The water and nutrient potential of brewery effluent for hydroponic tomato production

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Abstract

Brewery effluent that had undergone treatment in an anaerobic digester (AD) was used as an alternative water and nutrient source for hydroponic crop production. Brewery effluent was demonstrated to contain sufficient nutrients to support the growth, flowering and fruiting of *Lycopersicum escolentum* "Moneymaker" tomato crops. The adjustment of the effluent pH with phosphoric acid to between pH 6.0 and 6.5 increased the development of the crops by around 100% compared to crops grown in unaltered effluent. The pH adjusted effluent-grown plants grew to a mean height of 831.4 ± 21.1 mm and a dry biomass weight of 42.34 ± 2.76 g compared to the unaltered pH effluent plants which grew to a height of 410.6 ± 20.5 mm and a weight of 7.65 ± 0.68 g after 49 days. Effluent treatment in high-rate algal ponds (HRAP) was determined to have no positive effect on the nutritional potential of the effluent for Moneymaker production. The effluent-grown plants did not perform as well as plants grown in inorganic-fertilizer and municipal water. Plants grown in effluent grew taller but did not produce significantly more fruit when phosphoric acid (height: 1573.3 ± 50.4 mm, 19.4 ± 1.4 fruit per plant) was compared to nitric acid (height: 1254.1 ± 25.4 mm, 15.6 ± 1.5 fruit per plant) as the pH adjustment over 72 days. Direct and secondary plant stresses from effluent alkalinity, ammonium nutrition, nitrogen limitation, sodium concentrations and heat stress among other factors were probably confounding variables in these trials and require further investigation. Considering the raw effluent composition and manipulating the AD operation is a potential opportunity to improve overall AD performance, reduce chemical inputs in the effluent treatment process, reduce the final effluent alkalinity, and increase available nitrogen content in the final effluent. The anaerobic digester discharging >1000 m^3 of nutrient enriched effluent every day is a resource with considerable potential. The benefits of developing this resource can contribute to cost-reduction at the brewery, more efficient water, nutrient and energy management at the brewery, and offer opportunities for job creation and potentially benefit local food security.

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List of abbreviations

AD	Anaerobic digester
AWSS	Algoa water supply system
BOD	Biological oxygen demand
BER	Blossom-end rot
CCI	Chlorophyll concentration index
COD	Chemical oxygen demand
CW	Constructed wetland
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
FOGs	Fats, oils and greases
HFY	Healthy fruit yield
HRAP	High rate algal pond
IAPS	Integrated algal ponding system
LCFA	Long chain fatty acids
NMBM	Nelson Mandela Bay Municipality
NS	Nutrient solution
PFP	Primary facultative pond
PVC	Polyvinylchloride
SAB Ltd	South African Breweries Limited
STP	Standard temperature and pressure
TDS	Total dissolved solids

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Chapter 1: Introduction

1.1. Problem identification

South Africa is a water stressed country (Arnell 2004, WWF-SA 2013). Breweries are a major consumer of water resources and producer of nutrient rich wastewater (Brito *et al*. 2007). Currently this wastewater is an economic and environmental liability as the wastewater requires treatment before it can be discharged into the environment (Fillaudeau *et al*. 2006). This brewery pays to partially treat the effluent on-site before paying the municipality to polish the effluent to discharge standards (Mabuza *pers. comm.* 2012). The entire process is financially and energy expensive, with the post-treatment cost of discharge to the municipality alone costing the brewery R8.87 per kilolitre, with an annual discharge of around 390 000 kilolitres, roughly 65% of bought-in water (Mabuza *pers. comm.* 2012).

To date, no potential uses for brewery wastewater have been demonstrated. In a country facing severe water, energy and food supply challenges coupled with prolific unemployment and widespread pollution of water bodies there is an opportunity to develop a system that utilizes the productive water and nutrient potential available in brewery effluent. There is a need to identify potential alternative water, nutrient and energy resources and to develop the techniques needed to exploit their value. This need is felt in the local area where this project is based, as well as other water scarce, or water stressed, or developing areas around the world. Combining more efficient water-cycle management, with low carbon, pollution mitigating, treatment and beneficiation processes could yield great benefits for the brewing industry, broader society as well as the environment.

This project's main theme was efficiency: outlining a plan for a more resilient, multiple-use water cycle in an ecological design sense, and improving the brewery industry's water efficiency, carbon footprint and energy efficiency (Todd *et al*. 2003). With the brewery

effluent stream being the focus water resource, the principle method of using the water resource was hydroponics and the key knowledge areas were water management, plant nutrition, brewery effluent and anaerobic digestion.

1.2. Review of literature

Water has been identified as one of the key challenges that will define the future of most, if not every society on Earth (Arnell 2004). It has even been suggested that water is "emerging as a bigger crisis for humanity than oil" (Foundation for the Future 2010:1) *. Water in all its forms poses a unique series of fundamental challenges to humans as we strive to secure our own future while softening our impacts on the environment. In summary:

- humans are using water faster than our resources are being replenished (Foundation for the Future 2010);
- we have a severe imbalance in the access to safe water resources both in spatial and temporal distributions and reliability (Foundation for the Future 2010); and
- while depleting certain water resources we are polluting others, rendering them useless and dangerous (Foundation for the Future 2010).

One of the most commonly identified problems is water scarcity; there is too little water of suitable quality to meet the needs of society and the environment, particularly in developing countries (Turton 2000). This, as with many water related challenges, may be the result of any number or combination of complex contributing factors. We are slowly beginning to realise the importance of water in our lives and yet we are failing to take the necessary steps to ensure water security for people and the environment. The plethora of challenges we face as a society (population growth, poverty alleviation, environmental degradation among others) require that we develop and implement strategies and technologies that ensure

* References with page numbers indicate direct quotes from the source.

adequate supplies of water for all water users while minimizing environmental degradation and water resource pollution, particularly in South Africa (Turton 2000, DWA 2012).

Breweries are recognised as a significant global industry with global beer production around 1.8 billion hectolitres (HL) in 2010 (Ascher 2012). There is an average consumption of water between 4-6 litres of water per litre of beer produced (Fillaudeau *et al*. 2006). Thus breweries are an ideal target for the investigation of alternative wastewater management practices to meet the challenges mentioned above.

1.2.1. Local water context

The brewery is located in Port Elizabeth which receives its water from the Algoa Water Supply System (AWSS). A description of the AWSS is provided by the Department of Water Affairs (DWAF 2011: 1):

> "The Algoa Water Supply System (AWSS) consists of an intricate infrastructure system of dams, pipelines and canals linking the surface water resources of the Kouga and Krom rivers and some small local surface water sources in the West, the Orange River via the Fish and Sundays rivers, as well as groundwater resources to supply water to 1.1 million users of the Nelson Mandela Bay Municipality (NMBM) and several smaller towns as well as water for irrigation to the Gamtoos Irrigation Board and other irrigators".

The water in the catchment has to meet the needs of several water users including the NMBM and Port Elizabeth, numerous small towns, a major deciduous fruit industry in the upper catchment and other farming along the Gamtoos River and, importantly, maintain adequate environmental flows. The total water demand for the AWSS in 2009 was 158 million m^3 (DWAF 2011). This total water use was recorded before water use was severely curtailed due to restrictions introduced in 2010 (DWAF 2011). The Ibhayi brewery consumes around 10.5

million m^3 of water per year and discharges about 70% of this as wastewater (Jones *et al*. 2011). Even using the pre-drought peak water consumption in the catchment, the brewery is still a significant water user, responsible for around 7% of the total pre-drought water use.

The Algoa bay water supply exists in an extremely fragile state; the catchment water demands are in balance with water supply, but only if the catchment receives adequate rainfall. There are no safeguards or surplus supplies of water that can be employed in the event of a drought. This situation materialised in 2010 when a prolonged drought led to the implementation of extreme water-use restrictions throughout the region (DWAF 2011). It is important to understand that Nelson Mandela Bay is at risk of a water disaster (Figure 1.1). It must be considered that while struggling to meet current water demands, the region has also been the focus of major industrial investment. Port Elizabeth and the surrounding areas are the major economic driving forces in the province and the area has been identified as one of the key industrial development zones intended to grow the South African economy (DWAF 2004).

The value of water in the Kouga catchment, which serves as a tributary to the Gamtoos and supplies the AWSS, was $R0.74.m^{-3}$ at 2000 prices (Hosking & du Preez 2002). This was based on the cost of not having to transfer water into the catchment to meet water demands saved through water restoration projects (Hosking & du Preez 2002). This value was nearly four times higher than the next most valuable water source considered by the study. The implications of this finding are made clear once one realises that these same water resources are going to have to support major growth in both domestic and industrial consumption. What will the water cost of all the intended development be and where will this water come from? All water users must develop new technologies to maximise the potential of a unit of water. If we are to meet the socio-economic needs of the future we must look beyond a single-use and discharge system.

Figure 1.1 Roadside sign warning of water shortages seen on the N2 near Port Elizabeth.

1.2.2. The brewing process

Beer brewing makes use of three principle ingredients; malted barley, hops and water, followed by fermentation with yeast to convert the sugars to alcohol (Olajire 2012). Some brewers will make use of additional ingredients depending on the desired flavour, chemistry adjustments, style, or specific gravity of the individual brew such as: roasted barley, lactic acid, and/or other brewing adjuncts including maize, as is the case with this particular brewery (Mabuza *pers. comm.* 2012).

The wastes generated from the brewing and packaging processes are either liquid or solid waste (Fillaudeau *et al*. 2006). Solid wastes include spent grains, Keiselguhr sludge, trub, waste labels and packaging materials (Figure 1.2). The liquid fraction of brewery waste is comprised of either brewing or cleaning wastes, which have very different volumes and characteristics.

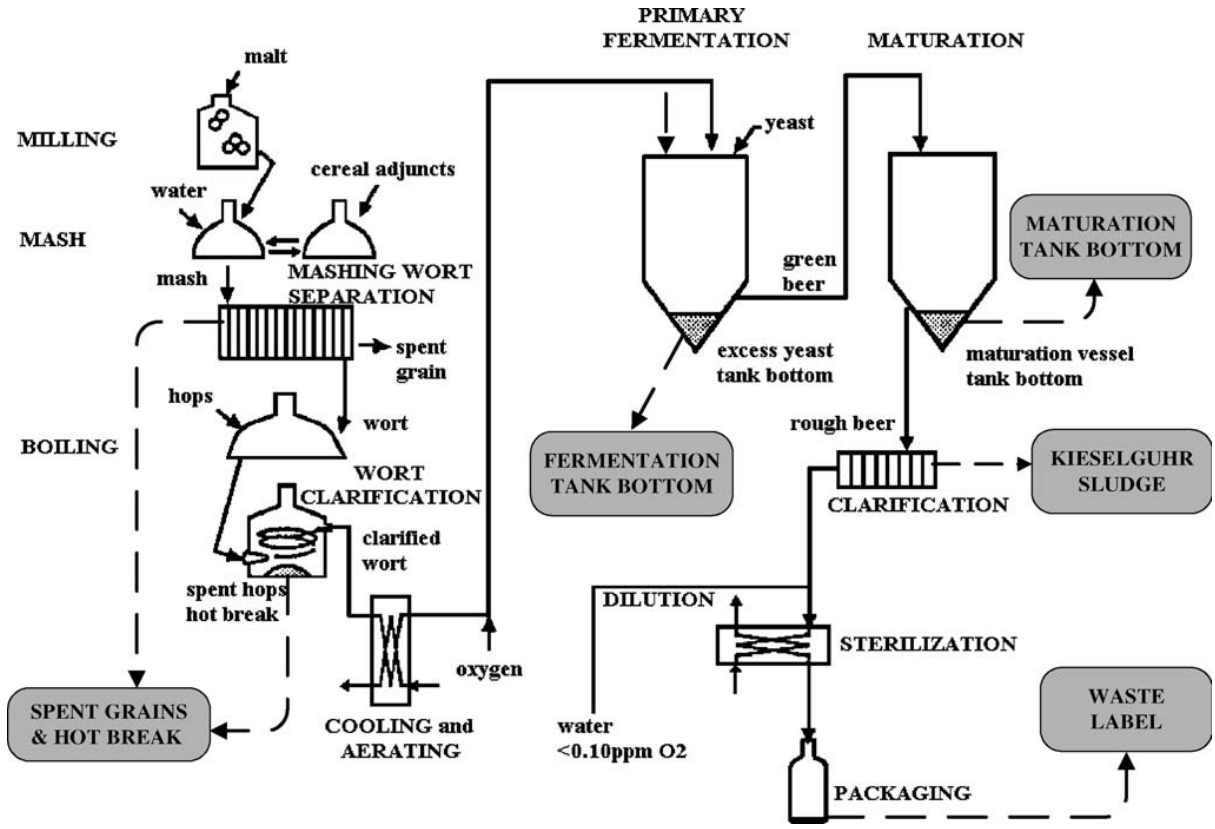

Figure 1.2 The brewing process, products and waste sources (Fillaudeau *et al*. 2006).

Bottle washing is typically a major contributor to wastewater volume but it produces very little organic waste (Simate *et al*. 2011). Brewing waste however, may only account for a small fraction (around 3%) of the total volume of wastewater, but it contributes around 97% of the total organic load in the effluent stream (Simate *et al*. 2011). The organic fraction in the effluent stream typically consist of sugars, soluble starch, ethanol, volatile fatty acids, lipids, etc. (Brito *et al*. 2007, Simate *et al*. 2011). The total chemical oxygen demand (COD) of brewery effluent can vary between 800 and 3500 $mg.L^{-1}$ (Fillaudeau *et al*. 2006). This COD is the main pollutant risk which requires treatment. The COD of the effluent can be portioned into biodegradable and non-biodegradable fractions with a general biodegradable fraction of between 0.6 and 0.7 of the total COD (Figure 1.3) (Ince *et al*. 1998, Olijare *et al*. 2012).

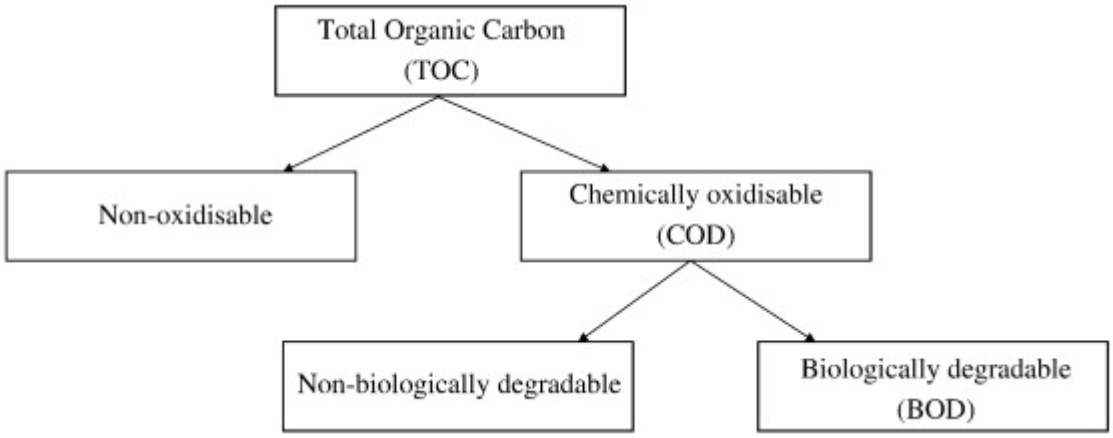

Figure 1.3 The division of total organic carbon, and subsequently total chemical oxygen demand (COD) as biodegradable biological oxygen demand (BOD) or non-biodegradable COD (Simate *et al*. 2011).

This biologically degradable COD can be treated with a variety of technologies, either aerobic, anaerobic or a combination of these. Anaerobic digestion is a widely used technology in many industries which produce effluents with high concentrations of organic, biodegradable waste and the technology is particularly favoured by the brewing industry (Brito *et al*. 2007). The main advantages of anaerobic versus aerobic treatment of organic wastes are; low energy inputs, low sludge volume production, and opportunities for energy generation through methane recovery (Brito *et al*. 2007).

1.2.3. Current water treatment system

The anaerobic digester (AD) currently processes around 2400 m^3 of effluent per day (Talbot & Talbot 2012). The waste stream is divided, with some water undergoing further treatment for on-site reuse, some drawn into the experimental system, and the rest discharged to the municipality (Figure 1.4). The total processing volume of the AD is larger than the net wastewater production from the brewery because some AD treated effluent is recycled back into the feed to help with raw effluent equalization and stabilisation. Of the roughly 700 m^3 of effluent processed by the activated sludge only about 200 m^3 is processed by the activated carbon. The final recovery for reuse is about 15% of the effluent volume with about 1091 m^3

of effluent being discharged to the municipality every day (Talbot & Talbot 2012, Mabuza *pers. comm.* 2012).

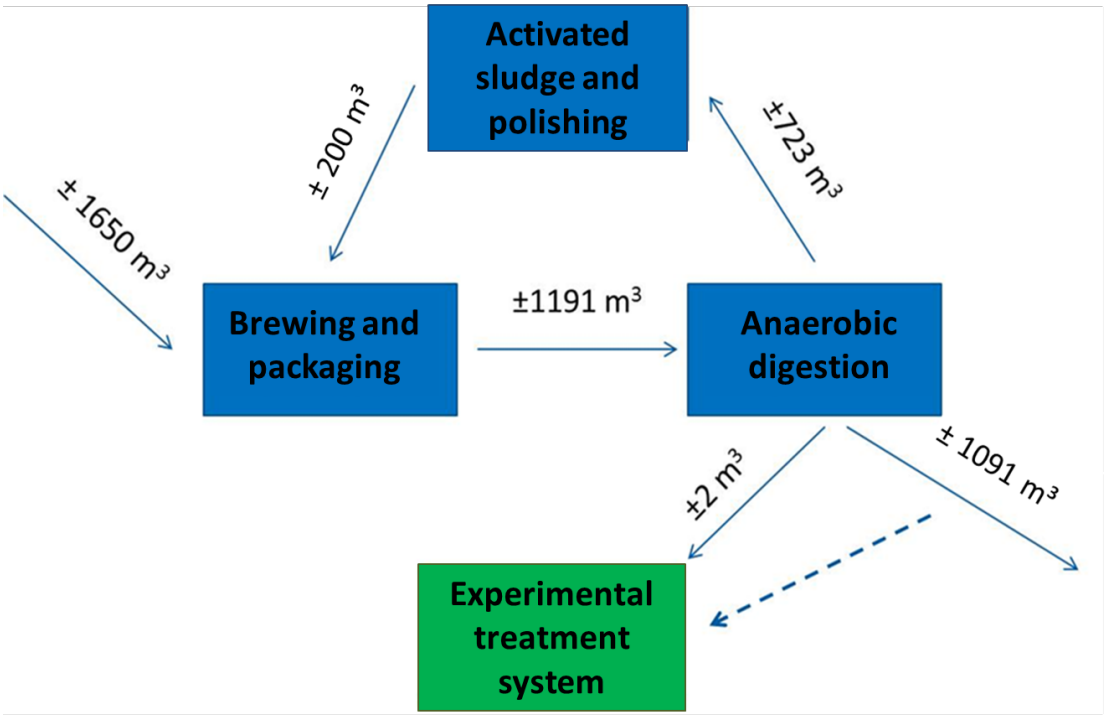

Figure 1.4 Wastewater flows in volumes per day. The dotted line represents the 'available' water that is currently being discharged to the municipality at a cost to the brewery.

The current integrated algal ponding system (IAPS) in operation at the Ibhayi brewery consists of the AD, a primary facultative pond (PFP), two series of high rate algal ponds (HRAP) and a constructed wetland (CW) (Jones *et al*. 2011). Brewery effluent is piped to the water treatment facility located on the brewery site. The effluent is passed through a screen filter which removes solid waste such as glass shards, paper and/or solid brewing waste (hops, grains etc.). The effluent then enters the bottom of the AD tank whereupon a consortium of anaerobic archaea breaks down the complex organic compounds in the effluent (Speece 1983, Angelidaki & Sanders 2004). A portion of the effluent leaving the AD was tapped off the main line and into the alternative treatment facility where it enters the PFP. The PFP acts as a settling pond and secondary anaerobic/anoxic treatment. The effluent was then fed into two pairs of 'raceway' algal ponds set up parallel to one another which are

mixed and aerated by paddlewheels. The effluent spills out of the HRAP system and was fed through two settling cones designed to remove most of the algae suspended in the effluent. The effluent was then fed into a storage tank where it could be released into the constructed wetland for further polishing (Figure 1.5). The post-HRAP effluent has been used for previous hydroponic growth trials (Jones *et al*. 2013).

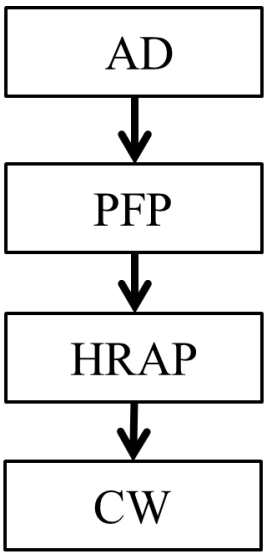

Figure 1.5 The process flow of the experimental treatment system. Effluent flows through the anaerobic digester (AD), the primary facultative pond (PFP), the high-rate algal ponds (HRAP) and finally the constructed wetland (CW).

1.2.4. Anaerobic digestion

Anaerobic digestion process is the key step in the water treatment system. Anaerobic digestion is "a biological conversion process without external electron acceptor such as oxygen as in aerobic processes or nitrate/sulphate as in anoxic processes" (Angelidaki & Sanders 2004). A consortium of chemoheterotrophic, non-methanogenic bacteria and methanogenic bacteria converts the organic carbon in a feedstock into methane, organic carbon in its most reduced state, and carbon dioxide in its most oxidised state (Speece 1983, Angelidaki & Sanders 2004). Anaerobic digestion is not a single reaction but rather a cascade of discrete but dependent or associated processes catalysed by different organisms which can

be divided into two main groups; biochemical reactions and physico-chemical reactions (Batstone *et al*. 2002: 66):

> "(a) *Biochemical reactions*. These are normally catalysed by intra or extracellular enzymes and act on the pool of biologically *available* organic material. Disintegration of composites (such as dead biomass) to particulate constituents and the subsequent enzymatic hydrolysis of these to their soluble monomers are extracellular. Degradation of soluble materials are mediated by organisms intracellularly, resulting in biomass growth and subsequent decay.
>
> (b) *Physico-chemical reactions*. These are not biologically mediated and encompass ion association/dissociation, and gas-liquid transfer. An additional reaction… is precipitation" (Batstone *et al*. 2002: 66).

The hydrolysis of complex organic molecules into smaller products is the first step in the cascade, and often the rate-limiting step (Angelikadi & Sanders 2004). The larger complexes are broken up into smaller products which are subsequently available for uptake and degradation by bacteria (Morgenroth *et al*. 2002). Following the disintegration of the larger molecules, acidogens convert "monosaccharide and amino acids to mixed organic acids, hydrogen and carbon dioxide" (Batstone *et al*. 2002: 66). Acetogenic bacteria convert long chain fatty acids, butyrate and valerate, and propionate into acetate, hydrogen and carbon dioxide (Batstone *et al*. 2002). Acetate and hydrogen are then consumed by aceticlasitc methanogens or hydrogenotrophic methanogens respectively to produce methane and carbon dioxide (Figure 1.6) (Batstone *et al*. 2002).

Various external factors can influence or inhibit the efficiency of the digestion process including temperature, redox conditions, pH, hydrogen, and free ammonia (Batstone *et al*. 2002, Angelikadi & Sanders 2004). The likelihood of a particular factor inhibiting or

encouraging a particular group of organisms depends on the operating environment. Temperature, for instance, has a strong influence on the metabolic rate of the organisms and anaerobic digesters can be operated in three different temperature ranges: psychrophilic (<20 °C), mesophilic (20-40 °C), or thermophilic (45-60 °C) (Angelikadi & Sanders 2004). The digester in this study maintains mesophilic conditions.

