria and utilise organic carbon in the wastewater to produce biomass, whereas the autotrophic micro-organisms present in the HRAP are able to utilise inorganic carbon (carbon dioxide) and sunlight to produce biomass (El Ouarghi *et al.* 2003, Tchobanoglous *et al.* 2003, Freeman 2005, Park *et al.* 2011). Therefore, HRAP reduces the anthropomorphic impact on global warming while AS contributes to it.

Brewery effluent grown algal and waste activated sludge (WAS) biomasses are suitable for use as an anaerobic digester feed stock. The specific gas yield of these substrates was similar with an average gas production of 241 ml/g volatile solids (VS) fed. The low C/N ratio (6.5-7.5) allowed the production of ammonia generated alkalinity which was able to maintain a stable liquor pH. A total solids feeding rate of 1-2 $g/l_{reactor}/d$ and a HRT between 15 and 35 days is recommended when operating continuously stirred anaerobic reactors fed sludge or algae from a brewery effluent treatment system. Under these conditions a VS reduction rate of 30-35 % can be expected.

Algae fed anaerobic reactors produced biogas with a significantly higher methane content and lower carbon dioxide content when compared to WAS fed reactors. The carbon dioxide to methane ratio of biogas depends on the oxidation state of the carbon in the substrate, with a positive relationship existing between the reduced carbon concentration of the substrate and methane production (Angelidaki and Sanders 2004). High rate algal ponds produced more biomass per volume of effluent treated than the AS system, with the algae containing a higher hydrogen concentration and a lower oxygen concentration than WAS. This resulted in the HRAP producing more methane per unit of effluent treated when the waste biomass is subject to AD.

6.5 Land application of anaerobically digested algae and sludge

One of the concerns when applying sludge from WWTPs to soil, is the risk of heavy metal and pathogen contamination of the soil and plants. The sludge and algal biomass produced from a brewery effluent treatment process was within the heavy metal limits for use on agricultural land. No *Escherichia coli.* was recorded in the biomass and soil or on the Swiss

chard leaves. Iron was the only metal present at a higher concentration in plants grown on sludge and algae fertilised soils when compared to soils receiving inorganic-fertiliser. Swiss chard has been shown to accumulate iron in its leaves at a higher concentration than cabbage, kale, potato, red beet and cauliflower when irrigated with domestic wastewater (Itana 1998, Itana 2002). However, the leaf iron concentration was still well within the limits for human consumption. Various authors have reported an increase in foliar heavy metal concentration when agricultural soils are fertilised with sludge (Hernandez *et al.* 1991, Morera *et al.* 2002, Bozkurt and Yarilgac 2003, Warman and Termeer 2005, Singh and Argawal 2008). The heavy metal concentration of crops grown on sludge fertilised soils needs to be monitored to ensure they do not become contaminated.

Biosolids have the potential to supplement or replace commercial fertilisers as they contain nitrogen, phosphorous and various micro-nutrients needed to support plant growth (Quilbe *et al.* 2005, Singh and Agrawal 2008). The yield of Swiss chard plants cultivated on soil fertilised with sludge or algae was significantly higher than on soil that had inorganic-fertiliser treatments. The majority of literature reports that agricultural crops fertilised with WAS had a similar yield to plants fertilised with a commercial fertiliser; however, the addition of inorganic phosphorous fertiliser has been shown to further increase crop yield (Chitdeshwari *et al.* 2002, Warman and Termeer 2005, Singh and Argawal 2008). Sludge and algae can be utilised as a inorganic fertiliser replacement when applied to the soil at the same nitrogen loading rate and can even increase crop yield, as is recorded in this study.

No difference was observed in the soil's physical fertility when algae or sludge were applied to the soil. Most literature reports an improvement in the soil's physical properties when sludge is applied to it (Tsadilas *et al.* 1995, Nielson *et al.* 1998, Singh and Argawal 2008,

Diacono and Montemurro 2010). The organic matter added to the soil from biosolids improved the bulk density, porosity and water holding capacity of the soil (Ramulu 2002, Ojeda *et al.* 2003, Diacono and Montemurro 2010). During this study no differences were noticed in the soil's physical characteristics due to the relatively short duration of the trial; however, prolonged application (>two years) should increase the soil's carbon concentration and physical structure as reported in the literature.