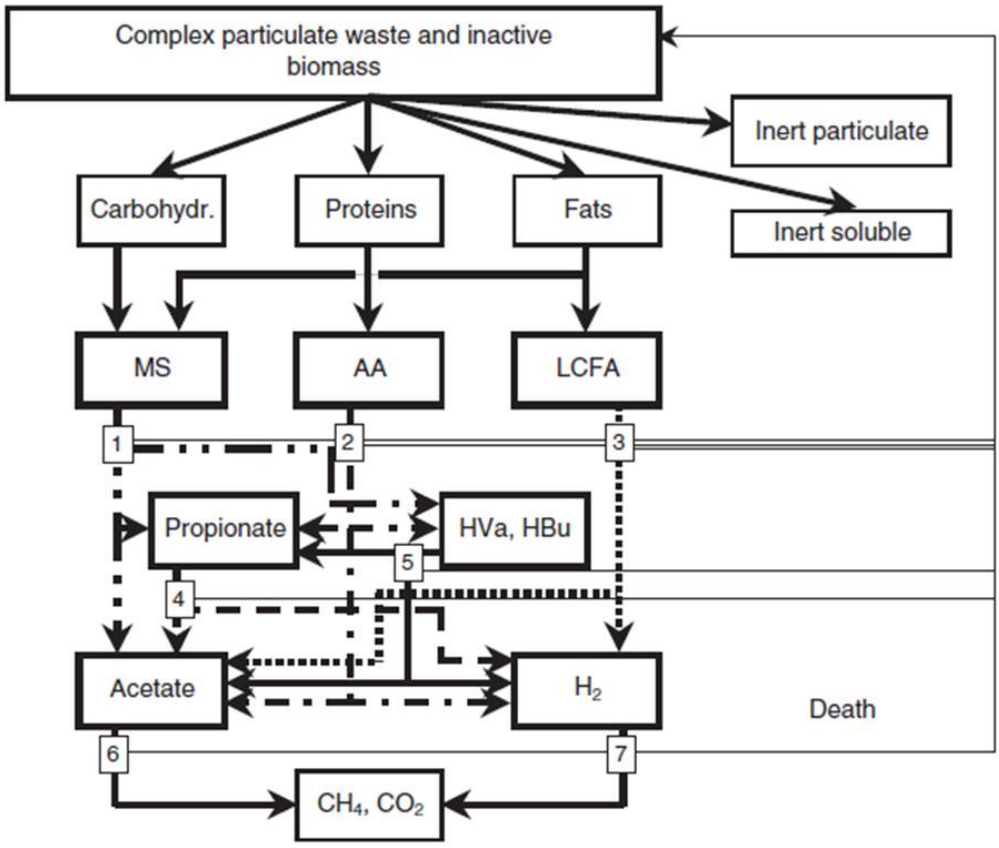

Figure 1.6 A representation of the anaerobic digestion biochemical processes described by the ADM1 (anaerobic digestion model 1): "(1) acidogenesis from monosaccharides (MS), (2) acidogenesis from amino acids (AA), (3) acetogenesis from LCFA (long chain fatty acids), (4) acetogenesis from propionate, (5) acetogenesis from butyrate and valerate, (6) aceticlastic methanogenesis, and (7) hydrogenotrophic methanogenisis" (Batstone *et al*. 2002).

Methane is a widely recognised and used fuel source which catered for about 20% of the energy requirements of the United States in 2001 (Chynoweth *et al*. 2001). The AD process thus combines wastewater treatment with the production of a highly valuable fuel source. The methane potential of brewery waste should be explored further for this particular brewery. It has also been acknowledged and demonstrated that anaerobically digested organic wastes can

provide the essential elements necessary for plant growth (Zhang *et al*. 2007, Jones *et al*. 2011). This demonstrates that there is considerable resource value in anaerobic digesters.

1.2.5. Alternative water treatment technologies

The brewery effluent at the Ibhayi plant has been successfully used to produce hydroponic lettuce as part of an integrated wastewater treatment system (Jones *et al*. 2011). Initial results indicate that the brewery effluent is a highly valuable nutrient source and its application in hydroponic crop production needs to be explored further (Jones *et al*. 2011). The initial development of this project was motivated by the Sustainability Objectives of South African Breweries Limited (South African Breweries (Pty) Ltd. 2012). A key strategy for implementing sustainable industrial practices is developing uses for industrial waste instead of disposing of it (Angenent *et al*. 2004). There is a clear opportunity for the brewery industry to develop technologies that capitalise on the nutrient and water potential available in brewery effluent. Implementation of such technology could have a number of simultaneous effects including; reducing its ecological footprint, reducing water treatment costs, producing valuable goods, generating income from what previously was discarded waste and creating employment opportunities (Todd *et al*. 2003). The water leaving the brewery should not be thought of as an effluent, but rather as a nutrient enriched water supply. The challenge is to determine the most effective strategy for managing this stream.

Wastewater return flows are a common source of irrigation water in South Africa with the importance of return flows predicted to increase in the future (DWAF 2011). Typically, wastewater treatment plants will discharge treated wastewater into a river whereupon it is available for downstream abstraction and use. That is the same principle that should be employed for significant water users in coastal regions, except that the effluent is treated and re-used on-site or locally because discharge generally occurs into the sea resulting in the water being lost. There is a significant amount of attention being directed towards natural

solutions for wastewater management with constructed wetlands receiving much attention (Kivaisi 2001, Todd *et al*. 2003, Konnerup *et al*. 2009, Zurita *et al*. 2009, Melián *et al*. 2010, Calheiros *et al*. 2012). Typically the wetlands aim to serve two purposes; (1) the treatment of wastewater to the chosen standard and (2) the production of a potentially valuable substance such as cut flowers (Konnerup *et al*. 2009, Zurita *et al*. 2009) or harvestable biomass (Kivaisi 2001). A hydroponic system would serve the same fundamental purposes. The plants would produce a harvestable product and simultaneously aid the polishing of the effluent through nutrient removal. The brewery effluent offers a unique opportunity to test the application of a hydroponic growth system as part of the integrated water treatment solution at the brewery.

1.2.6. Effluent characteristics

The water that has been used to irrigate the hydroponic system had already undergone treatment in the AD, PFP and HRAP systems. Samples of this water were collected and sent to a commercial analytical laboratory for analysis on two occasions with the results presented below.

Table 1.1 Post-high rate algal pond (HRAP) effluent analysis 2009-05-20 (BemLab 2009a).

Parameter	Value	Parameter	Value
pH	9.6	Magnesium ($mg.L^{-1}$)	10.9
Sulphate ($mg.L^{-1}$)	61	Phosphorus ($mg.L^{-1}$)	1.32
Conductivity (μS)	2080	Iron ($mg.L^{-1}$)	0.09
Boron ($mg.L^{-1}$)	0.2	Ammonium ($mg.L^{-1}$)	0.33
Sodium ($mg.L^{-1}$)	409.6	Chloride ($mg.L^{-1}$)	388.6
Manganese ($mg.L^{-1}$)	0.01	Nitrate ($mg.L^{-1}$)	0.01
Potassium ($mg.L^{-1}$)	19.1	Carbonate ($mg.L^{-1}$)	691.4
Copper ($mg.L^{-1}$)	0	Fluoride ($mg.L^{-1}$)	0.4
Calcium ($mg.L^{-1}$)	21.2	Bicarbonate ($mg.L^{-1}$)	129.9
Zinc ($mg.L^{-1}$)	0.31	Total dissolved solids ($mg.L^{-1}$)	1561

There are significant fluctuations in the composition and concentration of chemicals in the effluent (Tables 1.1 and 1.2). These variables are dependent on two factors; the incoming effluent from the AD and the performance of the algal ponds. The composition of the

brewery effluent is wholly dependent on the upstream actors i.e., the waste leaving the brewery (waste beer, cleaning water, spent grains, Kieselguhr etc.) and the functioning of the AD, the PFP and the HRAP ponds. All of which can vary significantly over time. While the above information cannot be used to accurately predict what nutrients will be available for hydroponic irrigation, the important factor to consider is that the water chemistry tests have revealed the presence of the essential elements for plant production. At least, the presence of the chemicals available in detectable quantities has been confirmed.

Table 1.2 Post-high rate algal pond (HRAP) effluent analysis 2010-02-19 (BemLab 2010a).

Parameter	Value	Parameter	Value
pH	9.8	Magnesium ($mg.L^{-1}$)	12.0
Sulphate ($mg.L^{-1}$)	22	Phosphorus ($mg.L^{-1}$)	16.6
Conductivity (μS)	3370	Iron ($mg.L^{-1}$)	0.31
Boron ($mg.L^{-1}$)	0.06	Ammonium ($mg.L^{-1}$)	2.46
Sodium ($mg.L^{-1}$)	652.9	Chloride ($mg.L^{-1}$)	375.3
Manganese ($mg.L^{-1}$)	0.04	Nitrate ($mg.L^{-1}$)	0.26
Potassium ($mg.L^{-1}$)	17.5	Carbonate ($mg.L^{-1}$)	1142.3
Copper ($mg.L^{-1}$)	0.01	Fluoride ($mg.L^{-1}$)	1.1
Calcium ($mg.L^{-1}$)	33.8	Bicarbonate ($mg.L^{-1}$)	252.1
Zinc ($mg.L^{-1}$)	0.11	Total dissolved solids ($mg.L^{-1}$)	2510

The concentration of copper recorded in the first test as 0.00 $mg.L^{-1}$ does not exclude the presence of copper (Table 1.2). It could mean copper is not present in sufficient quantities to be reflected by the tests. The low concentrations of copper in the effluent solution are not necessarily problematic as copper is only required in minute quantities by plants. It would be more problematic if copper were present in higher concentrations as it could lead to copper toxicity (Bouazizi *et al*. 2010). Given that plants have already grown successfully in the effluent we can safely assume that copper, along with the other essential micronutrients, is present in sufficient concentrations to enable plant growth (Jones *et al*. 2011). Further discussion on plant nutrient requirements and the role of each nutrient takes place in the following section as part of a review of hydroponic production.

Another influence on effluent chemistry is how algal metabolism affects the nutrient quality of the effluent. The production of algal biomass consumes the same essential elements as plant production and thus the effluent treated in the HRAP system has already supported one 'biomass growth' stage. The effect of algal metabolism is partly revealed in the water quality tests, mainly through the low ammonium, nitrite and nitrate concentrations (Table 1.3). Water quality data for pH, EC, phosphate, phosphorus, ammonia, chloride, nitrite, nitrate and chemical oxygen demand has been recorded on site with the results from the five months between November 2011 and March 2012 presented below.

Table 1.3 On-site post-HRAP water quality data for the period November 2011 – March 2012 (Scheepers *et al*. 2012).

Parameter	Min	Max	Mean
pH	9.35	11.16	9.80
Conductivity (µS)	>3999	>3999	>3999
Phosphate ($mg.L^{-1}$)	19.50	92.00	31.66
Phosphorus ($mg.L^{-1}$)	6.30	30.00	10.41
Ammonium ($mg.L^{-1}$)	0.26	2.95	1.22
Chloride ($mg.L^{-1}$)	970.00	1460.00	1169.67
Nitrite ($mg.L^{-1}$)	0.13	3.12	1.87
Nitrate ($mg.L^{-1}$)	3.10	53.8	19.03
Chemical oxygen demand ($mg.L^{-1}$)	108.00	215.00	150.7

Table 1.4 Post-primary facultative pond (PFP) effluent analysis 2010-02-19 (BemLab 2010a).

Parameter	Value	Parameter	Value
pH	8.20	Magnesium ($mg.L^{-1}$)	10.70
Sulphate ($mg.L^{-1}$)	24	Phosphorus ($mg.L^{-1}$)	6.95
Conductivity (µS)	2880	Iron ($mg.L^{-1}$)	0.49
Boron ($mg.L^{-1}$)	0.06	Ammonium ($mg.L^{-1}$)	24.72
Sodium ($mg.L^{-1}$)	476.63	Chloride ($mg.L^{-1}$)	285.20
Manganese ($mg.L^{-1}$)	0.04	Nitrate ($mg.L^{-1}$)	0.46
Potassium ($mg.L^{-1}$)	12.30	Carbonate ($mg.L^{-1}$)	165.30
Copper ($mg.L^{-1}$)	0.01	Fluoride ($mg.L^{-1}$)	1.20
Calcium ($mg.L^{-1}$)	50.90	Bicarbonate ($mg.L^{-1}$)	1066.60
Zinc ($mg.L^{-1}$)	0.04	TDS ($mg.L^{-1}$)	2150

The same data recording programme as described above was followed for the primary facultative pond. The data shows a very different chemical profile for the effluent before it undergoes treatment in the HRAP system (Table 1.4 and Table 1.5).

Table 1.5 On-site post-primary facultative pond (PFP) water quality data for the period November 2011 – March 2012 (Scheepers *et al*. 2012).

Parameter	Min	Max	Mean
pH	7.96	9.97	8.79
Conductivity (µS)	3372.00	3999.00	3868.33
Phosphate ($mg.L^{-1}$)	32.70	92.00	51.14
Phosphorus ($mg.L^{-1}$)	10.70	30.00	16.50
Ammonium ($mg.L^{-1}$)	2.65	66.00	37.37
Chloride ($mg.L^{-1}$)	580.00	910.00	791.25
Nitrite ($mg.L^{-1}$)	0.25	1.55	0.61
Nitrate ($mg.L^{-1}$)	3.80	22.40	8.41
COD ($mg.L^{-1}$)	93.00	139.00	119.75

The HRAP system influences nutrient form and availability in the effluent. However this may be a positive or negative effect. The algae may act as a buffer against harmful nutrient concentrations but they may also consume vital nutrients which are only available in a limited supply. The effect of the HRAP system was investigated as part of this research by irrigating plants with effluent from different stages of the treatment system.

1.2.7. Hydroponic production review

One potential option for making use of the available water and nutrients in the brewery effluent stream is hydroponic crop production. Hydroponic cultivation has been identified as an important opportunity for income generation or high-value crop or biomass production. Azad *et al.* (2010:1713) describe greenhouses as being "extremely successful in providing abundant, inexpensive, and high-quality produce by using resources (water, minerals, pesticides) with a very high economic efficiency". Hydroponic refers to a growth system that does not rely on soil as a growth medium and nutrient source (Roberto 2005). Instead the plant and its roots are provided with physical support by a neutral growth medium and the

nutrients required for the plant growth are delivered by means of a nutrient solution. Hydroponic systems can be 'open' or 'closed'. In an open system the nutrient solution washes over the roots once and is then discarded while in a closed system the nutrient solution that drains away from the roots is recycled. Closed systems are generally regarded as favourable as they minimize water loss and nutrient waste and reduce the environmental impacts of fertilizer discharge (Savvas & Gizas 2002, Bar-Yosef *et al*. 2009). All plants require a range of nutrients for growth with each nutrient performing a specific function. An advantage of using hydroponics in this scenario is that there is no soil nutrient contribution; plants grown hydroponically are restricted to making use of only the nutrients available in solution which enables an assessment of the effluent nutrient potential.

1.2.8. Plant nutrient requirements

Epstein & Bloom (2004) and Freeman (2005) describe how most vascular plants rely on 17 "essential elements" for life and in order to be defined as essential, each element must be required both for the plant to develop and reproduce normally, and it must fulfil a specific structural or metabolic role (Freeman 2005).

These essential elements are commonly described as either macronutrients (required in relatively large quantities for the production of nucleic acids, proteins, carbohydrates and phospholipids among other important molecules) or micronutrients which typically function as cofactors for specific enzymes and are required in much smaller quantities (Freeman 2005). The seventeen recognised essential elements are carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, potassium, calcium, magnesium, iron, manganese, zinc, copper, boron, molybdenum, chlorine and nickel (Epstein & Bloom 2004). A general guide is presented below with a summary of each nutrient describing the form in which it is available to plants and the characteristics of the nutrient-plant relationship. The information has been drawn from a variety of sources as indicated (Table 1.6).

Table 1.6 Essential elements for plant growth, their function, concentration in dry plant matter, and associated deficiency and toxicity symptoms.

Element	Function	Concentration in dry tissue	Deficiency symptoms	Toxicity symptoms
Carbon (CO_2)	Substrate for photosynthesis; major component of organic molecules (Freeman 2005).	45% (Freeman 2005)	Cell death (starvation) (Freeman 2005)	N/A
Oxygen (O_2, H_2O)	Function: Electron acceptor in cellular respiration; major component of organic compounds (Freeman 2005).	45% (Freeman 2005)	Cell death (suffocation) (Freeman 2005)	N/A
Hydrogen (H_2O)	Major component of organic compounds; electrical balance and establishment of electrochemical gradients (Freeman 2005). Water is also required to maintain turgor pressure in plants (Roberto 2005).	6% (Freeman 2005)	Cell death (dessication) (Freeman 2005)	N/A
Nitrogen (NO_3^-, NH_4^+)	Component of nucleic acids, proteins, hormones and coenzymes (Freeman 2005).	1.5% (Freeman 2005)	Plants will struggle to flourish or grow normally, leaves with signs of chlorosis or yellowing (Freeman 2005, Roberto 2005). Fruit may be "exceptionally well coloured" and older parts of plants may show signs of stress first as nitrogen is translocated to younger, active growing sites (Epstein & Bloom 2004).	Excessive nitrogen can lead to "overly vigorous growth, delayed fruit ripening and plants may also become more susceptible to pests" (Roberto 2005).

Table 1.6 (Continued) Essential elements for plant growth, their function, concentration in dry plant matter, and associated deficiency and toxicity symptoms.

Element	Function	Concentration in dry tissue	Deficiency symptoms	Toxicity symptoms
Potassium (K^+)	Cofactor for many enzymes, necessary for osmotic adjustment in cells and required for synthesis of organic molecules (Freeman 2005).	1% (Freeman 2005)	Chlorosis may appear in leaf margins and necrosis may occur in parts of the leaves. Plants may also appear much darker than normal, sometimes even taking on a blue-green appearance (Epstein & Bloom 2004). Growth is also significantly slower (Epstein & Bloom 2004, Roberto 2005).	Excessive potassium may result in a secondary magnesium deficiency (Roberto 2005).
Copper (Cu^+, Cu^{2+})	Cofactor and activator of enzymes, particularly in photosynthesis and respiration (Freeman 2005, Roberto 2005). Copper is also a component of lignin (Freeman 2005).	0.0006% (Freeman 2005).	The effects of copper deficiency are far more severe in the fruit, seeds and grain of plants than the vegetative growth of the plants (Marschner 1990). Deficiencies can appear in leaves as chlorosis, dark-green colouration or twisted and deformed leaf margins (Epstein & Bloom 2004, Freeman 2005).	Copper toxicity often causes iron deficiency but this can be dependent on the source of iron (Marschner 1990, Roberto 2005). Chlorosis can also appear (Marschner 1990) Excessive copper levels affect the root zone first and can lead to malformation of the root system which makes it important, if copper toxicity is suspected, to analyse both the shoots and the roots of the plant in question (Marschner 1990).
Nickel (Ni^{2+})	Nickel is a component of urease, an enzyme required for nitrogen metabolism (Freeman 2005 and Marschner	0.000005% (Epstein & Bloom 2004).	Nickel deficiency is rare but can present as small cupped leaves or leaf tip necrosis (Marschner 1990, Epstein & Bloom 2004).	Nickel toxicity may lead to zinc or iron deficiency and present as chlorosis (Marschner 1990).

1990).

Table 1.6 (Continued) Essential elements for plant growth, their function, concentration in dry plant matter, and associated deficiency and toxicity symptoms.

Element	Function	Concentration in dry tissue	Deficiency symptoms	Toxicity symptoms
Magnesium (Mg^{2+})	Chlorophyll component; activates many enzymes (Freeman 2005).	0.2% (Freeman 2005)	When a plant experiences a shortage of available magnesium it will transport magnesium already in the plant from the older parts of the plant to the younger, active growing sites (Epstein & Bloom 2004, Roberto 2005). Leaves will present signs of chlorosis and leaves will curl up or prematurely drop off the plant (Freeman 2005, Roberto 2005).	Magnesium toxicity is rare (Roberto 2005).
Phosphorus ($H_2PO_4^-$, HPO_4^{2-})	Used in energetic bonds (ATP); component of nucleic acids, phospholipids, and several coenzymes (Freeman 2005).	0.2% (Freeman 2005).	Plant growth will be stunted and leaves will discolour, appearing dark green with necrotic spots (Epstein & Bloom 2004, Freeman 2005). Fruit production and root growth can also be affected (Roberto 2005).	Excessive phosphorus can lead to copper and zinc deficiencies (Roberto 2005).
Sulphur (SO_4^{2-})	Component of protein coenzymes (Freeman 2005).	0.1% (Freeman 2005)	Sulphur deficiencies can present in a similar ways to nitrogen deficiencies except that the chlorosis caused by sulphur deficiency will present in the younger leaves (Epstein & Bloom 2004, Roberto 2005). Plants may also appear stunted or spindly (Epstein & Bloom 2004, Freeman 2005).	Slow growth and small leaves (Roberto 2005).

Table 1.6 (Continued) Essential elements for plant growth, their function, concentration in dry plant matter, and associated deficiency and toxicity symptoms.

Element	Function	Concentration in dry tissue	Deficiency symptoms	Toxicity symptoms
Chlorine (Cl^-)	Needed for water-splitting step of photosynthesis; functions in water balance and electrical balance (Freeman 2005).	0.01% (Freeman 2005).	Leaf tips can wilt, chlorosis or necrosis of leaves, or leaves will develop a bronze appearance characteristic of chlorine deficiency (Epstein & Bloom 2004, Freeman 2005).	Excessive chloride can cause calcium deficiencies (Freeman 2005).
Iron (Fe^{3+}, Fe^{2+})	Required for chlorophyll synthesis, aids in respiration, formation of coordination complexes (chelates) and the action of iron as a reversible oxidation-reduction reaction system (Marschner 1990, Roberto 2005). Iron serves as an enzyme cofactor and as a component of cytochromes and ferredoxin (Freeman 2005).	0.01% (Freeman 2005).	Iron deficiency will mainly present as chlorosis between the veins of young leaves (Epstein & Bloom 2004, Freeman 2005). Deficiency may also lead to blossom drop (Roberto 2005).	Iron toxicity is rare and hard to identify (Roberto 2005). However, iron toxicity, is "the second most severe yield-limiting factor in wetland rice" (Marschner 1990). It is unlikely that iron levels in the effluent will ever become toxic to the plants but plant tissue analysis will reveal the balance of available iron in each of the experiments and respective plants.

Table 1.6 (Continued) Essential elements for plant growth, their function, concentration in dry plant matter, and associated deficiency and toxicity symptoms.

Element	Function	Concentration in dry tissue	Deficiency symptoms	Toxicity symptoms
Manganese (Mn^{2+})	Required for photosynthetic oxygen evolution, important role in electron transfer and as an enzyme activator (Freeman 2005).	0.005% (Freeman 2005).	Leaves present with chlorosis between the veins and occasionally necrotic spots (Epstein & Bloom 2004 and Freeman 2005). Mn deficiency can also cause failed blooms (Roberto 2005).	Excessive manganese can lead to iron deficiency (Roberto 2005).
Zinc (Zn^{2+})	Zinc is involved in the synthesis of auxin, maintenance of ribosome structure and as a component in enzymes or enzyme activation, function, structure or regulation (Freeman 2005 and Marschner 1990).	0.002% (Freeman 2005).	Abnormal plant growth and small internodes which can cause 'rosette' syndrome or the appearance of successive nodes so close together that the leaves to grow in 'rosettes' (Epstein & Bloom 2004). Leaves may also appear small, distorted and/or chlorotic in some species (Epstein & Bloom 2004, Freeman 2005, Roberto 2005).	Zinc toxicity can cause the inhibition of root elongation, chlorosis in young leaves and restrict iron availability (Marschner 1990, Roberto 2005).
Calcium (Ca^{2+})	Regulatory functions in cells; role in cell wall structure; stabilizes membranes, controls movements; second messenger in signal transduction (Freeman 2005).	0.5% (Freeman 2005).	Calcium deficiency presents mainly as necrosis in the meristematic regions of plants and deformation of young leaves (Epstein & Bloom 2004 and Freeman 2005). Calcium deficiency is severely problematic in fruiting plants and will lead to blossom-end rot or "BER" on the growing fruit (Epstein & Bloom 2004, Roberto 2005). The effects of BER can be exacerbated by high temperatures (Roberto 2005). The roots of the plant will also be severely affected and the damaged roots can also become prone to infection by bacteria and fungi (Epstein & Bloom 2004, Freeman 2005). Nelson & Niedziela (1998) also noted that Ca deficiency in hydroponically grown tulips led to "topple and flower bud abortion".	Excessive calcium symptoms are hard to identify but calcium toxicity is extremely rare (Roberto 2005).

Table 1.6 (Continued) Essential elements for plant growth, their function, concentration in dry plant matter, and associated deficiency and toxicity symptoms.

Element	Function	Concentration in dry tissue	Deficiency symptoms	Toxicity symptoms
Boron ($H_2BO_3^-$)	Boron is required for lignification and xylem differentiation (Marschner 1990). It serves as a cofactor in chlorophyll synthesis, aids in the regulation of enzyme function and has a possible role in sugar transport (Freeman 2005).	0.002% (Freeman 2005).	Boron deficiency can restrict the lateral and longitudinal growth of roots causing them to appear "stubby and bushy" (Marschner 1990). Plant tissues may appear hard, dry and brittle with leaves becoming distorted and stems dry and cracked (Epstein & Bloom 2004). Growing tips may die and leaves may develop black necrotic spots (Epstein & Bloom 2004, Freeman 2005). Flowering is also severely affected and fruit may show similar dry, corky or cracking symptoms similar to those that appear in the stems (Epstein & Bloom 2004).	Boron toxicity may cause chlorosis on leaf margins or tips in mature leaves, often accompanied by necrosis (Marschner 1990, Roberto 2005).
Molybdenum (MoO_4^{2-})	Molybdenum acts as a cofactor in nitrogen reduction and it is essential for nitrogen fixation especially in plants that rely on nitrogen fixing bacterial symbionts (Epstein & Bloom 2004, Freeman 2005).	0.00001% (Freeman 2005).	Common symptoms of deficiency include "whiptail"; necrotic leaf blades disintegrate or do not form normally and only a small strip remains along the leaf midrib (Epstein & Bloom 2004). Chlorosis will also occur in older leaves thus the simultaneous appearance of "whiptail" in younger leaves and chlorosis in older leaves is a strong indicator of molybdenum deficiency (Marschner 1990).	Molybdenum toxicity is noted to affect animals, particularly ruminants, sooner and more severely than plants (Marschner 1990). However, in rare cases, molybdenum toxicity can cause leaves to turn yellow (Roberto 2005).

The nutrient supply in any effluent will always be a complex challenge as the variability in composition and flow of the effluent, as well as the efficiency and effects of the water treatment processes will influence both the nutrient presence and availability of the key elements described above (Table 1.6). The visual stress symptoms can play a useful role in identifying various nutrient stresses (Table 1.6), whether deficiencies or toxicities, in the effluent stream. Combining this information with other assessments will deepen the understanding of the effluent nutrient dynamics and direct the way forward to optimising nutrient management in the effluent stream.

1.2.9. Potential effluent problems and toxicities

The variable concentrations of the nutrients and characteristics of the brewery effluent stream require a thorough understanding of how each variable could potentially affect plant function or nutrient availability in the solution. The symptoms of nutrient deficiencies and general toxicities have already been covered in the review of the essential elements for plant growth. Some potential problems and toxicities that may be present in the effluent can be identified based on the current knowledge of the effluent stream.

The various stages of the integrated treatment system all influence the chemical composition and characteristics of the effluent stream. Microbial activity, algal metabolism, evaporative water loss and the nitrification process all affect the chemical composition of the effluent (Muñoz & Guieysse 2006, Musvoto *et al* 2008, Rawat *et al*. 2011). Some notable changes that occur include the change in the predominant form of available nitrogen, increasing salinity and the consumption of various macro and micronutrients by algae (Jones *et al*. 2011). For example, water in the PFP has high ammonium levels and low nitrate levels while the post-HRAP water has virtually no ammonium and relatively high nitrate concentrations (Tables 1.1 to 1.5). The form of available nitrogen in the nutrient solution has been shown to

influence plant growth (Borgognone *et al*. 2012). The effluent drawn from different stages of the treatment process has a different chemical profile and thus a potentially different nutritional value when employed as a nutrient source for plant growth (Jones *et al.* 2011).

Monitoring the changes in water chemistry in the hydroponic system shed light on the 'appetites' of the plants and therefore aid our understanding of their suitability for use in such a system and the potential limiting aspects of the brewery effluent solution. If plant production is to be incorporated into a wastewater management system, the relationship between the plants and the chemistry of the effluent irrigation solution must be understood with an eye towards effective nutrient and effluent management.

1.2.10. Nutrient availability and pH

Brewery effluent is a nutrient rich water source and not a tailored hydroponic nutrient solution. This raises the crucial question of whether or not the effluent can supply an adequate amount of each one of the seventeen essential elements previously mentioned to support normal plant growth (Freeman 2005). While the previous lettuce trials and the performance of the wetland indicate that the effluent can supply enough nutrients to support plant growth the limits of this nutrient potential need to be explored. It is also important to test whether the effluent in its "normal" state (particularly high pH) is limiting plant growth in any way.

High pH in hydroponic irrigation solutions and the associated effects on nutrient availability may restrict plant productivity (Tyson *et al*. 2007). Tyson *et al*. (2007: 904) state that "precipitation of Fe^{2+}, Mn^{2+}, phosphate (PO_4^{3-}), calcium (Ca^{2+}) and Mg^{2+} to insoluble and unavailable salts can occur in nutrient solution culture at pH levels above 7". This is of particular concern for this project because the post-HRAP water has an average pH of 9.80

(Table 1.3). This high pH could restrict the ability of plants to take up nutrients and thus overall plant productivity and yield may be reduced (Tyson *et al*. 2007).

Bar-Yosef *et al*. (2009) showed that concentrations of P, Ca and Mn in rose leaves decreased as the nutrient solution pH varied between 7.8 and 8.5. The pH of the effluent was not monitored in the previous lettuce growth trials so the effects of pH on the nutrient availability in the effluent are not known (Jones *et al*. 2013). The body of literature on hydroponic crop production places considerable emphasis on the importance of pH in hydroponic systems (Lucas & Davis 1961, Epstein & Bloom 2004, Tyson *et al*. 2007, Zhao *et al*. 2013). According to the accepted theory, if adjustments were made to the pH of the effluent solution, the plants may be able to sequester the nutrients they require more freely from the effluent resulting in better growth performance (Lucas & Davis 1961, Epstein & Bloom 2004). Epstein & Bloom (2004) suggest that the optimum pH range for most plants is between 5.0 and 7.0. Based on the recommended pH for hydroponic systems found in the literature, it is suggested that correcting the pH of the effluent will boost plant productivity (Figure 1.7).

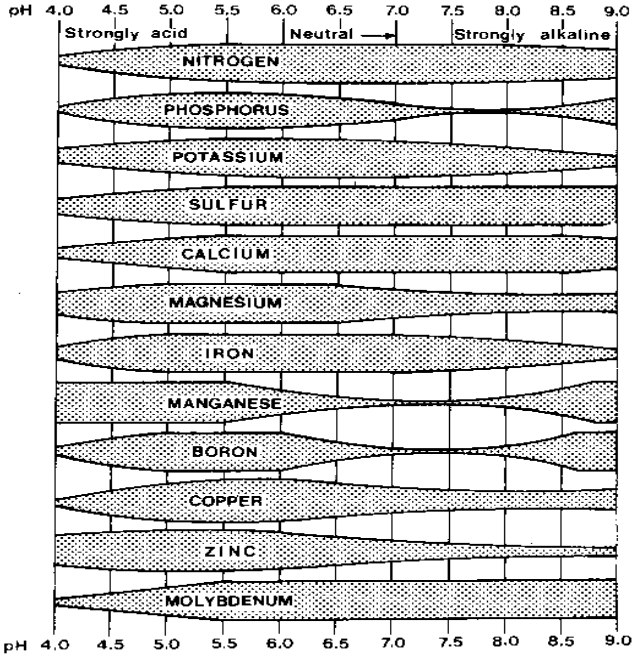

Figure 1.7 The availability of different essential elements as influenced by pH (Lucas & Davis, 1961).

1.2.11. Nutrient concentrations

It is acknowledged that a nutrient that acts as a fertilizer in a given concentration may, as the chemical accumulates in the plant or nutrient solution, begin to inhibit growth of the plant or accumulate in the plant tissue and become toxic to the being that consumes the plant (Marschner 1990, Roberto 2005, Tyson *et al*. 2007, Lastra *et al*. 2009, Bouazizi *et al*. 2010). It is therefore important to monitor the levels of nutrients in the effluent frequently for any fluctuations outside of beneficial concentrations and to react accordingly. Some notable problems identified in the literature which may potentially become problems in this system are discussed below.

1.2.11.1. Ammonium

Ammonium has been cited as an important source of inorganic nitrogen for plants when the supply of ammonium is combined with nitrate (Strojny 1999, Lastra *et al*. 2009). The growth of four varieties of lettuce in four different nutrient solutions containing different ratios of available nitrogen was shown to vary significantly (Lastra *et al*. 2009). The test was designed to determine the most suitable concentration of NO_3^--N and NH_4^+-N in a nutrient solution as measured by growth performance and foliar nitrate concentration (Lastra *et al*. 2009). Two of the lettuce varieties performed the best in the lowest NO_3^--N concentration with no NH_4^+-N supplied while the other two varieties performed better as the concentrations of NO_3^--N and NH_4^+-N increased. Additionally, the roots of two of the varieties showed signs of N toxicity in the solutions with high NO_3^--N and NH_4^+-N concentrations (Lastra *et al*. 2009).

The results of their trials showed that the genetic disposition of the cultivar determines the growth performance of plant and not necessarily the available nitrogen in the solution (Lastra *et al*. 2009). The cultivar specific preference for nutrient concentrations was also observed by Strojny (1999). This is significant for this research in two ways. Firstly, the treated brewery effluent leaving the HRAP system has very little NH_4^+-N, due to the nitrification processes in

the ponds, and lower concentrations of NO_3^--N than the solutions used in the Lastra *et al*. (2009) trial; ±19 mg.L^{-1} NO_3^--N in the effluent versus levels of 100 to 400 mg.L^{-1} N as NO_3^--N and NH_4^+-N in the Lastra *et al*. (2009) trials. Secondly, it means that there will be a plant that possesses the genetic or physiological properties that allow it to outperform others in the effluent based nutrient solution. The treated brewery effluent may not be suitable as a hydroponic solution for all plants. It is a matter of finding the right plant with the appropriate physiological preferences or nutrient requirements that will best suit the brewery effluent. Any observed growth deficiencies in plant trials can be attributed to the unsuitability of the plant as much as the apparent nutrient limitations of the effluent.

1.2.11.2. Nitrite

Nitrite concentrations have been shown to damage the root tips of tobacco plants when concentrations in the nutrient solution exceeded 5.0 mg.L^{-1} (Hamilton & Lowe 1981). Nitrite production occurs as an intermediate product of the nitrification of ammonia-nitrogen (NH_3-N) into nitrate (NO_3^-) (Tyson *et al*. 2007). Nitrification is therefore a fundamental aspect of the water treatment process that is performed by the HRAP system. The highest recorded nitrite level in the post-HRAP water was 3.12 mg.L^{-1} and the average level was 1.87 mg.L^{-1} (Scheepers *et al*. 2012). These are the highest levels recorded in the system. While these levels are within the identified toxicity level for tobacco roots, they may affect other plants differently and more severely. The benefit of a closed hydroponic system in this case would be that if nitrite levels are seen to be rising towards toxic levels, the effluent solution could be discharged into a constructed wetland and fresh solution can be added to the hydroponic system.

1.2.11.3. Nitrate

Foliar nitrate concentration has been identified as a significant source of NO_3^- in human diets and therefore it is a potential complication in crops intended for human consumption (Lastra

et al. 2009). Vegetable consumption can account for between 72% and 94% of nitrate concentrations in the human body (Shen *et al*. 1982, Dich *et al*. 1996). Excessive nitrate intake is believed to contribute to health problems including 'blue baby' syndrome and cancers (Taiz & Zeiger, 1998, Wang *et al*. 2008). The European Commission Legislation limits for nitrate content in greenhouse lettuce are 3500 $mg.kg^{-1}$ for lettuce harvested in summer and 4500 $mg.kg^{-1}$ for lettuce harvested in winter (Lastra *et al*. 2009). The foliar nitrate concentration was related to the growth performance of the lettuce and not the N concentration in the nutrient solution (Lastra *et al*. 2009). Forcing a higher nitrogen load in the irrigation solution may not improve the performance of the crop but it may contribute to foliar nitrate concentrations. Clearly the better suited to a particular nutrient solution a plant is, the greater its productivity. Therefore a suitable plant will efficiently assimilate nutrients from the solution regardless of the concentration of the nutrient solution.

Lettuce from two trials with treated brewery effluent was analysed by an independent analytical laboratory (BemLab, South Africa). The highest nitrate content of the lettuce harvested in winter 2010 was 4.16% of foliar weight (4160 $mg.kg^{-1}$) while the lettuce harvested in summer 2009 showed a maximum foliar nitrate concentration of 3.27% (3270 $mg.kg^{-1}$) (BemLab 2009b, BemLab 2010b). Therefore the effluent has been producing a crop suitable for human consumption according to European legislated limits on foliar nitrate concentration.

1.2.11.4. Salts

The presence of salts in an irrigation fluid has been comprehensively studied for both traditional agriculture and hydroponic production (Savvas *et al*. 2007, Savvas *et al*. 2008, Varlagas *et al*. 2010, Azad *et al*. 2010, Rivelli *et al*. 2010). Salts are particularly concerning in closed-cycle hydroponic systems where the ions that are "sparingly" taken up by plants will accumulate in the nutrient solution (Savvas *et al*. 2007, Varlagas *et al*. 2010). The

accumulation of salts can cause serious problems for the plants as many plants are not well adapted to coping with high levels of salinity.

Grattan & Grieve (1999) reviewed the effects of salinity on mineral availability and uptake in horticultural crops. Salinity induced nutritional disorders with a plant can result from "the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant" (Grattan & Grieve 1999: 127). Because this study aims to test the nutrient potential of the effluent with as few alterations or supplements as possible, the effects of salinity must be thoroughly understood and monitored. Chloride has been shown to reduce nitrate uptake while sodium has been shown to reduce calcium availability and reduce calcium transport within the plant (Grattan & Grieve 1999). Sodium and chloride are both present in the effluent stream and will persist and concentrate as water is lost through evaporation and/or transpiration. Sato *et al*. (2006) found that the quality and taste of tomato fruit improved when grown hydroponically in a salt-treated nutrient solution. The salt levels could be controlled in an effluent-based hydroponic system by establishing a suitable nutrient solution replacement routine and flushing the system when salt levels were determined to be too high.

1.3. Conclusion

This review has described the need for new water management practices particularly in regions prone to water stress such as South Africa. The brewery is a significant consumer of water, most of which leaves the brewery as nutrient enriched wastewater. The value of this wastewater has, until now, largely been overlooked. The brewery effluent has been used successfully to produce hydroponic lettuce but further investigation into its potential as a hydroponic nutrient solution and the effects of hydroponic production on water quality are needed. Recognising the value of industrial effluent instead of adhering to current disposal practices can have major impacts on South African water management, the economy and the

environment. Brewery effluent could hold the potential to provide the nutrients and water necessary to support a productive industry, creating jobs, stimulating the economy and reducing the ecological footprint of the brewery all at the same time. The appropriate application of industrial wastewater towards alternative industries could contribute towards meeting some of the socio-economic goals this country so desperately strives for. This project was based on the application of tested principles in a manner which could see them serve additional purposes, in particular the hydroponic production of valuable crops as a part of an alternative integrated wastewater management system.

The goal of this investigation was not to grow the *ne plus ultra* specimen of a particular plant but to test whether a plant would grow successfully in the brewery effluent. Successful growth can therefore be defined as the absence of fatal or severe nutrient deficiencies, restricted growth or unsuccessful flowering and fruiting. This is a nascent research area and as such the trials were intended to further the understanding of the nutritional potential and limitations of the effluent. Developing an understanding of the relationships and consequences of the brewing and water treatment processes was a key part of this research.

Chapter 2: Experiment 1 - Effluent based hydroponic nutrition

2.1. Introduction

The challenges posed by scarce water resources and the need for development motivate investigations into developing alternative water resources that can contribute to social, economic and environmental sustainability in South Africa (DWA 2012, Chapter 1 - Section 1.2.1.). Breweries produce large volumes of nutrient enriched wastewater that requires treatment before the effluent is disposed of (Fillaudeau *et al*. 2006, Simate *et al*. 2011, Chapter 1). With the current model of effluent treatment at Ibhayi brewery (SAB Ltd) in Port Elizabeth both the nutrient and water resource potential of the effluent is lost. The brewery produces about 390 000 kilolitres of effluent annually (Mabuza *pers. comm.* 2012). The effluent is pretreated by an on-site anaerobic digester before being discharged to the municipal water treatment works for final treatment after which it is discharged into the ocean (Mabuza *pers. comm.* 2012, Chapter 1). This experiment begins to demonstrate the potential value in the brewery effluent system. Hydroponic crop production offered an opportunity to evaluate the productive potential of the effluent in its current form, as well as to assess the effects of effluent manipulation on productive potential.

Brewery effluent has a high organic load which is reduced by treatment in the anaerobic digester (Simate *et al*. 2011). This treatment process breaks down complex organic molecules into mineralized, potentially nutritious compounds including phosphate and ammonia (mainly present as ammonoium) (Speece 1983, Angelidaki & Sanders 2004, Fillaudeau *et al*. 2006, Brito *et al*. 2007, Simate *et al*. 2011, Chapter 1). Combined with the water, these nutrients could hold significant, untapped potential for crop production. Another characteristic of the effluent is its high pH, particularly after treatment in the high-rate algal ponds (HRAP) in the experimental treatment facility, which was observed to be around 9.80 (Scheepers *et* al. 2012,

Chapter 1 - Section 1.2.6.). High pH is known to restrict nutrient bioavailability and reduce plant performance (Lucas & Davis 1961, Tyson *et al*. 2007, Zhao *et al*. 2013). The high pH of the brewery effluent is a potential obstacle in making effective use of the nutrient value of the effluent.

The experiment was designed to test the nutrient potential of the effluent, the effect of the HRAP on the effluent as a nutrient solution, and the effect of reducing the effluent pH on the performance of the plants. The nutritional benefit of the brewery effluent was compared to that of a commercial hydroponic inorganic-fertilizer through physical, visual and chemical analyses of the plants and nutrient solutions. The effect of the algal ponds was assessed by comparing the performance of plants grown in effluent drawn from two points in the treatment system, post-primary facultative pond (PFP) or post-treatment in the HRAP (Chapter 1 - Figure 1.5). The effect of the pH adjustment was assessed by comparing effluent and control solutions to identical nutrient solutions with or without the addition of phosphoric acid to reduce the pH of the solution to between 6.0 and 6.5. This was a baseline assessment as there is no published literature available on the use of brewery effluent as a hydroponic nutrient solution for tomato production.

Aims and objectives

The aim of this research was to identify potentially valuable alternative methods of brewery effluent management. The main focus of this body of work was the application of brewery effluent as a nutrient source and water supply for the production of *Solanum lycopersicon* "Moneymaker*"* tomatoes as a test crop. The investigation also examined the ability of a crop production platform to serve as part of the water treatment/recovery system. This was a practical demonstration of a new approach to water management as endorsed by the Department of Water Affairs which includes identifying uses for wastewater. The broad aim

of this work was to contribute to the sustainability of industrial practices in South Africa with particular focus on energy and water consumption.

The objectives were to:

1. compare the effect of brewery effluent pH on growth performance of plants between unaltered brewery effluent and brewery effluent that has received phosphoric acid as a pH adjustment treatment;
2. compare nutritional potential of brewery effluent as a hydroponic nutrient solution (NS) drawn from different stages of the treatment process (before or after the HRAP) to a commercial hydroponic nutrient solution;
3. evaluate the effect of hydroponic crop production on brewery effluent water chemistry; and
4. evaluate the overall potential for crop production in the brewery effluent.

The experimental programme was designed to address various gaps in the understanding of the potential of the effluent to serve as a nutrient and water source in plant production.

2.2. Methods and materials

2.2.1. Experimental system

The experimental system consisted of 30 identical recirculating hydroponic growing systems each containing five pots (Figures 2.1, 2.2 and 2.3). The system was a variation of the Dutch bucket hydroponic system (Roberto 2005). Each channel was made of one 1500 mm long 160 mm diameter polyvinylchloride (PVC) pipe with a series of 110 mm holes drilled in the top to accommodate the pots (Figure 2.1). Each pot was a 120 mm common plastic garden pot which was extensively perforated with a 5.0 mm drill bit. The pots were filled with rinsed

and dried 10 mm diameter crushed gravel which served as physical support for the plants but provided no nutritional benefit to the plants.

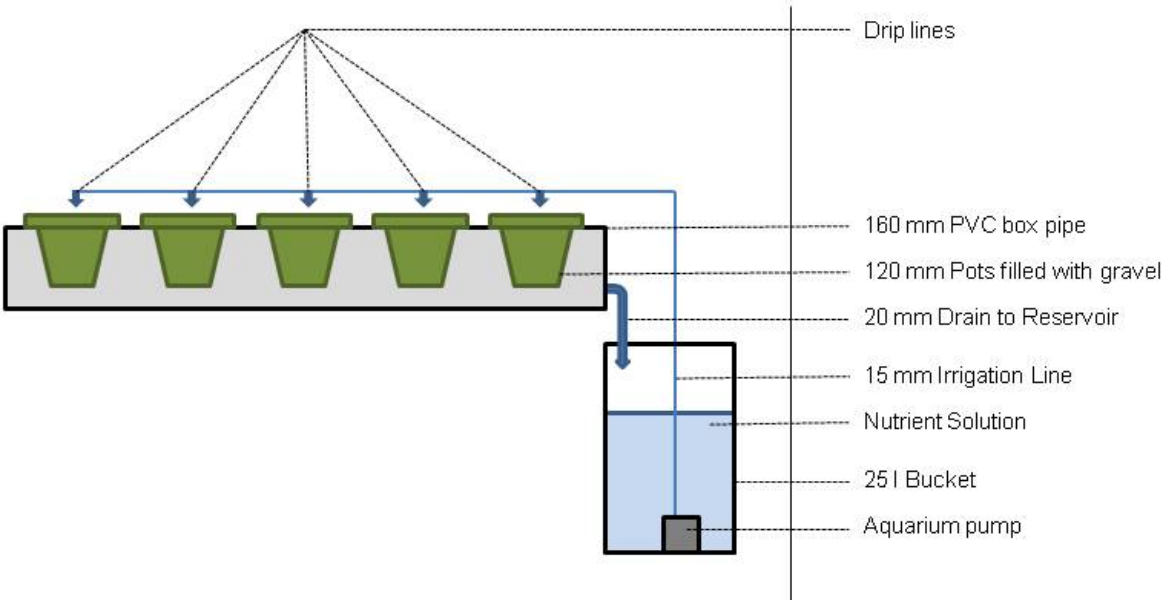

Figure 2.1 Schematic cross-section of an individual growth channel with pots in which plants were grown, nutrient solution sump (drain reservoir), pump and irrigation line (not drawn to scale).

The nutrient solution for each channel was contained in a 30 L plastic bucket stored on the ground at the foot of each channel. An 18 watt submersible aquarium pump (Resun®, Model: SP-2500, China) fed the nutrient solutions up through a 15 mm irrigation line along the length of the channel. A 5.0 mm spaghetti tube, fitted with a micro-valve to ensure even discharge along the pipe, connected the 15 mm main line to each of the gravel filled pots.

A 20 mm drain hole was cut into the end of each channel. A 20 mm PVC pipe was fitted into this hole and extended both into and out of the 160 mm channel. A 20 mm plastic elbow joint was fitted on either end of the 20 mm PVC pipe. The elbow inside the channel was orientated upwards to raise the drainage level of the channels to roughly 50 mm and to create a submerged root zone for the plants (Figure 2.2). The outer elbow was orientated downwards, towards the nutrient solution reservoir. The outer elbow was connected to the reservoir by a 20 mm plastic hose. This created a closed, recirculating system.

Figure 2.2 Views of the completed growth channels including reservoir, pump, irrigation connections, gravel-filled pots, and drain.