Sludge derived organic fertiliser can be advantageous over inorganic fertilisers as a portion of their nitrogen is in an insoluble form and only becomes available to plants after microbiological decay in the soil, thus resulting in less nitrogen being leached into surrounding water bodies (Sanger *et al.* 2011, Walsh *et al.* 2012, Withers *et al.* 2014). Future studies should collect and analyse the leachate from waste sludge fertilised soil to determine the amount of nitrogen lost via leaching, when WAS or algae are used as fertilisers.

Sodium contamination of agricultural soils is the leading cause of rendering soils unsuitable for agriculture (Qadir *et al.* 2003, Muyen *et al.* 2011). This is the first field trial which has reported a significant increase in the sodium concentration and sodium absorption ratio (SAR) of soils fertilised with algae or sludge from WWTPs. The application of sewage sludge to potting soil planted with olive trees, resulted in an increase in soil sodium concentrations, SAR and decreased plant growth (Gasco and Lobo 2006). The effect of sludge applied to soils from WWTPs that treat effluent with a relatively high sodium concentration (>600 mg/l) needs to be documented as its land application could result in an increase the soil's SAR which can be accompanied by a deterioration in the soil's physical fertility (Gasco and Lobo 2006, Muyen *et al.* 2011). Regulations on the application of sludge on agricultural soils

should be altered to consider the limit values for sodium and not only heavy metals (Gasco and Lobo 2006). Future LCAs' on the agricultural land application of WWTP derived solids should incorporate the possibility of soil contamination through sodium build-up.

The HRAP scenario had higher impacts on terrestrial environments, when the waste biomass was applied to the soil, due to the higher heavy metal content of algae and greater biomass production. The HRAP system recovered more heavy metals in the algal biomass and when this biomass is applied to the soil it will have a accumulative effect over time, resulting in higher terrestrial ecotoxicity (Tangsubkul *et al.* 2005, Arashiro *et al.* 2018). In the AS scenario a greater portion of the heavy metals remain in the effluent and are released into the environment, (heavy metal concentration below discharge standards) where they are diluted into the receiving water body. This results in the AS system having a lower ecotoxicity score. This interpretation can be misleading, as from a mass balance perspective, the amount of heavy metals released into the environment is the same for both systems, with a greater portion being applied to the land in the HRAP scenario and discharged into fresh water in the AS scenario. If the waste biomasses from both systems are continuously applied to the same land the HRAP system will have a higher terrestrial ecotoxicity impact (as represented in this scenario), however, if the biomass is applied to various sites this impact will be reduced. If technologies are available to remove the heavy metals from the algal biomass prior to its application to agricultural land this will also reduce the ecotoxicity impacts of the HRAP.

6.6 Conclusion

High rate algal ponds (HRAP) and activated sludge (AS) effluent treatment technologies are effective at treating post-anaerobically digested (AD) brewery effluent for reuse, however

each system has its disadvantages and advantages. The biomass generated from HRAP and AS is suitable for AD, with a positive energy gain when comparing energy recovered from biogas production to energy consumption from anaerobic digester operation. Biogas from algae fed digesters had a higher methane content when compared to sludge fed digesters, due to the higher hydrogen and lower oxygen concentration of algal biomass.

The digestate from sludge or algae fed digesters can be utilised as an inorganic fertiliser replacement when applied to the soil at the same nitrogen loading rate and can even increase crop yield. The fertiliser value of algal and sludge biomass needs to be exploited as it will add value to these waste products; this will aid in decreasing agriculture's dependence on fossil fuel based fertilisers and the associated environmental impacts from its production. No difference was observed in the soil's physical fertility when algae or sludge was applied to the soil; however, an extended trial needs to be done to understand the long-term impacts. The application of sludge or algae on soil increased the soil's sodium concentration and SAR; therefore, regulations on the application of sludge on agricultural soils should consider the limit values for sodium. Future LCA models dealing with the agricultural land application of wastewater treatment plant derived solids needs to incorporate the possibility of soil contamination by sodium.

Life cycle analysis of both treatment systems indicate that electrical consumption, emissions to water and land application of waste biomass cause the major environmental impacts of both treatment systems. High rate algal ponds have a lower freshwater and marine ecotoxicity, climate change effect and fossil fuel depletion due to their lower energy consumption. While AS systems have a lower terrestrial ecotoxicity, marine eutrophication and metal resource depletion due to the smaller biomass produced, lower nitrite