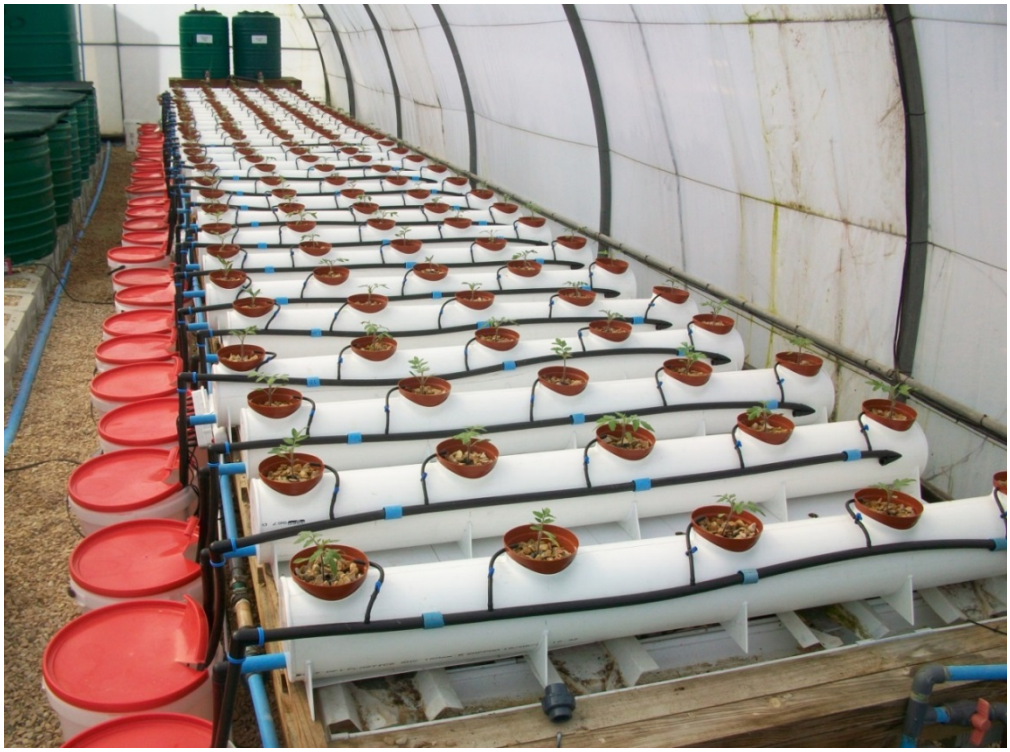

Figure 2.3 The complete 30 channel experimental system with plants at the start of the first trial.

The growth channels were placed across the width of the existing hydroponic table with the drain end of the channels being placed in alternate directions so as to accommodate the buckets on the floor (Figures 2.2 and 2.3).

2.2.2. Plant selection

Three cultivars of tomato seeds (Moneymaker, Heinz 1370 and Rodade, Starke Ayers, South Africa) were germinated in commercially available Jiffy-7 (Jiffy®, Canada) peat pellets. The purpose was to determine whether tomato plants would grow in the brewery effluent before embarking on the full scale trial. The plants were grown in a custom variant of the Dutch bucket system made out of a single 60 L container with all the plants grown in the same container and irrigated with unaltered post-HRAP effluent (Figures 2.4 and 2.5).

After the pre-trial growth test Moneymaker was chosen as the trial cultivar as it is widely available variety with relatively rapid growth rates and no specific or exceptional resistances or stress-tolerance and was the first cultivar to produce fruit (Figure 2.5).

Figure 2.4: The pre-trial growth test with tomato cultivars Rodade, Moneymaker and Heinz 1370 plants in order from left to right.

Figure 2.5: The pre-trial growth trial confirmed that the tomato plants could grow, flower and fruit successfully enough for trial purposes. This is a small Moneymaker fruit.

Moneymaker tomato plants were germinated from commercially available seed. At least two seeds were sown in the 36 mm diameter, peat pellets (Jiffy-7, Jiffy®, Canada) with at least 400 seeds sown across 200 pellets. The pellets were distributed evenly between three miniature plastic greenhouses (Jiffy®, JiffyPro 70 Self-Watering Greenhouse, Canada). The pellets were soaked in municipal water before the seeds were sown. Two weeks after sowing, the smaller germinating seedling was cut to allow the larger seedling to thrive. Four weeks after germination the seedlings were transplanted into the 12 cm pots and surrounded with gravel. Thirty plants were randomly selected and analysed to determine the mean starting root mass and, stem height and diameter, and stem and leaf mass. The remaining plants were randomly allocated to the various treatments and exposed to their particular nutrient solutions for the first time.

2.2.3. Plant productivity monitoring

Plant height and stem diameter were recorded at the start of the trial and once a week for 49 days. Plant height was measured to the nearest millimetre on the tallest unsupported stem.

Basal stem diameter was measured with callipers once a week and when the plants were harvested at the end of the trial.

Nutrient deficiency or toxicity symptoms described in the literature review were identified and recorded. Supplementary information including the date the symptom appeared and which areas of the plant were affected was recorded. Photographs were taken of suspected stress symptoms which were used to gather secondary opinions from botanists or other experts, if necessary.

Plant biomass was separated at the end of the growing period into 'aboveground' biomass and roots. Fruit was harvested and counted with damaged fruit identified and recorded. The samples were weighed then oven dried at 80 °C for 72 hours to determine dry weights of the plants (Borgognone *et al*. 2012). All weights were determined using a four digit analytical balance (AS 220.R2 analytical balance, Radwag, Poland). Due to limited drying space and time constraints it was only possible to determine the dry root weights of four replicates from each treatment. All the root and shoot samples were dried and analysed.

The total number of fruit produced per plant was recorded. Fruit specific deficiencies and toxicities such as blossom-end rot were recorded as they occurred. A proportion of healthy fruit produced compared to the total number of fruit was determined. This ratio was compared between individual systems or separate treatments. All weights were determined using the four digit analytical balance.

The chlorophyll concentration index (CCI) of the leaves of the plants was recorded with a chlorophyll content meter (CCM-200 Plus Chlorophyll Content Meter, Opti-Sciences Inc., USA). Samples were recorded on one of the uppermost fully expanded leaves of each plant. One reading was taken on each plant in the trial.

Samples from fruit and leaf tissues were sent to a commercial analytical lab and analysed for their nitrogen, phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, copper, zinc and boron contents (BemLab (Pty) Ltd., Strand, South Africa).

2.2.4. Experimental treatments

A multi-factor experiment was designed to determine if plant growth was influenced by different nutrient sources (i.e. conventional inorganic-fertilizer, post-PFP and post-HRAP; factor 1), with and without pH adjustment (factor 2; Table 2.2). The treatments were each replicated five times.

Table 2.1 The 6 treatments (i.e. T1 to T6) that formed a multi-factor experiment where three different nutrient solutions (factor 1) were tested with and without pH adjustment (factor 2).

Nutrient Solution	pH Not Adjusted	pH Adjusted to 6
Commercial NS	T1	T4
Post-PFP Effluent	T2	T5
Post-HRAP effluent	T3	T6

The following hypotheses were tested:

1: H_0= Plant productivity is not influenced by an interaction between nutrient source, either with or without pH adjustment.

2: H_0= Plants grown in brewery effluent at different pH values will have the same productivity.

3: H_0= Plants grown in effluent with different forms of available nitrogen will have similar growth performance.

2.2.5. Water chemistry

Water quality was monitored in all the trials to determine the plant's consumption of nutrients and the ability of a hydroponic system to serve as a water treatment. Water samples were

collected from each batch of fresh nutrient solution and the replaced solution prior to the replacement of solutions in the treatments. These samples were taken each time the NS was replaced. Each sample was tested for EC, pH, NO_3^-, NO_3^--N, NH_4^+, NH_4^+-N, PO_4, PO_4-P, NO_2^-, NO_2^--N concentrations, and chemical oxygen demand (COD). Samples were analysed using a spectrophotometer (Merck Spectroquant Pharo 100 spectrophotometer, product number 100706, Darmstadt, Germany).

Each sample was filtered through an eight µm paper filter prior to analysis. Water quality analyses were conducted using the standard reagents and methods supplied by Merck (Pty) Ltd. for each of the selected parameters:

- High-range ammonia cell tests (product: 1.14559.0001)
- Low-range ammonium test (product: 1.14752.0001)
- Nitrite test (product: 1.14776.0001)
- Nitrate test (product: 1.09713.0001)
- Phosphate test (product: 1.14842.0001)
- Chemical oxygen demand cell test (product: 1.14895.0001)

It was not possible to determine dilution ratios for each of the parameters and each of the treatments when the tested parameter exceeded the range of the test because of the time required for each test and the number of samples that required testing. Values that were above the maximum range of the test were recorded as the maximum as shown in the results section.

Electrical conductivity (EC) and pH were measured in each of the treatment reservoirs with the fresh solutions. The readings were measured with a pH/EC/TDS probe (Hanna, HI 991300, United Kingdom).

The average pH values were calculated by converting the pH readings to H^+ concentrations with the formula below where x is the recorded pH value.

$$H^+ = 10^{-x}$$

The H^+ concentration values were then averaged for each treatment and the mean H^+ value was converted back to a pH value using the following formula:

$$pH = -\log(H^+)$$

2.2.6. Nutrient solution irrigation regime

The solutions used in the treatments were municipal water with added commercial hydroponic nutrients, post-PFP water and post-HRAP water. The nutrient solution was replaced every seven to ten days depending on the plants' consumption of the solution. The intention was to expose the plants to the solutions for as long as possible in order to maximise the nutrient removal. The solutions were replaced when the liquid level had dropped to less than a quarter of the volume of the reservoir or every ten days, whichever was soonest. Each reservoir contained 20 L of solution so each fully replicated treatment in the experiment required 80 L of irrigation solution. The pH of selected treatments was corrected to between 6.0 and 6.5 using 80% phosphoric acid (Protea Chemicals (Pty) Ltd., South Africa). The NS was replaced according to the schedule of the trial and treatment with the intention of maximising both nutrient supply and removal.

The control solution was comprised of commercially available Hygrotech® hydroponic inorganic-fertilizer (Registration number K5709; Act 36 of 1947), and calcium nitrate with a composition of 11.7% nitrogen and 16.6% calcium, mixed in a ratio of 1:0.8 and dissolved in municipal water to achieve an EC of 2000 µm (Table, Hygrotech (Pty) Ltd., South Africa).

Table 2.2 The elemental composition of the Hygrotech® hydroponic fertilizer used in the control treatments (Hygrotech (Pty) Ltd., South Africa).

Composition in %							Composition in $mg.kg^{-1}$					
N	P	K	Ca	Mg	Na	S	Fe	Mn	Zn	Cu	B	Mo
6.8	4.2	20.8	-	3	-	6.4	1254	299	149	22	373	37

2.2.7. Statistical analysis

Raw data were tested for homogeneity of variance (Levene's test; $p<0.05$) and normality of the residuals (Shapiro-Wilk W-test; $p<0.05$); if the data did not meet these assumptions these tests were carried out on the log-transformed data. A multi-factor analysis of variance (multi-factor ANOVA) of the growth indices mentioned above was used to establish if there were interactions between factors (factors: nutrient solution and pH regime), at $p<0.05$. If there were no interactions, each factor was analysed separately with a one-way analysis of variance (ANOVA) and Tukeys multiple range analysis were used to compare means for each factor or treatment, at $p<0.05$. If the data did not meet the assumptions of an ANOVA (Levene's test; $p<0.05$ and Shapiro-Wilk W-tests; $p<0.05$) after being transformed then the treatments were compared using a non-parametric Kruskal-Wallis ANOVA, $p<0.05$. A repeated measures ANOVA was used to compare data collected over the course of the trial ($p<0.05$). All statistical analyses used Statistica 64 version 11 (StatSoft Inc., Tulsa, United States of America).

2.3. Results

2.3.1. Water chemistry

The electrical conductivity (EC) of the treatments varied according to each nutrient solution (Figure 2.6).

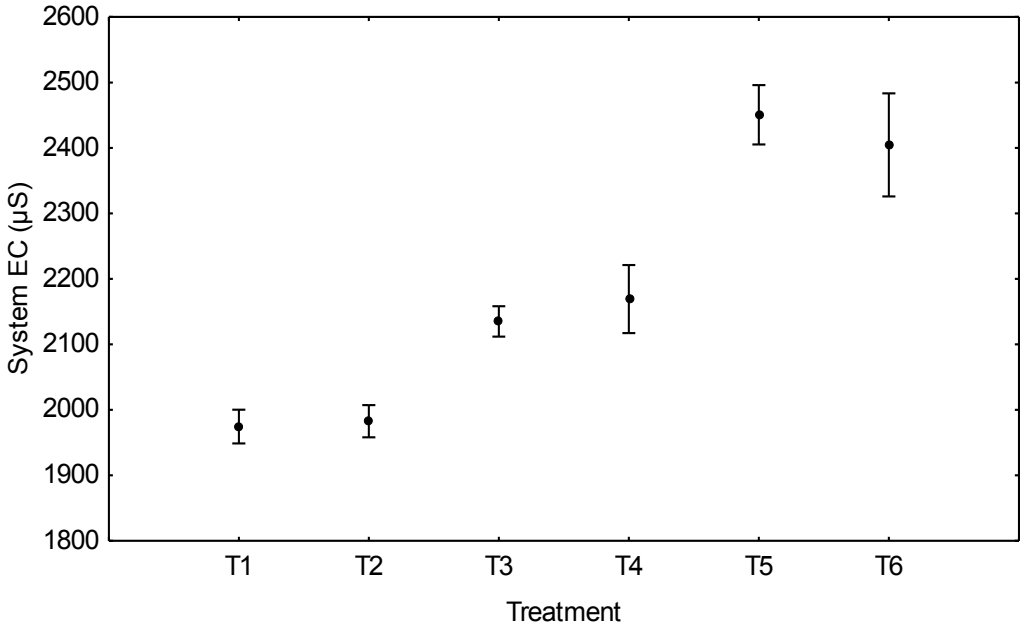

Figure 2.6 The mean electrical conductivity (EC) levels (± standard error) at the start of each solution replacement cycle. The treatments are indicated as follows: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

The EC was highest in the post-HRAP effluent and post-PFP effluent was consistent with EC levels only slightly higher (between 2100 and 2200 µS) than those of the inorganic-fertilizer systems. The HRAP treatment produced effluent with higher EC readings that those of the PFP effluent with average readings above 2400 µS (Figure 2.6). The EC remained constant within each treatment over the course of the experiment.

The pH levels of the irrigation solutions differed as was the intention of the experiment (Table 2.3). The pH corrected treatments (T2, T4 and T6) had similar corrected mean pH values within the target range of 5.8 to 6.5. The alkalinity of the uncorrected effluent was apparent with the T3 pH ranging from 8.71 to 9.43 with a mean pH of 8.91. The T5 pH ranged from 8.33 to 9.86 with a mean pH of 9.35 (Table 2.3).

Table 2.3 The mean, maximum and minimum pH values for the individual treatments. The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

Nutrient solution		pH adjusted	Mean	Max	Min
T1	Control	No	7.38	8.05	6.90
T2	Control	Yes	5.97	6.98	5.50
T3	Post-PFP	No	8.91	9.43	8.71
T4	Post-PFP	Yes	6.16	7.03	5.71
T5	Post-HRAP	No	9.35	9.86	8.33
T6	Post-HRAP	Yes	6.16	6.98	5.65

The brewery effluent, both post-PFP and post-HRAP, required ten times the volume (around 25 mL) of 80% phosphoric acid to lower the pH to between 6.0 and 6.5 compared to the municipal water (2.5-3.0 mL) for each 25 L of replacement solution.

The post-PFP effluent contained the highest levels of ammonium-nitrogen and the post-HRAP treatments contained the lowest (Figure 2.7). The two highest NH_4^+-N recording samples for T4 from irrigation solutions just prior to being replaced, were the first samples taken from the first solution replacement cycle (Figure 2.7 B). The T3 systems did not achieve the same level of nitrification and/or ammonium uptake by the plants compared to the T4 systems.

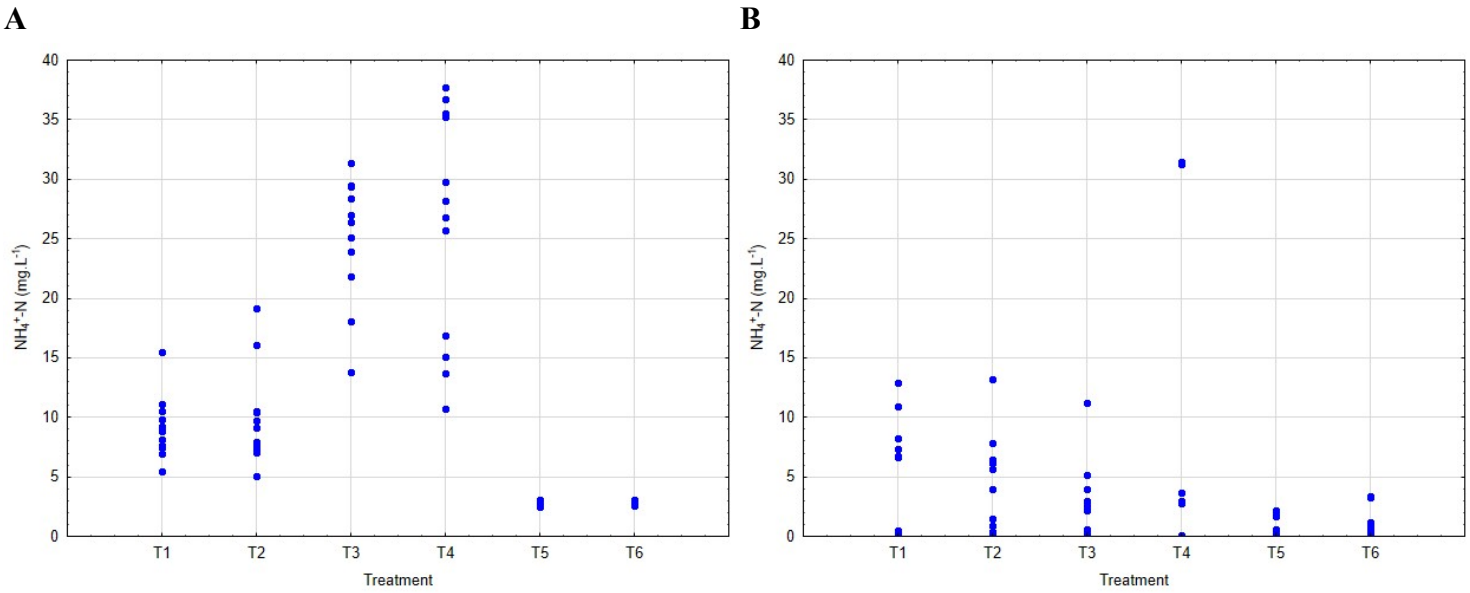

Figure 2.7 Ammonium-nitrogen (NH_4^+-N mg.L^{-1}) levels from fresh samples (A) and samples from irrigation solutions just prior to being replaced (B) (n=12 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

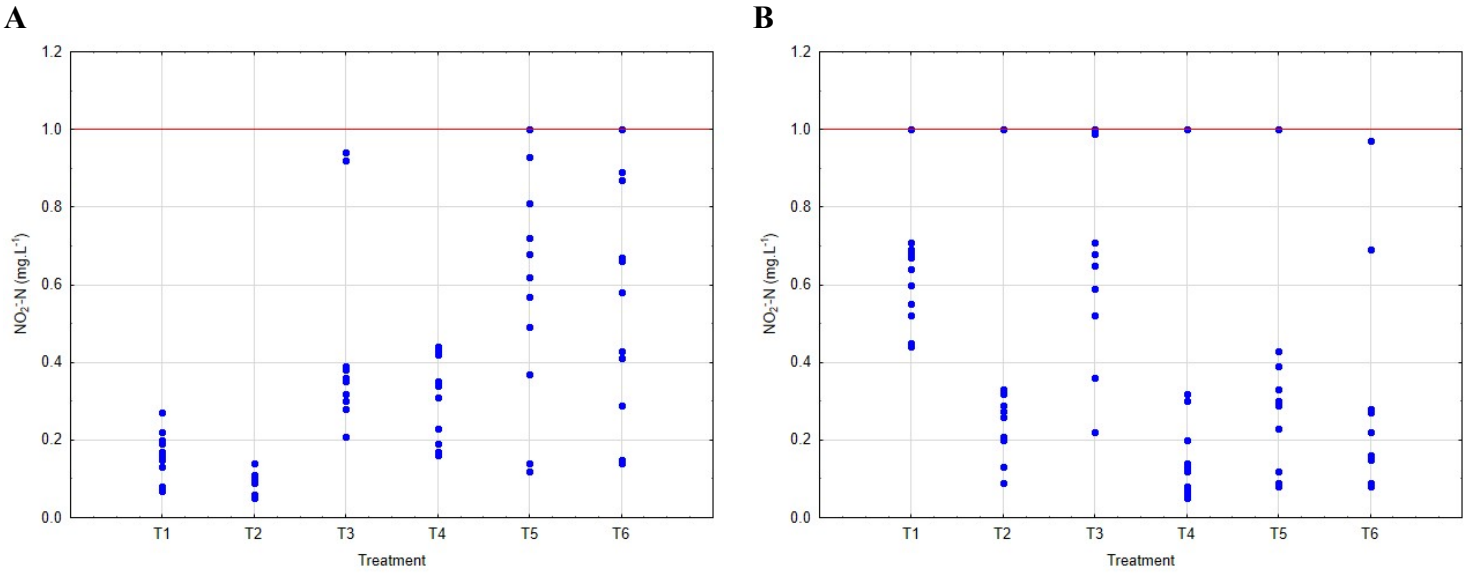

Figure 2.8 Nitrite-nitrogen (NO_2^--N mg.L^{-1}) levels from fresh samples (A) and samples from irrigation solutions just prior to being replaced (B) (n=12 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid. Red lines on graphs indicate the upper concentration limit of the test.

Nitrite-nitrogen values appeared to increase in treatments T1, T2 and T3. They remained low and appeared to decline in T4, while in T5 and T6 the nitrate-nitrogen values consistently decreased (Figure 2.8).

The inorganic-fertilizer systems (T1 and T2) contained abundant nitrate-nitrogen throughout the trial (Figure 2.9). Nitrate-nitrogen levels increased in T3, remained constant in T4 and T5 and decreased in T6. The plants in each nutrient solution were exposed to different ratios of available nitrogen as ammonium-nitrogen or nitrate-nitrogen (Figure 2.7 and 2.9). The plants in the post-PFP treatments (T3 and T4) were predominantly exposed to ammonium while plants in the other treatments were mainly exposed to nitrate (T1, T2, T5 and T6).

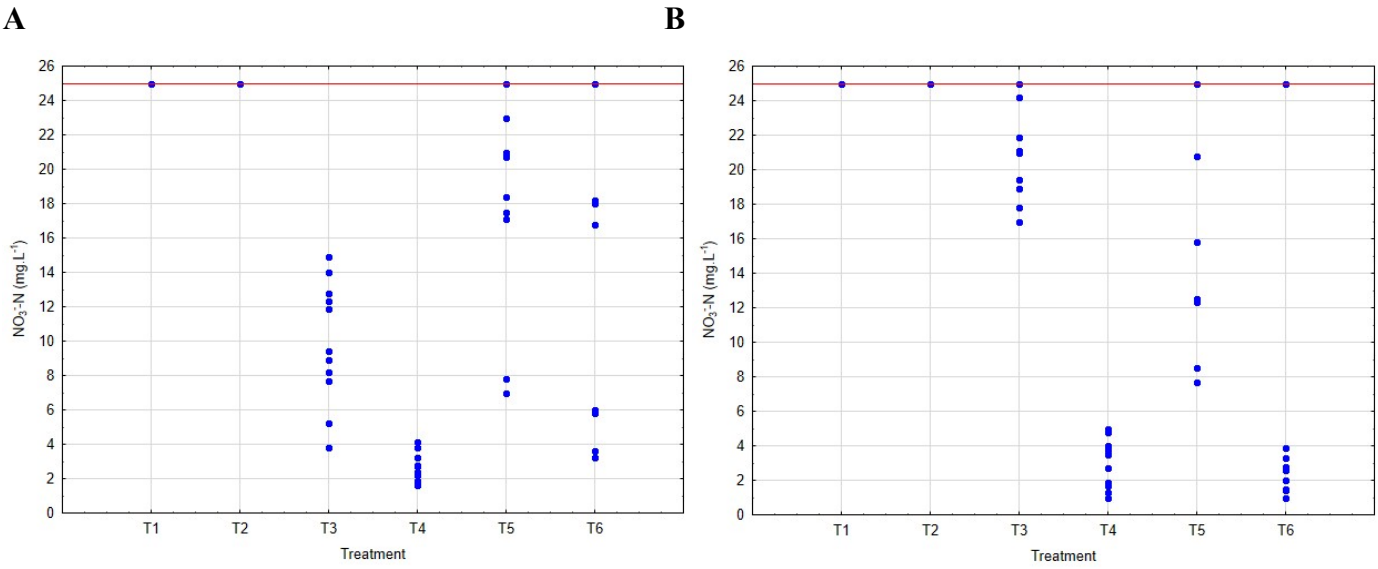

Figure 2.9 Nitrate-nitrogen (NO_3^--N $mg.L^{-1}$) levels from fresh samples (A) and samples from irrigation solutions just prior to being replaced (B) (n=12 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid. Red lines on graphs indicate the upper concentration limit of the test.