concentration in treated effluent and nitrogen emission to the air as nitrogen gas rather than ammonia. The higher biomass produced from the HRAP results in more fertiliser and energy production (from the AD of the biomass) per volume of treated effluent, and this also resulted in the HRAP system having a higher terrestrial ecotoxicity, according to the LCA. However, the mass of heavy metals released into the environment was the same for both systems with a greater portion being applied to the land in the HRAP scenario and discharged into fresh water in the AS scenario. It is recommended that future LCAs' should take into account site specific situations such as risk of contamination via pollutant build up in an ecosystem (e.g. will the biomass be disposed at one site for an extended period of time or be disposed over multiple sites) and sensitivity of the ecosystem to released pollutants being emitted to water or land. Since HRAP recovered more heavy metals into biomass, they could be more suitable for the treatment of toxic wastewater (acid mine drainage water, and other effluent containing heavy metals) especially in tropical climates where space is not an issue. Life cycle analysis should be utilised as a tool to identify processes which could be addressed to decrease the environmental impact of the studied systems (removal of heavy metals from algal biomass, and decreasing ammonia volatilisation in HRAP).

The results of LCA studies comparing the environmental impacts of various wastewater treatment technologies must be interpreted with caution; where the inventory data used was theoretical or from systems that treat different effluents. Environmental impacts associated with construction, energy utilisation and air emissions were similar between this study and Arashiro *et al.* (2018), who compared a theoretical HRAP system to a real life AS system. The results from LCA that compared HRAP to AS technologies that treated different effluents, in different geographical locations had similar impacts related to energy

consumption, construction and air emissions. On the contrary, environmental impacts caused by emissions to land and water were different as these emissions are influenced by effluent type and geographical location. It is critical that future LCAs' report water emissions as detailed as possible because this had an influence on the eutrophication impacts of the studied systems. This project demonstrated, for the first time, that reporting effluent as total nitrogen does not produce an accurate representation of downstream impacts; as such, nitrogen emissions should be reported by their species concentration (e.g. ammonia, nitrite and nitrate) in future LCA on wastewater treatment systems.

When using a LCA to compare biological effluent plants such as AS and HRAP systems, it is important to model similar biomass disposal options (especially if they have a similar chemical composition) as this will allow for a better comparison of their environmental impacts. A LCA comparing wastewater treatment systems where the biomass from a HRAP system is used for fertiliser production while it is incinerated from another, can have misleading results with regards to the impacts of the treatment system. The marine eutrophication impact category should take into account site specific interactions as emissions close to the marine environment will have a greater impact than a source that is farther away. It is recommended that a marine eutrophication sensitivity co-efficient be included in future LCA models which accounts for the vulnerability of the marine environment to eutrophication.

Both HRAP and AS effluent treatment systems have their advantages and disadvantages which will influence their suitability for certain scenarios. Activated sludge systems are favourable for situations where space is a concern, there are limited options for biomass disposal (biomass not be used in agriculture or AD) and electricity is generated from a

renewable source. Whereas HRAP are more suitable for circumstances where skilled labour is scarce, electricity production (fossil fuel deprived) carries a high environmental impact and where options are available to use the biomass for economic gain such as biogas and fertiliser production.

The concept of a single-use, treatment and discharge system will not be able to address the water resource needs of society in the future. This study demonstrates that HRAP and AS treatment technologies, coupled with AD of their waste biomass and followed by the application of digestate on agricultural lands allows the recovery of nutrients and carbon contained within effluents. The HRAP have a 50 % lower energy consumption and can sequester atmospheric carbon dioxide, resulting in lower climate change, photochemical oxidant formation, freshwater and marine ecotoxicity and fossil fuel depletion impact categories. The major disadvantages of HRAP are their large surface area requirement and raising pH and conductivity of the effluent, rendering it less suitable for irrigation or treatment where increasing effluent conductivity is a major concern. Activated sludge systems produce less waste biomass than HRAP and are more suitable for urban situations where space is limited and transport of waste biomass to agricultural lands is further than 100 km. High rate algal ponds assimilate a greater portion of nitrogen into their biomass, making them more suitable for nitrogen recovery and reuse in agriculture.

Future research needs to focus on the long-term sustainable utilisation of waste biomass from WWTPs as this process has economic potential and reduces the environmental impact associated with current disposal practices. This thesis contributes towards a zero-waste brewery effluent treatment process, where its three major resources that can be exploited:

- carbon content of the primary effluent and waste solids from HRAP and AS has the potential for biogas production;
- the macro- and micro-nutrients assimilated in the solids can be used as an agricultural fertiliser; and
- the water component of the effluent can be used for irrigation purposes or industrial use.

The benefits of developing this energy, nutrient and water resource could contribute to cost-reductions, and more efficient water, nutrient and energy management at breweries and other industries that produce a similar effluent stream.

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