Phosphate-phosphorus levels were only reduced in T1. The addition of phosphoric acid in T2, T4 and T6 resulted in off the scale phosphate concentrations throughout the trial. T3 and T5 phosphate-phosphorus levels tended to increase (Figure 2.10).

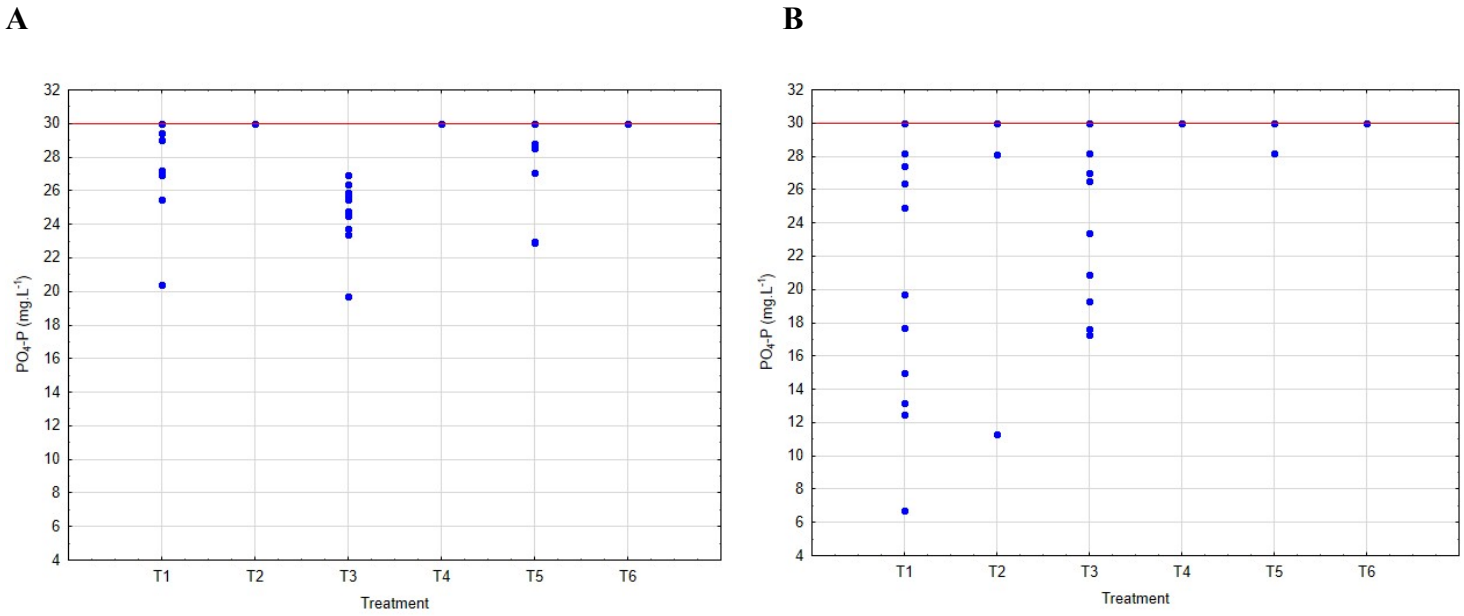

Figure 2.10 Phosphate-phosphorus (PO_4-P mg.L^{-1}) levels from fresh samples (A) and samples from irrigation solutions just prior to being replaced (B) (n=12 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid. Red lines on graphs indicate the upper concentration limit of the test.

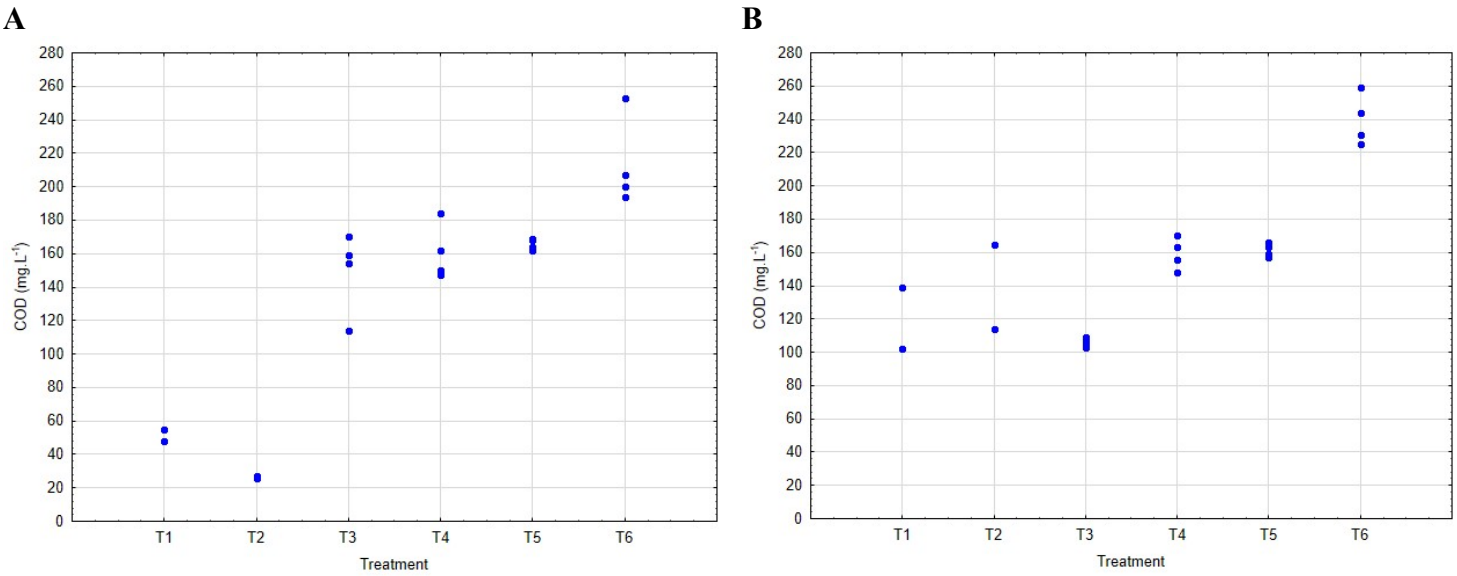

Figure 2.11 Filtered chemical oxygen demand (COD) (mg.L^{-1}) levels from fresh samples (A) and samples from irrigation solutions just prior to being replaced (B) (n=12 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid (n=2 for T1 and T2, and n=4 for T3 through T6).

There was no reduction in effluent COD in any of the treatments. The municipal systems (T1 and T2) showed an increase in COD levels (Figure 2.11).

2.3.2. Physical development of the plants

Basal stem diameter development was influenced by a significant interaction between nutrient solution and pH adjustment (Multifactor ANOVA, $F_{(2,24)}$=10.59, p=0.0005; Figure 2.12). The inorganic-fertilizer control treatments had similar basal stem diameters to one another and significantly larger basal stem diameters than the effluent treatments, irrespective of whether acid was added or not (T1: 11.2 ± 0.2 mm, T2: 11.3 ± 0.4 mm; Figure 2.12). The pH adjustment resulted in significantly larger stem diameters within each effluent nutrient solution (Figure 2.12). The pH adjusted effluent treatments resulted in plants with similar stem diameters to each other, as did the pH unaltered effluent treatments (Figure 2.12). The HRAP system had no significant impact on the nutritional potential of the effluent as it affected basal stem diameter with the pH correction having a significant effect (Figure 2.12).

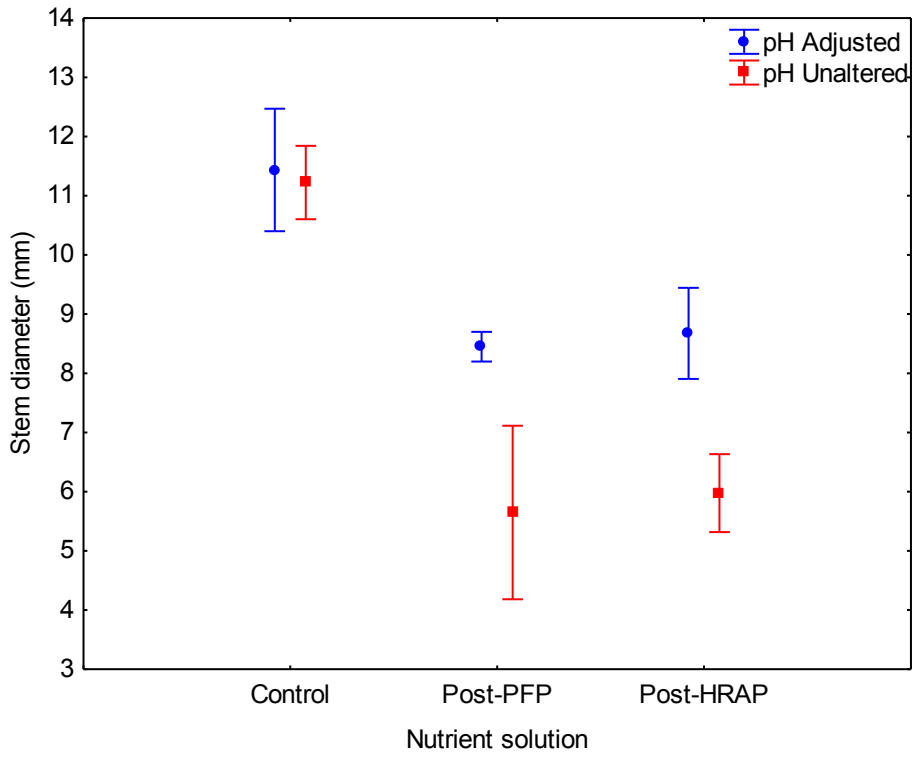

Figure 2.12 The mean (±95% confidence interval) stem diameter of *Moneymaker* tomato plants subject to treatments of municipal water and fertilizer (Control), or brewery effluent drawn from different points in the experimental treatment system Post-primary facultative pond (Post-PFP) and Post-High Rate Algal Pond (Post-HRAP) for 49 days (Multifactor ANOVA, $F_{(2,24)}$=10.59, p=0.0005). Duplicate treatments were subject to pH correction with phosphoric acid.

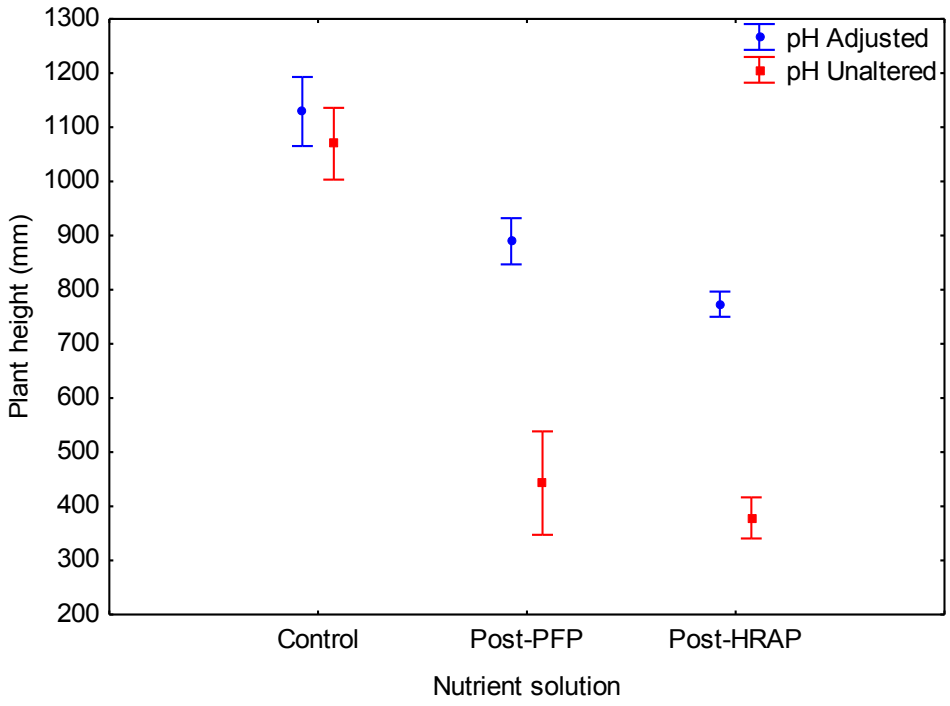

Figure 2.13 The mean (±95% confidence interval) plant height of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent. T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP) (Multifactor ANOVA, $F_{(2,24)}$=47.78, p<0.00001). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid for 49 days.

Plant height was affected by a significant interaction between nutrient solution and pH adjustment (T3: 443 ± 34 mm versus T4: 890 ± 15 mm, and T5: 378 ± 14 mm versus T6: 773 ± 8 mm, Multifactor ANOVA, $F_{(2,24)}$=47.78, p<0.00001; Figure 2.13). There was a significant difference in the mean plant height of the pH corrected effluent systems (T3: 443 ± 34 mm versus T4: 890 ± 15 mm, and T5: 378 ± 14 mm versus T6: 773 ± 8 mm), whereas the addition of acid did not have a significant effect on the mean plant height of the inorganic-fertilizer control treatments (Figure 2.13). There was no significant difference between the plant height of the pH uncorrected effluent treatments (Figure 2.13). The pH adjusted post-PFP plants grew significantly taller than the plants grown in pH adjusted post-HRAP effluent (T4: 890 ± 15 mm versus T6: 773 ± 8 mm; Figure 2.13).

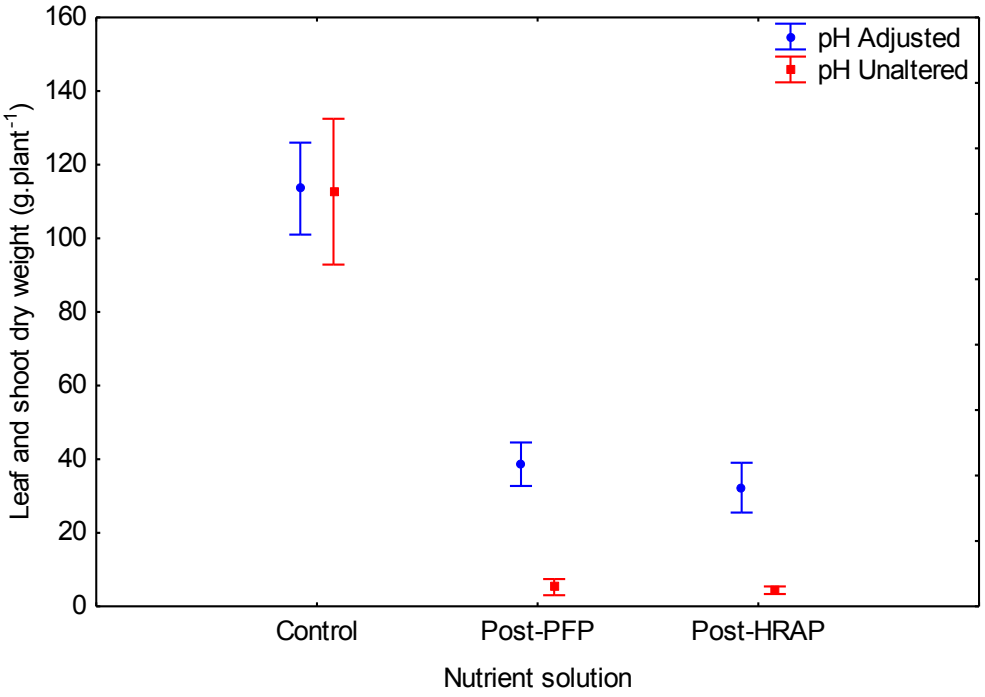

Figure 2.14 The mean (±95% confidence interval) leaf and shoot dry weight Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent. T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP) (Multifactor ANOVA, $F_{(2,24)}$=11.06, p=0.00039). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid for 49 days.

There was a significant interaction between pH adjustment and nutrient solution as they influenced the accumulation of leaf and shoot dry biomass (Multifactor ANOVA, $F_{(2,24)}$=11.06, p=0.00039; Figure 2.14). There was significantly more plant leaf and shoot dry weight in the pH corrected effluent systems compared to the uncorrected effluent (T3: 5.23 ± 0.79 g.plant^{-1} versus T4: 38.62 ± 2.12 g.plant^{-1}, and T5: 4.39 ± 0.375 g.plant^{-1} versus T6: 32.26 ± 2.44 g.plant^{-1}, Figure 2.14). The addition of acid did not have a significant effect on the leaf and shoot dry weight of the inorganic-fertilizer treatments (Figure 2.14). There was no significant difference between the effluent sources when compared under pH corrected or uncorrected conditions (Figure 2.14). The HRAP system did not significantly reduce plant leaf and shoot dry matter accumulation (Figure 2.14). All of the effluent systems developed significantly less dry mass than the inorganic-fertilizer control systems (Figure 2.14).

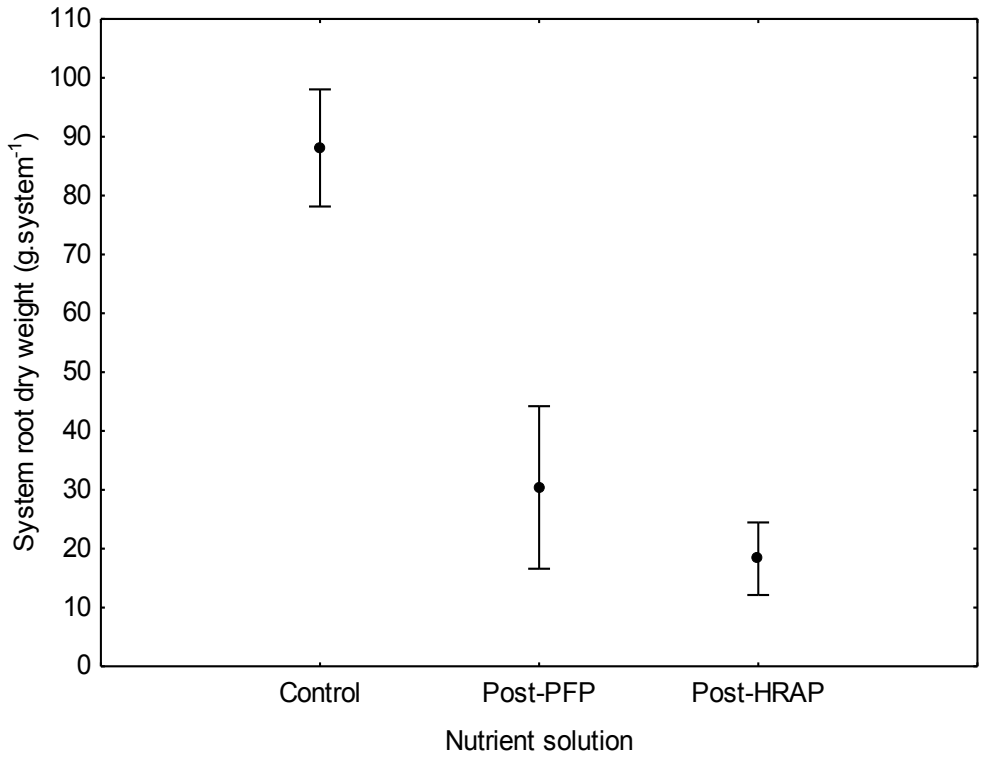

Figure 2.15 The mean (±95% confidence interval) system root dry weight of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent. T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP) (ANOVA, $F_{(2,18)}$=186.05, p<0.00001). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid for 49 days.

There was no interaction between pH adjustment and nutrient solution in the accumulation of dry root biomass (Multifactor ANOVA, $F_{(2,18)}$=2.36, p=0.12). The grouped mean of the control systems developed significantly more root biomass than the effluent sources (ANOVA, $F_{(2,18)}$=186.05, p<0.00001; Figure 2.15). The addition of phosphoric acid was also a significant factor with the pH adjusted treatments developing significantly more root biomass than the pH unaltered systems (pH adjusted 54.54 ± 9.14 g.system^{-1}, pH unaltered 36.64 ± 9.77 g.system^{-1}; ANOVA, $F_{(1,18)}$=32.14, p=0.00002).

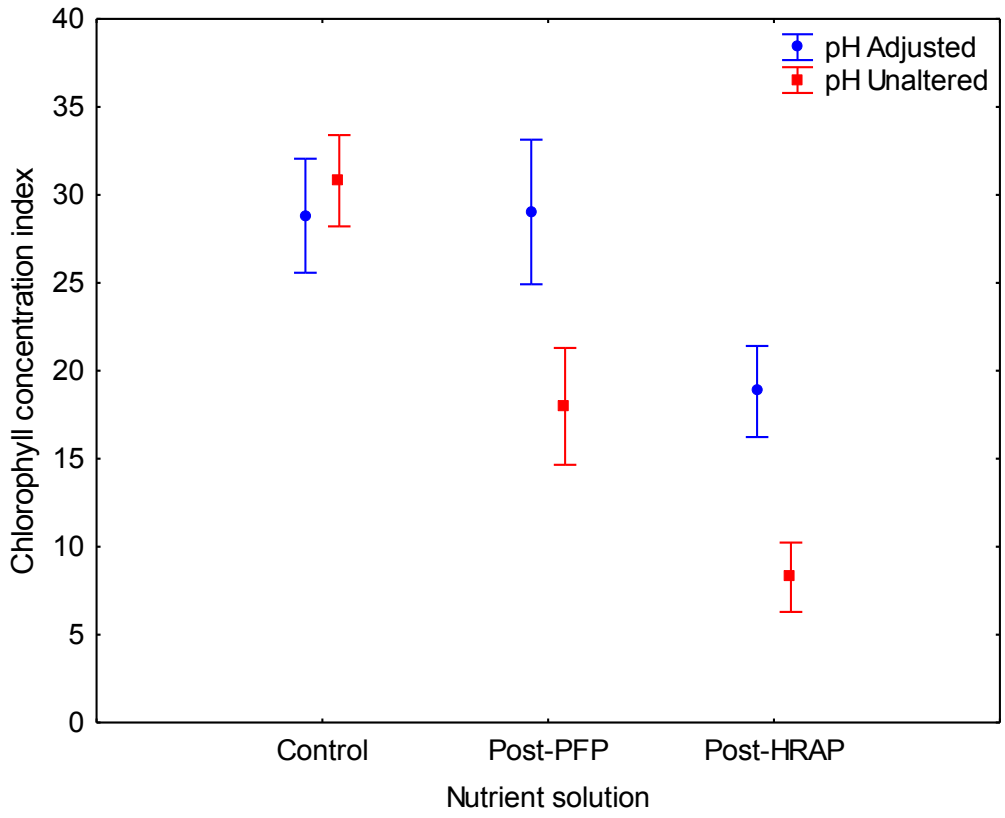

Figure 2.16 The mean (±95% confidence interval) chlorophyll concentration index (CCI) of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent. T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP) (Multifactor ANOVA, $F_{(2,24)}$=22.66, p<0.00001). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid for 49 days. This graph shows the values recorded on day 42 only.

Chlorophyll concentration index was influenced by a significant interaction between nutrient solution and pH adjustment (Multifactor ANOVA, $F_{(2,24)}$=22.66, p<0.00001; Figure 2.16). There was no significant difference in the mean CCI measured at the end of the trial between the two inorganic-fertilizer treatments (T1 and T2) and the pH corrected post-PFP effluent treatment (T4). The pH correction in the effluent treatments resulted in a significantly higher CCI value compared to the plants grown in the same effluent, but without pH correction (T3: 17.97 ± 2.67 versus T4: 29.03 ± 3.13, and T5: 8.26 ± 1.59 versus T6: 18.82 ± 2.09; Figure 2.16, Multifactor ANOVA, $F_{(2,24)}$=22.66, p<0.00001). T4 had significantly higher CCI values than any of the other effluent treatments (Figure 2.16). The HRAP system significantly reduced the CCI of the effluent plants.

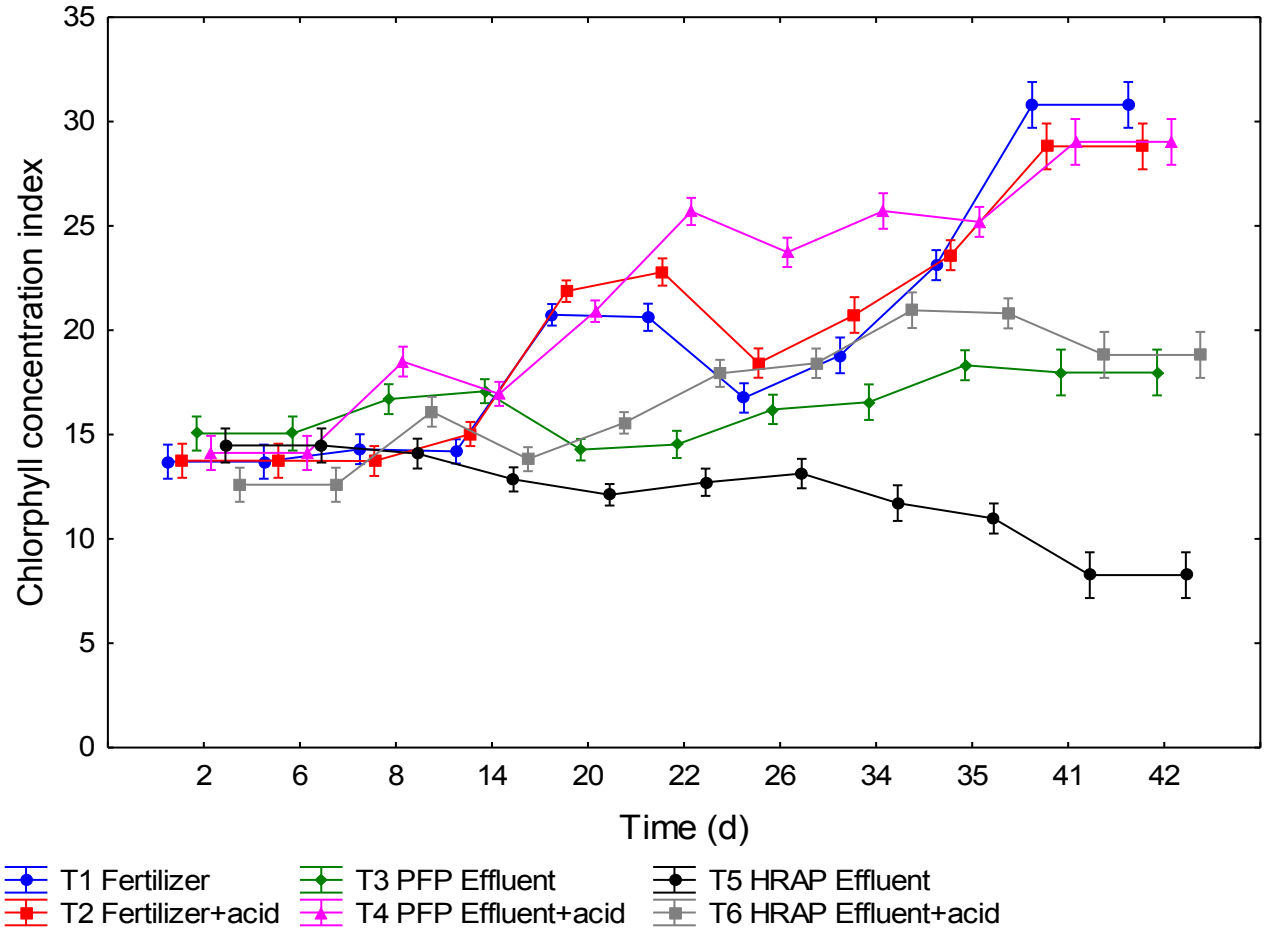

Figure 2.17 The mean (± standard error) chlorophyll concentration index (CCI) of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent. T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP) (Repeated measures ANOVA, $F_{(20,240)}$=9.36, p<0.00001). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid for 49 days.

The mean chlorophyll concentration index (CCI) of each treatment followed distinct and significantly different patterns over the course of the trial (Repeated Measures ANOVA, $F_{(20,240)}$=9.36, p<0.00001, Figure 2.17). Treatment T5 (uncorrected post-HRAP effluent) had a significantly lower CCI than all of the other treatments. Treatments T3 (uncorrected post-PFP effluent) and T6 (pH corrected post-HRAP effluent) followed a roughly similar pattern to each other over the course of the trial.

The CCI readings are a ratio of leaf thickness to chlorophyll concentration. The result must therefore be read with the understanding that the CCI value is a function of the two factors and not an absolute measure of chlorophyll content.

Figure 2.18 A visual comparison of plant development from the beginning of the trial (top) and the same systems on the 31st of October 2012, after 35 days (bottom). In these images, the treatments are arranged randomly from left to right: T3, T6, T4, T2, T1 and T5. The treatment solutions indicated are as follows: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 received pH adjustment with phosphoric acid.

The difference in growth between the experimental treatment systems is shown above (Figure 2.18). The plants in T5 and T6 (post-HRAP), and in T3 and T4 (post-PFP), are being irrigated with effluent from the same source, yet they have developed differently. This is the result of the pH alteration with the phosphoric acid.

2.3.3. Fruit development and chemical content

There was no significant interaction between nutrient solution and pH for the number of fruit developed on each plant at the end of the trial (Multifactor ANOVA, $F_{(2,24)}$=2.51, p=0.127). Nutrient solution had a significant influence on fruit development (ANOVA, $F_{(2,24)}$=21.00,

p=0.0001; Figure 2.19). The control treatments of municipal water and inorganic-fertilizer produced significantly more fruit per plant than the effluent treatments (Figure 2.19).

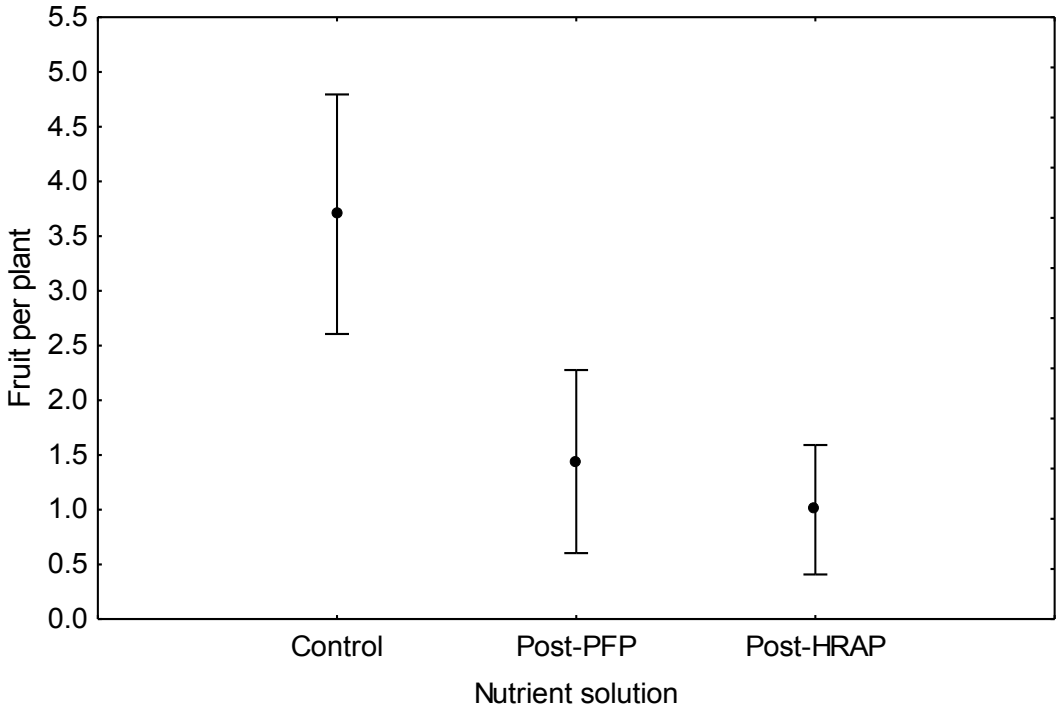

Figure 2.19 The mean (±95% confidence interval) number of fruit developing per plant in each system after 49 days (n=5). The treatment solutions indicated municipal water and inorganic-fertilizer (Control), or brewery effluent. T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP) (ANOVA, $F_{(2,24)}$=21.00, p=0.0001). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid for 49 days.

Only one fruit was sent for analysis from T3 and T5, because the sample fruit were harvested before the end of the trial, which allowed for other fruit on those plants to develop further. This graph also excludes fruit which were affected by blossom-end-rot, and were thus unsuitable for chemical analysis as only healthy fruit were selected for analysis (Figure 2.19, Figure 2.20). See Figure 2.23 for more information on the occurrence of blossom-end-rot.

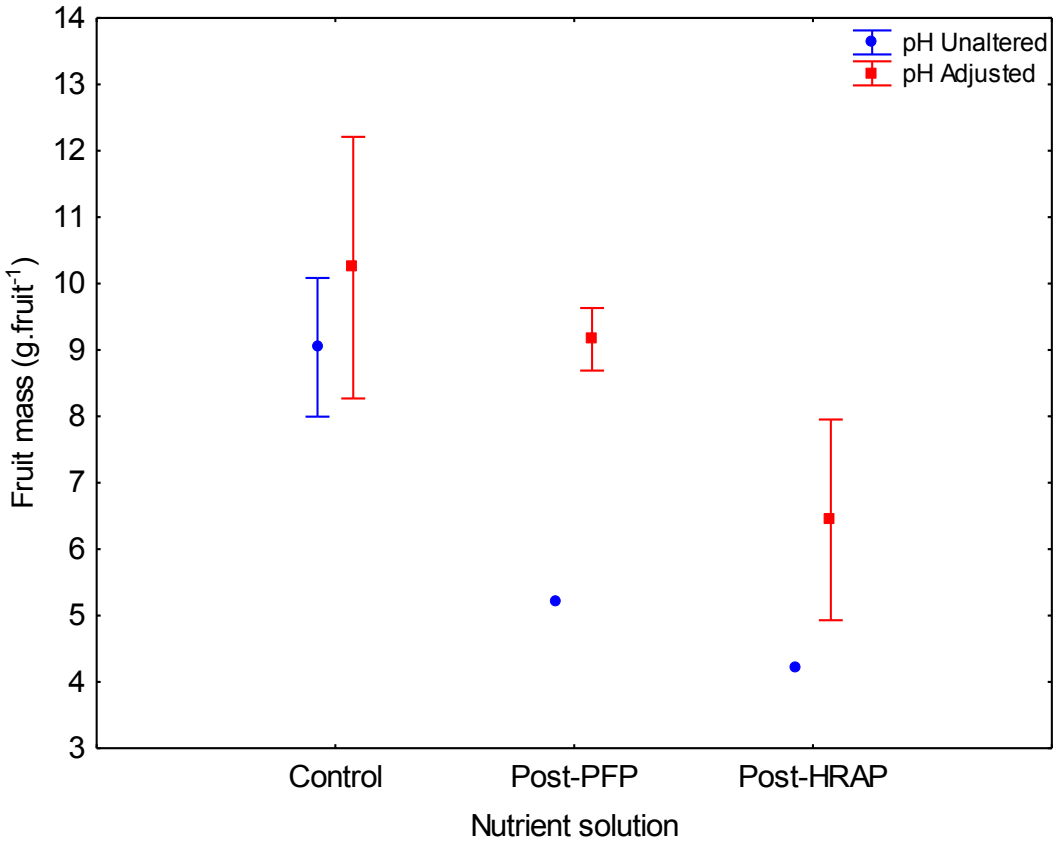

Figure 2.20 The mean mass (± standard error) of individual fruit harvested and sent for chemical analysis at the end of the trial. These samples were collected before the end of the trial which is why only one fruit was available for testing in treatments T3 and T5 (n: T1=5, T2=5, T3=1, T4=5, T5=1, T6=5). The treatment solutions indicated are; Control: T1 and T2 – municipal water and inorganic-fertilizer, post-PFP: T3 and T4 – post-primary facultative pond effluent, and post-HRAP: T5 and T6 – post-high rate algal ponds effluent. Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

No significant differences in elemental concentration were observed between the fruit analysed across all the treatments and factors for the following elements; calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus and zinc (Table 2.4).

Table 2.4 The mean (± standard error) fruit chemical concentration for the individual treatments (Multifactor ANOVA, $p<0.05$). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

		Treatment							
Element	Unit	T1	T2	T3*	T4	T5*	T6	$F_{(2, 16)}$ value	P value
Calcium	$mg.100g^{-1}$ fresh mass	6.62 ± 1.29	6.46 ± 0.80	7.10	5.66 ± 0.15	6.60	10.94 ± 4.20	0.33	0.72
Copper	$mg.kg^{-1}$ fresh mass	0.38 ± 0.06	0.44 ± 0.02	0.30	0.26 ± 0.04	0.20	0.60 ± 0.28	0.45	0.64
Iron	$mg.kg^{-1}$ fresh mass	4.16 ± 0.96	3.74 ± 0.32	3.00	4.10 ± 0.23	4.00	8.16 ± 2.90	0.58	0.57
Magnesium	$mg.100g^{-1}$ fresh mass	10.52 ± 0.56	10.62 ± 0.62	12.40	9.40 ± 0.20	16.20	15.98 ± 3.60	0.17	0.84
Manganese	$mg.kg^{-1}$ fresh mass	0.96 ± 0.10	1.04 ± 0.05	1.00	0.92 ± 0.04	1.00	1.20 ± 0.29	0.13	0.88
Nitrogen	$mg.100g^{-1}$ fresh mass	221.40 ± 12.39	239.60 ± 7.76	234.00	189.60 ± 4.37	195.00	250.20 ± 49.10	0.65	0.54
Phosphorus	$mg.100g^{-1}$ fresh mass	39.18 ± 1.56	44.43 ± 1.07	39.61	45.88 ± 0.86	38.37	50.95 ± 7.17	0.24	0.79
Zinc	$mg.kg^{-1}$ fresh mass	2.80 ± 0.23	2.82 ± 0.24	2.90	2.32 ± 0.14	3.30	4.16 ± 1.20	0.22	0.80

*Treatment 3 and Treatment 5 had only developed one fruit at the time of sampling, hence the lack of error values.

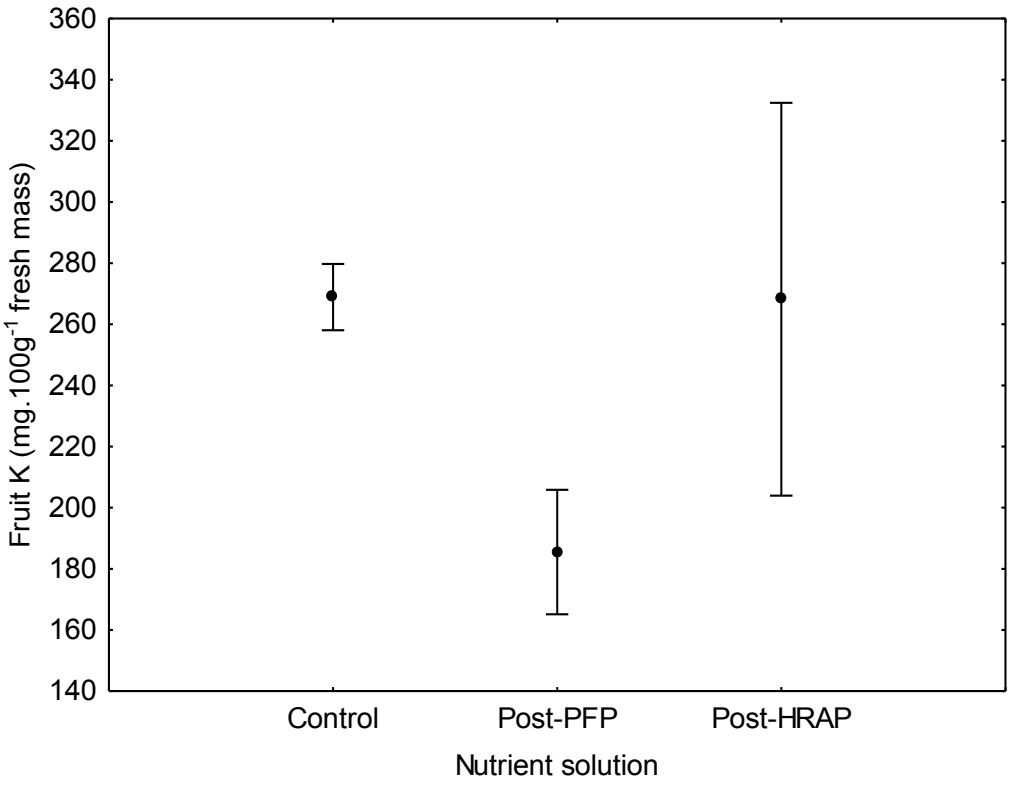

Figure 2.21 Fruit potassium content (mg.100g^{-1} of fresh fruit mass) (ANOVA, $F_{(2,16)}$=5.10, p=0.02). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer (Control group), T3 and T4 – post-primary facultative pond effluent (post-PFP group), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP group). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

There was no interaction between nutrient solution and pH adjustment acid affecting fruit potassium content (Multifactor ANOVA, $F_{(2,16)}$=1.23, p=0.32). The potassium analysis showed that the grouped mean of the post-PFP nutrient solution grown fruit samples had significantly lower concentrations of potassium (185.5 ± 7.92 mg.100g^{-1} fresh mass) than the control (268.9 ± 4.79 mg.100g^{-1} fresh mass) and the post-HRAP (268.17 ± 24.99 mg.100g^{-1} fresh mass) samples (ANOVA, $F_{(2,16)}$=5.10, p=0.02; Figure 2.21). There was no significant effect of pH adjustment on fruit potassium content (ANOVA, $F_{(1,16)}$=0.22, p=0.89).

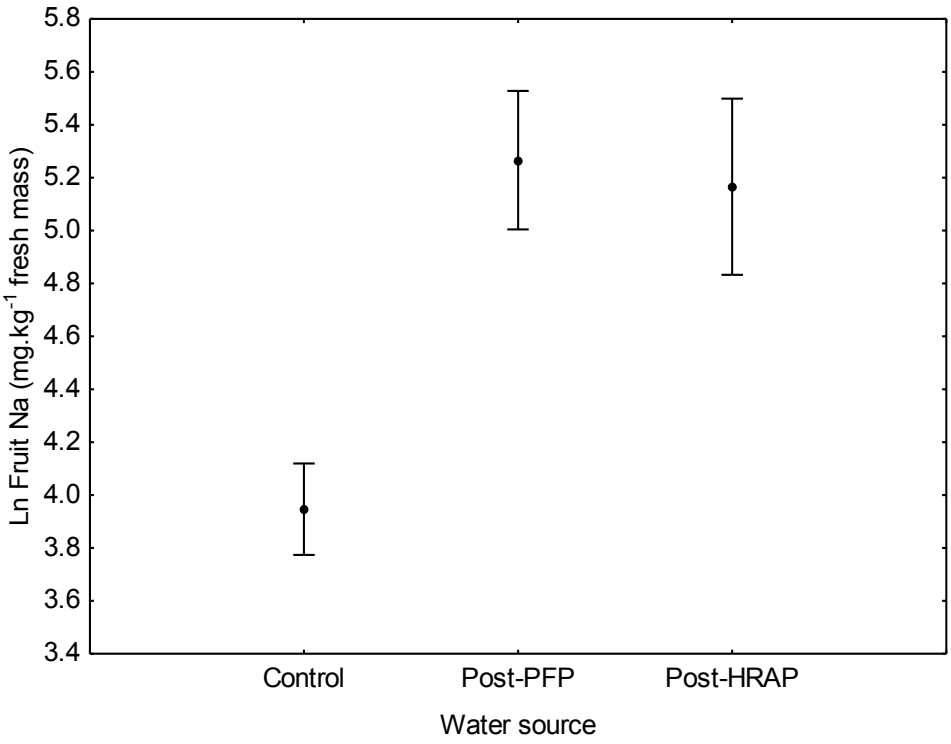

Figure 2.22 The Ln fruit sodium content (mg.kg^{-1} of fresh fruit mass) (ANOVA, $F_{(2,16)}$=53.90, p<0.00001). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer (Control group), T3 and T4 – post-primary facultative pond effluent (post-PFP group), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP group). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

There was no interaction between pH correction and nutrient solution for the log transformed sodium concentrations (Multifactor ANOVA, $F_{(2,16)}$=1.62, p=0.23). However, comparison between nutrient solutions, regardless of pH adjustment found that fruit in the effluent systems all had higher sodium concentrations (post-PFP: Ln 5.266 ± 0.102 mg.kg^{-1} fresh mass, post-HRAP: Ln 5.166 ± 0.13 mg.kg^{-1} fresh mass) than the fruit in the inorganic-fertilizer systems (Ln 3.947 ± 0.076 mg.kg^{-1} fresh mass) (ANOVA, $F_{(2,16)}$=53.90, p<0.00001; Figure 2.22). The pH regime had no effect on log transformed sodium content of the tomatoes in the respective treatments (ANOVA, $F_{(1,16)}$=1.18, p=0.29).

2.3.4. Fruit development disorder - Blossom end rot

The fruit development of each treatment system as an average per plant, as well as the system average for fruit affected by blossom-end rot (BER) varied according to nutrient solution and

pH adjustment. The pH alteration in T4 and T6 improved the fruit yield of the effluent systems (Figure 2.23).

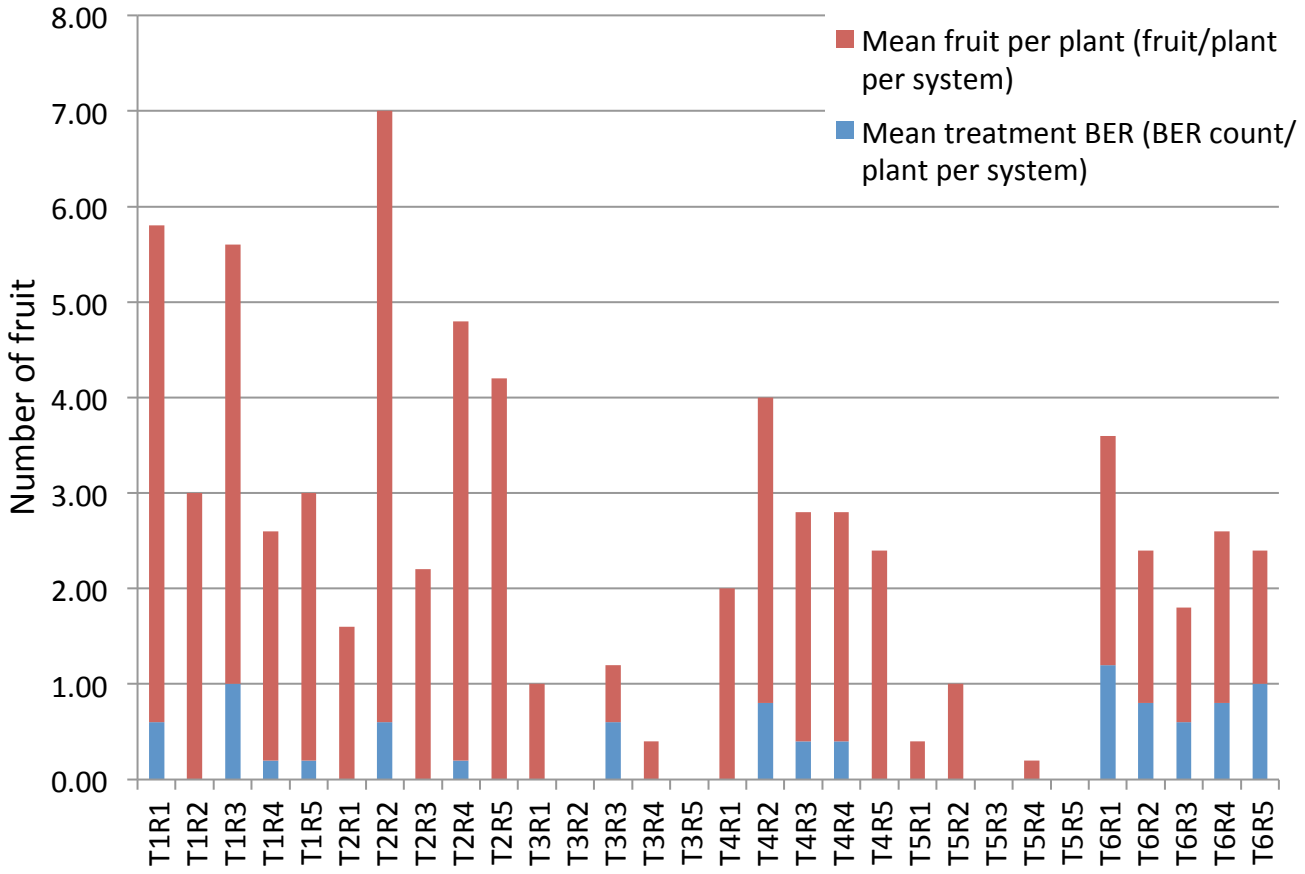

Figure 2.23 The mean number of fruit (red) and the mean number of fruit affected by blossom-end-rot (BER) (blue) per plant in each of the thirty systems. The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid. The R values in the X-axis labels refer to the replicate number of each treatment.

2.3.5. Leaf tissue chemical analysis

Plant leaf concentrations of boron were similar across all the treatments (Kruskal-Wallis, $H_{(5, N=30)}$=19.67 p=0.14; Figure 2.24). Treatments 3 and 5, the unaltered pH effluent treatments, had wider ranges of boron content up to 250 $mg.kg^{-1}$ while the other treatments had maximum values around 100 $mg.kg^{-1}$ (Figure 2.24).

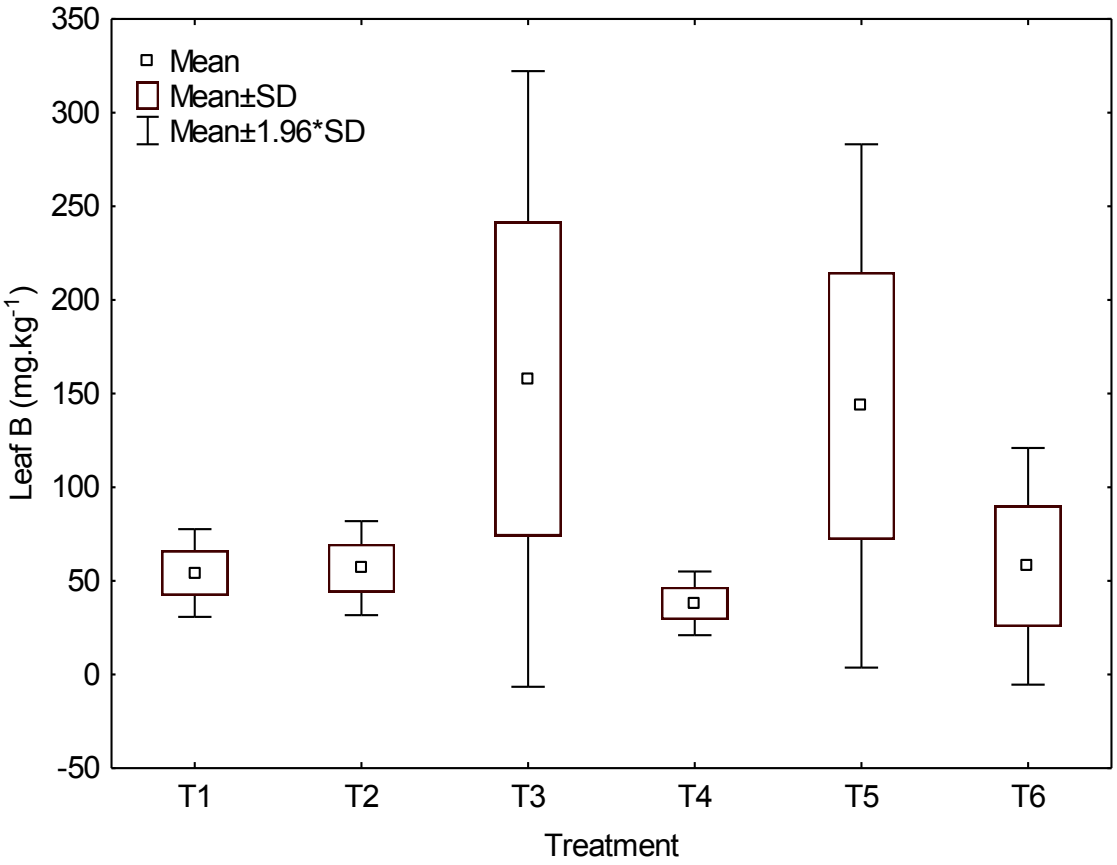

Figure 2.24 Leaf boron content (mg.kg^{-1} of fresh fruit mass) (Kruskal-Wallis test, $H_{(5, N=30)}$=19.67 p=0.14). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

Plant leaf concentrations of calcium were similar across all the treatments (Multifactor ANOVA, $F_{(2,24)}$=1.952, p=0.164; Table 2.5).

Table 2.5 The mean (± standard error) foliar calcium concentration (%) for the individual treatments (Multifactor ANOVA, $F_{(2,24)}$=1.952, p=0.164).

Treatment	Nutrient solution	pH adjusted	Mean %	Standard error
T1	Control	No	3.950	± 0.573
T2	Control	Yes	5.454	± 1.011
T3	Post-PFP	No	5.652	± 0.894
T4	Post-PFP	Yes	3.208	± 0.519
T5	Post-HRAP	No	5.194	± 1.411
T6	Post-HRAP	Yes	4.678	± 1.256

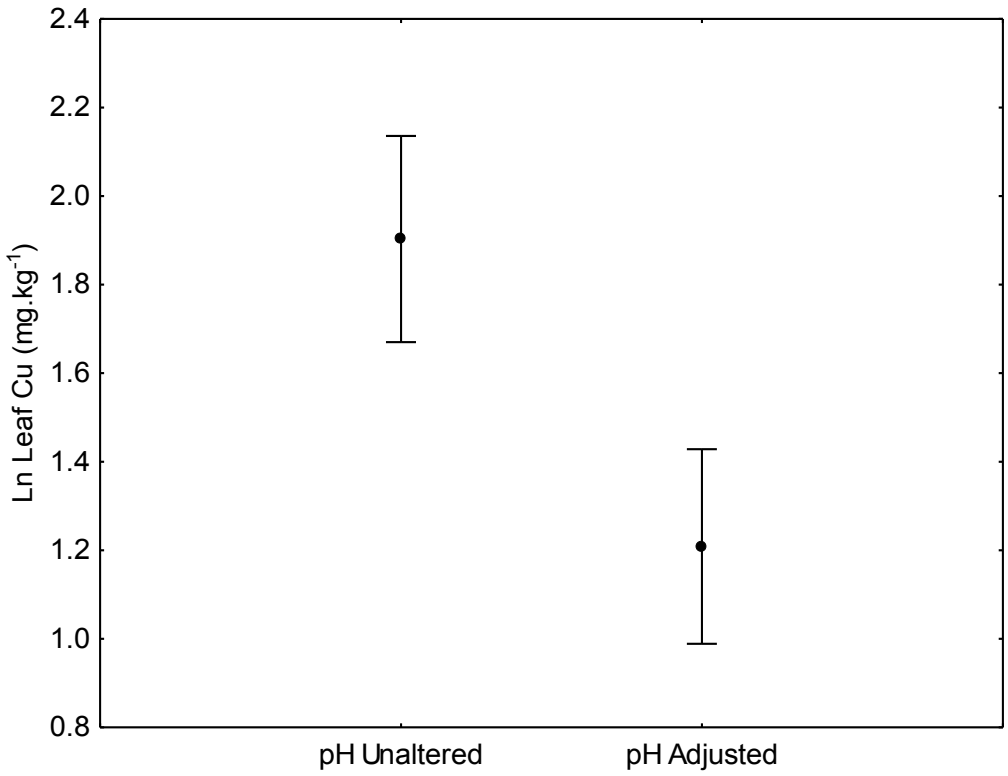

Figure 2.25 The mean (±95% confidence interval) Ln leaf copper content ($mg.kg^{-1}$kg of fresh fruit mass) of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent drawn from different points in the treatment system post-primary facultative pond (post-PFP) and post-high rate algal pond (post-HRAP) (ANOVA, $F_{(1,28)}$= 21.63, p=.00007). Duplicate treatments were subject to pH correction with phosphoric acid. Treatments are grouped according to pH adjustment.

There was no interaction between nutrient solution and pH adjustment across the treatments for the log transformed foliar copper content (Multifactor ANOVA, $F_{(2,24)}$=3.26, p=0.557), and no significant difference between nutrient solutions (ANOVA, $F_{(2,24)}$=0.57, p=0.572). However pH adjustment was observed to be a significant factor influencing the foliar copper concentrations with the pH adjusted treatments having a log transformed copper concentration of 1.2 ± 0.1$mg.kg^{-1}$ while the unaltered treatments had a log transformed copper concentration of 1.9 ± 0.1 $mg.kg^{-1}$ (ANOVA, $F_{(1,28)}$= 21.63, p=.00007, Figure 2.25).

There was a significant interaction between nutrient solution and pH adjustment for the log transformed leaf potassium content (Multifactor ANOVA, $F_{(2,24)}$=3.43, p=0.49; Figure 2.26). The brewery effluent solutions, regardless of pH adjustment, all had significantly lower log transformed foliar potassium content than the control plants (Figure 2.26).

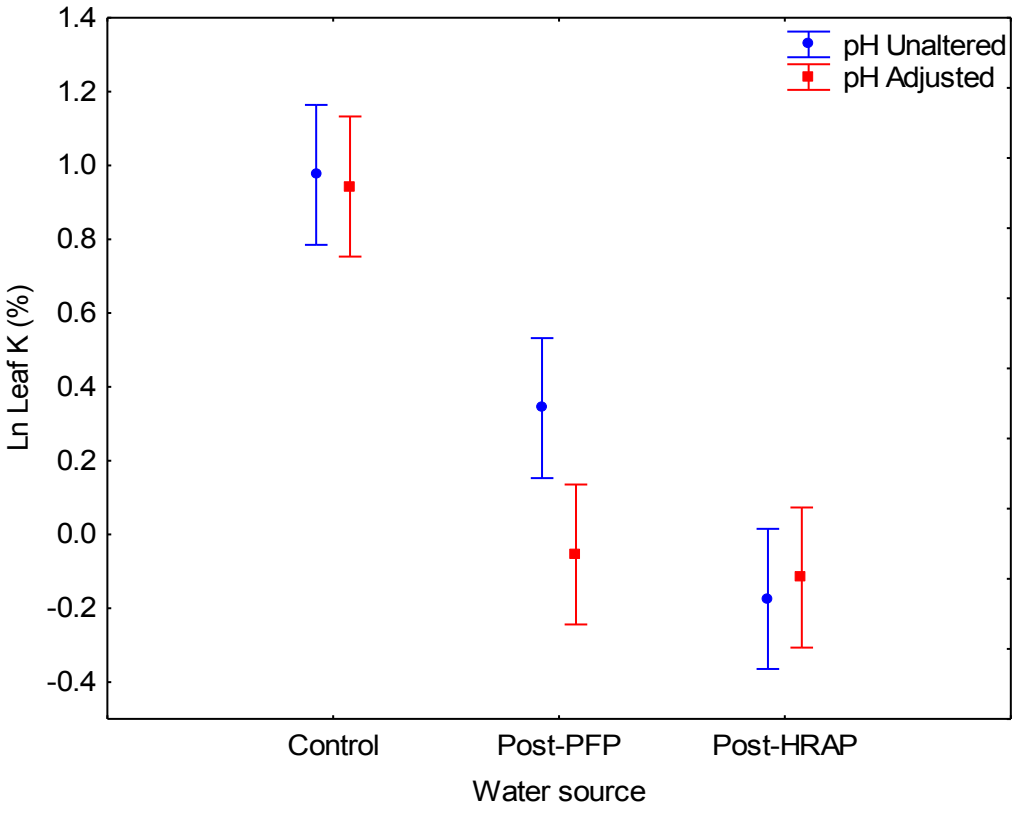

Figure 2.26 The mean (±95% confidence interval) leaf potassium content (% of fresh fruit mass) of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent drawn from different points in the treatment system post-primary facultative pond (post-PFP) and post-high rate algal pond (post-HRAP) (Multifactor ANOVA, $F_{(2,24)}$=66.45, p<0.00001). Duplicate treatments were subject to pH correction with phosphoric acid.

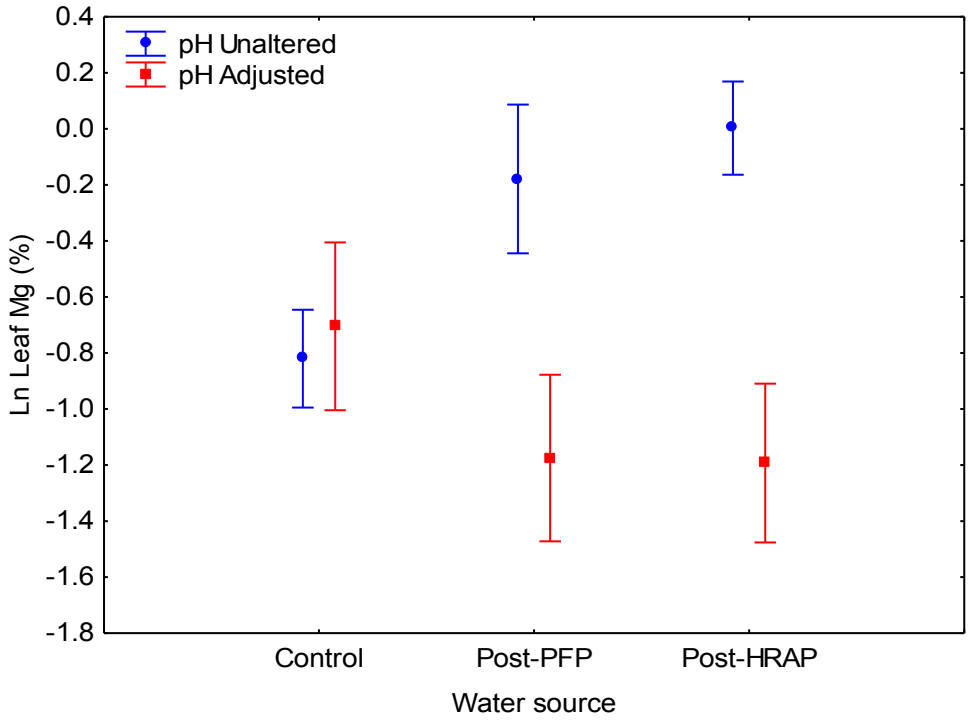

Figure 2.27 Mean (± 95% confidence interval) Ln leaf magnesium content (% of fresh fruit mass). The treatment solutions indicated are: T1 and T2 – 'control' municipal water and fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid (Multifactor ANOVA, $F_{(2,24)}$=29.82, p<0.00001).

There was a significant interaction between nutrient solutions and pH adjustment with the log transformed foliar magnesium content (Multifactor ANOVA, $F_{(2,24)}$=29.82, p<0.00001; Figure 2.27). The two inorganic-fertilizer treatments had similar log transformed magnesium content in their leaves (T1: -0.820 ± 0.063 % and T2: -0.705 ± 0.107 %). The pH adjusted effluent systems, and the pH uncorrected effluent systems had respectively similar magnesium content to their corresponding pH group treatment but not to their corresponding nutrient solution showing that pH adjustment resulted in significantly lower leaf magnesium content in boththe effluent systems (T4: -1.175 ± 0.107 %, T6: -1.193 ± 0.102 % versus T3: -0.178 ± 0.096 %, T5: 0.003 ± 0.06 %; Figure 2.27).

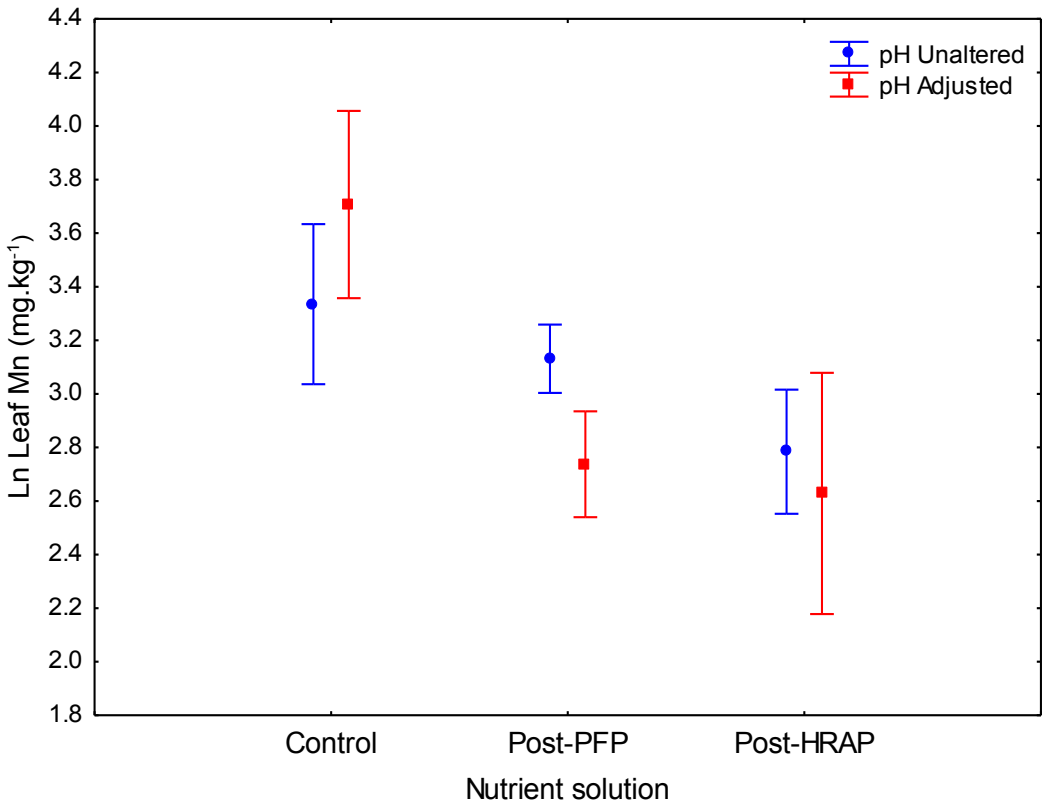

Figure 2.28 Ln Leaf manganese content (mg.kg^{-1} of fresh fruit mass) (Multifactor ANOVA, $F_{(2,24)}$=6.79, p=0.0046). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer (Control group), T3 and T4 – post-primary facultative pond effluent (post-PFP group), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP group). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

There was an interaction between nutrient solution and pH adjustment in the log transformed leaf manganese content (Multifactor ANOVA, $F_{(2,24)}$=6.79, p=0.046 Figure 2.28). The pH adjustment caused a significant difference in the log transformed magnesium content between the post-PFP effluent systems (T3 and T4; Figure 2.28).

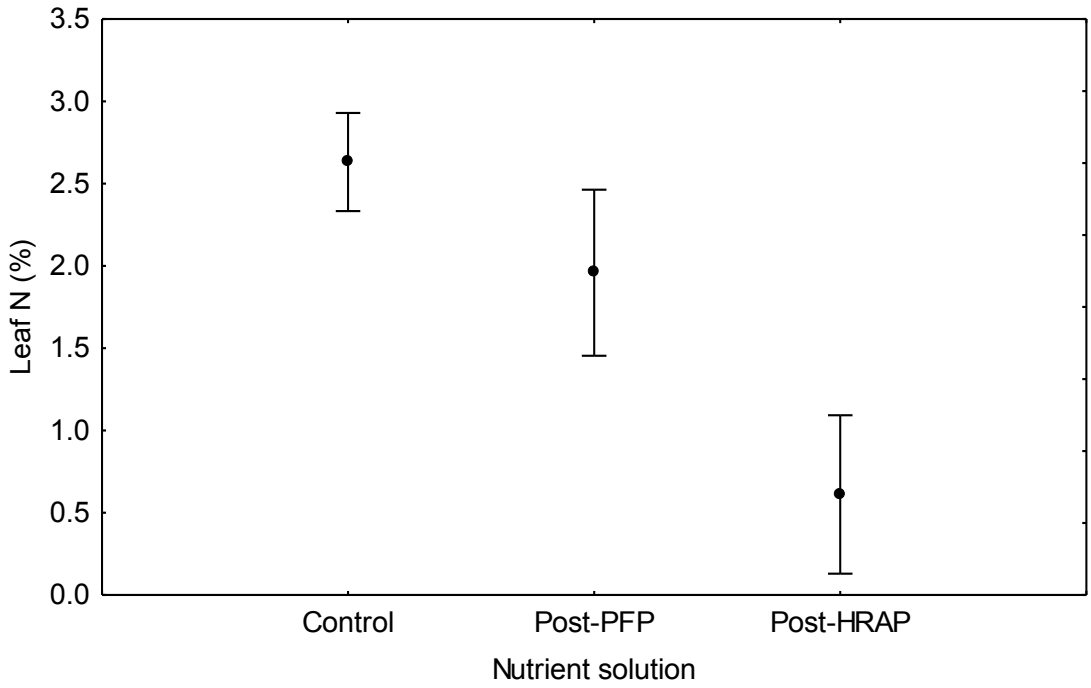

Figure 2.29 Mean (± 95% confidence interval) foliar nitrogen content (% of fresh fruit mass). The treatment solutions indicated are: Control (T1 and T2 – municipal water and inorganic-fertilizer), post-PFP (T3 and T4 – post-primary facultative pond effluent), and post-HRAP (T5 and T6 – post-high rate algal ponds effluent) (ANOVA, $F_{(2,23)}$=26.28, p<0.00001). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

There was no significant interaction between nutrient solution and pH adjustment in leaf nitrogen content (Multifactor ANOVA, $F_{(2,23)}$=0.39, p=0.68). The pH adjustment had no significant effect on leaf nitrogen content (ANOVA, $F_{(1,23)}$=1.40, p=0.25). There was a significant difference between the foliar nitrogen content (%) of the grouped nutrient solution means with post-HRAP 0.61 ± 0.209 % N, treatments having less nitrogen than the other two nutrient solutions; Control 2.631 ± 0.132% N, and post-PFP 1.95 ± 0.223% N (ANOVA, $F_{(2,23)}$=26.28, p<0.00001; Figure 2.29). One sample result for the N analysis was not returned from the analytical laboratory, hence the change in the $F_{(x,y)}$ statistic.

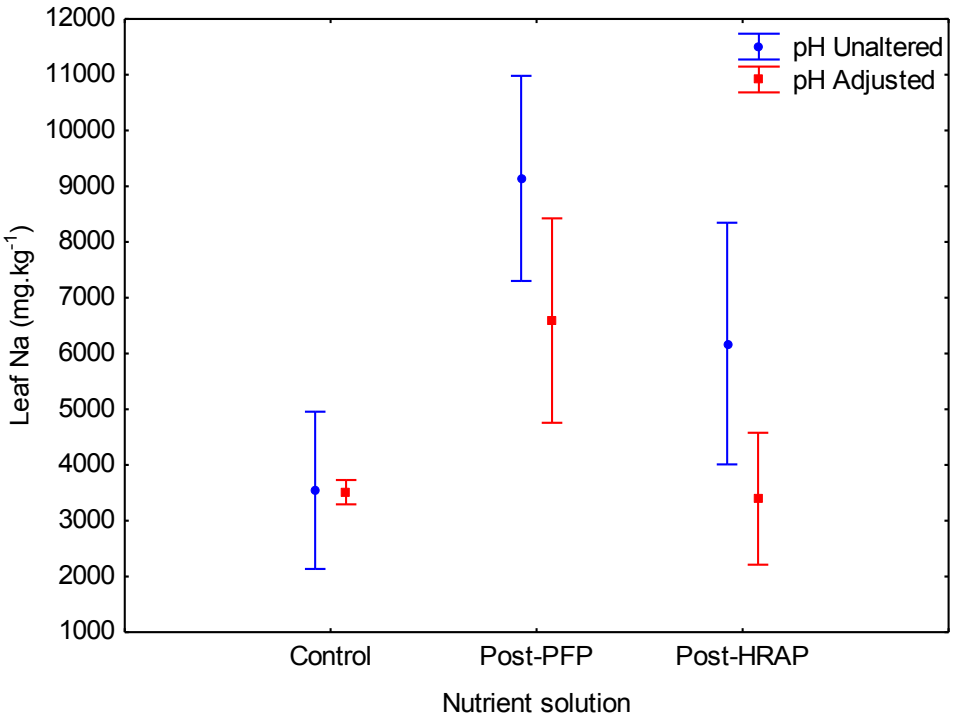

Figure 2.30 Mean (± 95% confidence interval) foliar sodium content (mg.kg^{-1}) (Multifactor ANOVA, $F_{(2,24)}$=3.61, p=0.043). The treatment solutions indicated are: Control (T1 and T2 – municipal water and inorganic-fertilizer), post-PFP (T3 and T4 – post-primary facultative pond effluent), and post-HRAP (T5 and T6 – post-high rate algal ponds effluent). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

There was a significant interaction between nutrient solution and pH adjustment in foliar sodium content (Multifactor ANOVA, $F_{(2,24)}$=3.61, p=0.043; Figure 2.30). The inorganic-fertilizer control systems had similar sodium content to the pH adjusted post-HRAP effluent system (T1: 3545.4 ± 508.17 mg.kg^{-1} and T2: 3511.6 ± 78.28 mg.kg^{-1} and T6: 3393.8 ± 426.17 mg.kg^{-1}; Figure 2.30). The highest sodium concentrations were found in the post-PFP effluent systems (T3: 9140.2 ± 662.50 mg.kg^{-1}, T4: 6590.6 ± 66.38 mg.kg^{-1}; Figure 2.30).

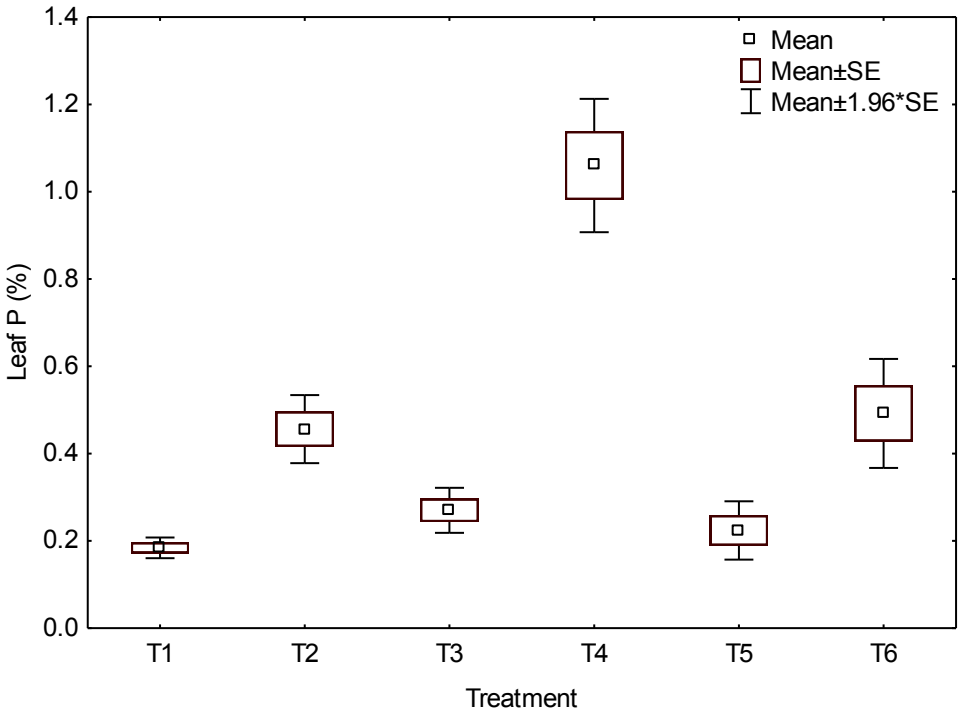

Figure 2.31 Leaf phosphorus content (% of fresh fruit mass) (Kruskal-Wallis, $H_{(5,\ N=30)}$=25.44, p=0.0001). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), and T5 and T6 – post-high rate algal ponds effluent (post-HRAP). Treatments T2, T4 and T6 were subject to pH adjustment with phosphoric acid.

Foliar phosphorus concentration was similar across all the treatments with the exception of T4 which had a significantly higher phosphorus concentration (Kruskal-Wallis, $H_{(5,\ N=30)}$=25.44, p=0.0001; Figure 2.31).

Table 2.6 The foliar zinc content ($mg.kg^{-1}$) in each treatment (Multifactor ANOVA, $F_{(2,24)}$=2.26, p=0.13).

Treatment	Nutrient solution	pH adjusted	Mean	Standard Error
T1 [a]	Control	No	40.8 ±	10.0
T2 [a]	Control	Yes	35.0 ±	6.0
T3 [a]	Post-PFP	No	38.4 ±	13.1
T4 [a]	Post-PFP	Yes	23.8 ±	7.7
T5 [ab]	Post-HRAP	No	64.6 ±	13.1
T6 [ac]	Post-HRAP	Yes	19.2 ±	6.1

There was no significant interaction between nutrient solution and pH adjustment on foliar zinc concentrations (Multifactor ANOVA, $F_{(2,24)}$= 2.26, p=0.13; Table 2.6). There was no

significant effect between water sources (ANOVA, $F_{(2,24)}$= 0.62, p=0.54). The pH adjustment had a significant effect on foliar zinc concentrations with the pH adjusted samples having lower concentrations than the unaltered pH samples (ANOVA, $F_{(1,24)}$= 7.55, p=0.01).

2.4. Discussion

Brewery effluent is an industrial effluent that requires treatment before disposal or reuse. The composition and quantity of the effluent will always be dependent on upstream factors which include, but are not limited to, the business cycle, the product and process management of the brewery, and the operational goals and performance of the wastewater treatment system. The unavoidable business caveat for any potential beneficiation scheme is that its success relies entirely on the approval of the brewery and therefore cannot create financial, social or operational risks for the brewery. These risks include any possible health risks of consumable products, unsound business models that could saddle the brewery with infrastructural waste or other collateral from a failed venture, and/or the risk to compliance with final effluent standards.

When considering the results of this trial, it is important to acknowledge and account for the environment in which this project exists. These results confirm an opportunity for using brewery waste as an alternative hydroponic water and nutrient source. However there are complex dynamics with the water chemistry of the anaerobic effluent, including the quantity of certain nutrients and the alkalinity of the effluent. The nutrient dynamics and the alkalinity challenge will be discussed in detail.

2.4.1. Making "Moneymakers"

The Moneymaker tomato plants grew in the effluent drawn from both points in the treatment system, whether the pH was altered or not. Adjusting the pH with phosphoric acid significantly improved the growth and development of the effluent-grown plants confirming

the effect of pH on crop performance (Tyson *et al*. 2007, Zhao *et al*. 2013). Hydroponic solutions at a pH of 10, compared to the control of pH 7, were shown to negatively affect the plant height, leaf area and plant crown width of *Paeonia lactiflora* (Zhao *et al*. 2013). The performance of the crops is conditional on the presence of nutrients as well as their availability, which is governed by pH (Lucas & Davis 1961). The data show that the effluent fed plants did not grow as well, or develop as many fruit during the trial period as the inorganic-fertilizer plants. Two of the questions that arise are; why did the effluent plants perform worse than those in the inorganic-fertilizer treatments? Secondly, does an analysis of data suggest management techniques that could improve the growth and yield of a crop, without breaching any of the previously mentioned conditions for a beneficiation project?

The physical-chemical characteristics of the effluent must be considered. The high pH of the anaerobically digested effluent was identified as a potential obstacle in hydroponic production in the experimental design phase. Accounting for the high pH of the effluent is something that requires an analysis of the anaerobic digestion process, the nature of the brewery effluent, the operation of the anaerobic facility and the post-digestion physico-chemical processes (van Rensburg *et al*. 2003). A further investigation into the anaerobic process and its implications for hydroponic crop production follows in Chapter 4.

The experiment tested whether using phosphoric acid as a pH correcting agent would improve the performance of crops grown in brewery effluent. The effluent treatments which received phosphoric acid correction produced crops which grew significantly better than the crops in the effluent that did not receive the phosphoric acid adjustment. In considering this result the question arises: does the addition of mineral phosphorus as a nutrient, or the pH effect of the acid enable to plants to perform better? There are three datasets available for considering the effect of the additional phosphorus: the water quality data, fruit phosphorus concentration, and leaf phosphorus concentration.

In the treatments which received phosphoric acid dosing (T2, T4 and T6) the initial PO_4-P concentrations were all above the range of the test (30 $mg.L^{-1}$). Given the volume of acid that was needed to correct the effluent systems (±15 mL of 80% phosphoric acid per 25 L of fresh effluent) compared with the inorganic-fertilizer and municipal water systems (<5 mL of 80% phosphoric acid per 25 L of solution), the results of the initial phosphate tests were expected. The high buffering capacity of the effluent is likely due to the generation of carbonate alkalinity and the stripping of carbonic acid when the effluent is exposed to normal atmospheric partial pressure after leaving the AD (van Rensburg *et al*. 2003). The pH of anaerobic supernatants has been shown to increase with aeration of the liquor (Musvoto *et al*. 2000a). This buffering capacity and the increase in pH will be discussed in more detail later on but it is a potential problem for brewery effluent reuse and nutrient bioavailability.

The plants were not able to consume phosphorus quickly enough to reduce the concentrations in the solutions. The fresh unaltered solutions had reasonably similar distributions of phosphate-phosphorus. However, it is in the waste solutions and the tissue analysis that we start to see that there was no nutritional benefit from the additional phosphorus. Only T4 plants (post-primary facultative pond effluent with phosphoric acid) had elevated phosphorus concentrations in the sampled leaf tissues compared to the other treatments. This did not result in the T4 plants developing more rapidly or massively than the T6 plants (post-HRAP effluent with phosphoric acid addition). Studies suggest that the plant removal of phosphate in constructed wetlands is relatively small compared to removal through physical processes such as adsorption and sedimentation (Merlin *et al*. 2002, Martín *et al*. 2013). The kinetic rate of phosphate uptake also varies between different plants (Yao *et al*. 2011). The "Moneymaker" tomato may have an inherently low phosphate uptake capacity, at least insufficient to consume all of the phosphate in the experimental solutions. This suggests that the benefit the plants derived from the addition of phosphoric acid was not due to the addition

of phosphorus but because of the acid lowering the pH of the effluent and the associated effects on the water chemistry of the irrigation solutions and nutrient bioavailability (Lucas & Davis 1961, Tyson *et al*. 2007, Zhao *et al*. 2013). The amount of phosphoric acid required to lower the pH of the effluent to a more suitable level makes it an unsuitable acid as it creates a problem with downstream phosphate pollution. An alternative solution for managing effluent pH is required.

It was shown that both fruit development, and blossom end rot (BER) increased with pH adjustment in the effluent. The plants in T4 and T6 developed far faster and more prolifically than their respective unaltered effluent counterparts. The inference is that the more fruit a plant develops, the more likely BER is likely to occur, especially given that these plants were not grown in an ideal, nutrient, temperature and humidity controlled environment, any one of which could contribute to BER (Saure 2001, Epstein & Bloom 2004, Roberto 2005, Magán *et al*. 2008). While much of the literature points to calcium deficiency as being the cause of BER, plant stress through heat and water stress, ammonium activity, and high salinity are also recognised as contributing factors (Saure 2001, Epstein & Bloom 2004, Roberto 2005, Magán *et al*. 2008). Given that the fruit analysis showed no significant differences between the calcium concentration in the sampled fruit across the trial suggests that some other stress may have been affecting the incidence of BER. The BER figures may also be skewed slightly as the trial was terminated before the plants had fully developed their harvests. A longer trial period may have shown that when T3 and T6 did develop fruit, and if they developed fruit, the fruit may have been more susceptible to BER. Rapid plant development is a quality and should be seen as a positive outcome in the effluent systems.

2.4.2. Nutrition - demand versus supply and bioavailability

Plant nutrition management is a balance between what is present in the treated effluent and what a given plant needs to grow successfully. This section deals with nitrogen supply,

specifically the implications of an ammonium-rich solution as is the case with the post-primary facultative pond effluent, and its implications for plant-water biochemistry, micronutrient supply and plant stress. It is not sufficient to consider ammonium only in its role as a nitrogen source; its effect on other nutrient dynamics must also be considered. Ammonium and nitrate are the two main sources of nitrogen for plants and the ratio in which they are present has been shown to affect plant growth and micronutrient uptake (Britto & Kronzucker 2002, Horchani *et al*. 2010 and Borgognone *et al*. 2012). Ammonium has been identified as potentially toxic in some cases where it is the sole source of nitrogen or where the plant species is particularly sensitive to ammonium (Britto & Kronzucker 2002).

Ammonia (pKa 9.25) is one of the products of the anaerobic digestion of proteins (Angelikadi & Sanders 2004). It is mainly present in its ionized form of ammonium in the brewery effluent at pH below 9.25, above this level the proportions invert and ammonia is present in a greater proportion. Protonated ammonium causes an increase in the alkalinity of the effluent through the abstraction of an H^+ and the resulting OH^- molecule (Musvoto *et al*. 2000b, Hafner & Bisogni 2009).

$$NH_3 + H_20 \leftrightarrow NH_4^+ + OH^-$$

This cationic form has been linked with specific nutrient uptake restriction in crops, specifically other cations (Britto and Kronzucker 2002, Bar-Yosef *et al*. 2009, Borgognone *et al*. 2012). Ammonium toxicity is generally measured by supplying plants with various ratios of inorganic nitrate: ammonium in the feed solutions and observing the responses (Strojny 1999, Tabatabaei *et al*. 2008, Borgognone *et al.* 2012). These tests are usually done under sterile conditions with carefully tailored nutrient supplies, which is not the case when working with brewery effluent. This ammonium form is not stable in the brewery effluent. The aeration of the brewery effluent once it has left the anaerobic digester encourages the biological nitrification of ammonium to nitrate (Tyson *et al*. 2007). The reduction of

ammonium concentration has two effects; the first being that nitrification should reduce the overall ammonium stress on the plants, the second being that the effluent loses ammonium alkalinity (Gallert *et al*. 1998, Britto & Kronzucker 2002). However, even after nitrification and thus nearly complete loss of ammonium alkalinity in the algal ponds, the effluent pH is at its highest. This effect on effluent alkalinity cannot be ignored as it relates to the broader challenges with pH management and suggests we need to look elsewhere to discover the source of effluent alkalinity. The carbon acid/base system and its contribution to effluent alkalinity, along with sodium hydroxide addition as a pH buffer in the raw effluent are the most likely contributors to effluent alkalinity and are discussed in Chapter 4 (van Rensburg *et al*. 2003, Whittington *pers. comm.* 2013).

Ammonium induced cation deficiency has been suggested to present secondary stresses in, among others, three essential elements; potassium, calcium, and magnesium in numerous studies and plants (Kirkby 1968, Salsac *et al*. 1987, van Beusichem *et al*. 1988, Boxman *et al*. 1991, Hölldampf & Barker 1993, Troelstra *et al*. 1995, Gloser & Gloser 2000). Fruit calcium and magnesium content in this trial were not significantly different when compared across pH adjustment or nutrient solution factors. The fruit grown in post-PFP effluent (T3 and T4) had significantly lower concentrations of potassium in their fruit tissue than those grown in the other nutrient solutions. These findings suggest a relationship between the high proportion of ammonium in the post-PFP pH adjusted (T4) solution compared to the high nitrate levels, and virtually no ammonium, in the post-HRAP (T6) solutions. Ammonium induced potassium exclusion has been noted in solutions containing NaCl, similar to the brewery effluent (Nieves-Cordones *et al*. 2007). The ammonium-fed post-PFP plants (T3 and T4) did not present higher proportions of blossom-end-rot affected fruit, a key indicator of calcium deficiency, which suggests other stresses on fruit development (Epstein & Bloom 2004).

Ammonium induced magnesium deficiency is not clearly demonstrated by the magnesium chemical analysis however that may be the result of the small sample size. The pH corrected effluent plants had significantly less magnesium than their pH uncorrected counterparts but showed no differences across the nutrient solutions. There were no significant differences in fruit magnesium concentration. In plants subject to magnesium stress, magnesium is transported away from older parts of the plants and to the newer, active growing sites (Epstein & Bloom 2004). This can lead to the chlorosis, curling up, and even premature dropping of older leaves from the plant (Yeh *et al*. 2000, Epstein & Bloom 2004). Considering Figure 2.18, yellowing and curling of the oldest leaves on the plants nearest the camera in the pH adjusted effluent plants (T4 and T6) can be seen. The brewery effluent itself may not contain sufficient magnesium, or there is some other action inhibiting magnesium uptake within the plants as the pH uncorrected effluent plants (T3 and T5) do not show the same symptoms. This suggests that ammonium is not the only influence in magnesium deficiency in the leaf tissue of these plants and future work should consider other stresses and exclusion effects.

The analysis of potassium stress could indicate the influence of ammonium on potassium exclusion. While leaf samples showed similar potassium levels for the effluent treatments, fruit potassium levels were significantly lower in the ammonium-rich post-PFP effluent than in the other fruit samples from the other nutrient solutions. This may be explained by the effect that ammonium has been suggested to have on potassium levels in plants (Britto & Kronzucker 2002). However, the ammonium stress did not have any apparent effect on fruit development as the pH adjusted post-PFP plants, with the low fruit potassium content, produced more fruit with fewer incidences of blossom-end-rot than the pH adjusted post-HRAP plants (Figure 2.23). This suggests broader stresses and other potential deficiencies must be examined. This may indicate that there is some synergistic or additive stress

occurring with the combination of ammonium nutrition and sodium concentrations in the irrigation medium.

The PFP effluent irrigated plants had significantly lower concentrations of potassium in their fruit and leaf tissue than compared to the control treatments. Sodium concentrations in irrigation solutions have been linked to increased sodium and chloride content and decreased potassium content in plant tissue (Sacher *et al*. 1983, Tal & Shannon 1983). The HRAP irrigated plants (T5 and T6) showed lower concentrations of potassium in leaf tissue but not in fruit tissue compared to the control plants. It has been suggested that tomato plants can alleviate potassium stress by substituting sodium for potassium in its role as an enzyme activator or vacuolar solute (Walker *et al*. 2000). If the brewery effluent is potassium deficient, the presence of sodium may be fortuitous if tomato plants are chosen as the crop for future work.

The salinity of the effluent, as sodium and chloride, may have a direct effect on sodium concentrations in the plant tissue, as well as reduce root and shoot growth (Cuartero & Fernández-Muñoz 1999). The water quality tests in this trial, previous effluent elemental analyses, and the tissue analyses all confirmed the presence of sodium and chloride in the effluent stream. The effluent plants were also shown to have accumulated less biomass and to have been affected by a higher incidence of blossom-end rot. These relationships are complex and sodium may contribute to direct or indirect stress on the plants, or alleviate potassium stress to some degree (Cuartero & Fernández-Muñoz 1999, Walker *et al*. 2000). Salinity can improve fruit quality by increasing both sugar and acid concentrations in the fruit (Cuartero & Fernández-Muñoz 1999), however no assessment of fruit quality was made in this experiment. The complexity of the relationships in and between these ion processes requires more detailed analysis to determine what effects, if any, they may have.

The leaf and fruit tissue analyses revealed that the effluent plants had higher sodium concentrations in fruit tissue than those in the inorganic-fertilizer systems (Figure 2.22). This could indicate a sodium stress in the effluent systems which may affect the availability and/ or transport of calcium in the plants which can contribute to apparent calcium deficiency (Grattan & Grieve 1999). Sodium toxicity can present in the form of secondary calcium stress and/or contribute to the incidence of blossom-end rot (BER) (Cuartero & Fernández-Muñoz 1999 and Grattan & Grieve 1999). This could have contributed to the incidence of blossom-end-rot in the effluent plants which was observed. The balance between sodium mitigating potassium deficiency, ammonium-induced cation uptake reduction, and sodium stress is difficult to establish but warrants further investigation. Sources of sodium in the brewery effluent stream need to be identified and solutions to mitigate or minimise sodium stress devised.

While brewery effluent can serve as a nutrient source for tomato production, the nutrient dynamics associated with the effluent need careful consideration in order to optimise the productivity of the system. The presence of nutrients alone is not enough to guarantee that their effectiveness is not impeded by an imbalance in the chemical spectrum or some other effect of the water treatment process or associated water chemistry dynamics. The presence and biochemical dynamics between, sodium, ammonium, magnesium, phosphorus and calcium among other nutrients and/or stresses make for a complex plant-effluent relationship that requires further investigation.

2.4.3. Chemical oxygen demand

In general, the COD levels for the effluent systems either remained stable or increased. Evaporative losses concentrating non-biodegradable COD, plant water consumption, and microbiological products could have all contributed to an increase (either relative or absolute) in COD levels in the systems (Figure 1.3) (Ince *et al*. 1998, Barker & Stuckey 1999, Kasapgil

Ince *et al*. 2000, Simate *et al*. 2013). Without information on what contribution these recalcitrant and/or volatile fractions make to the total COD in this brewery stream, it is not possible to assess the true oxygen demand of this effluent. A biological oxygen demand (BOD5) test would be a better indicator of biologically accessible COD in this situation and could suggest more appropriate methods for final COD reduction in future research. Quantifying the volatile and inert fractions of the effluent would be useful in order to assess the efficacy of any water quality treatment system be it algae, wetland or hydroponic. This should be determined in future research.

2.4.4. Chlorophyll concentration

The presence of ammonium in the effluent may have increased the chlorophyll concentration index (CCI) readings of the post-primary facultative pond (post-PFP) irrigated plants. The readings over the course of the trial showed that the ammonium-rich post-PFP effluent plants had equal or higher CCI values than the other treatments. A positive correlation has been demonstrated between external ammonium concentration and the chlorophyll concentration in tomato plants (Horchani *et al*. 2010). The effluent contains less nitrogen than the inorganic-fertilizer mixture, which would explain the lower CCI readings for post-high rate algal ponds (post-HRAP) effluent plants than those in the control systems. The slightly elevated CCI values in the unaltered pH post-PFP treatment (T3) are likely due to the uptake of ammonium, however the high pH stress on nutrient uptake meant that the T3 plants could not assimilate nitrogen as was seen in the wastewater quality results, and they were probably experiencing other pH-induced nutrient stresses (Lucas & Davis 1961).

The pH adjusted post-PFP effluent treatment (T4) CCI values matched or exceeded those of the inorganic-fertilizer treatments over the course of the trial. While the T4 plants had similar CCI readings to those in the inorganic-fertilizer systems, one must take into account the effect of ammonium on increasing leaf chlorophyll concentrations, fluorescence and

photosynthetic activity (Horchani *et al*. 2010). The higher CCI readings show the effects of nitrogen form on plant development but do not indicate that there is a difference in nutritional value between the effluent and inorganic-fertilizer systems however the physical development results do.

Based on the T4 CCI readings and the consumption of available nitrogen in the pH adjusted effluent systems, it is hypothesised that the effluent is probably nitrogen deficient. This does not preclude the possibility that there may be other stresses affecting the plants besides a nitrogen limitation but, in order to optimise the performance of the plants, it is worth considering additional nitrogen inputs, so long as the intervention does not cause downstream nitrogen pollution. The CCI values of plants can be used in future trials to determine whether an adjustment to the nitrogen supply in plants causes an increase in chlorophyll concentration, fluorescence or photosynthetic activity and to determine whether other nutrient stresses have been alleviated by a chosen intervention.

2.5. Conclusions

The use of brewery effluent as a hydroponic nutrient solution is possible as the effluent contains all of the required macro- and micronutrients required for plant growth. While the effluent does support the growth, flowering and fruiting of Moneymaker tomato plants, it is an inferior nutrient source compared to commercial inorganic-fertilizer. The first null hypothesis; "*plant productivity is not influenced by an interaction between nutrient source, either with or without pH adjustment*" was rejected as there was a significant interaction between water source and pH adjustment across the treatments (Multifactor ANOVA, $p<0.05$). The effluent alkalinity and the balance of the chemical profile, specifically ammonium-cationic stress and sodium toxicity, which make the removal of nitrogen and phosphorus problematic, inhibit the growth of the plants, and contribute to plant and fruit

development disorders needs further investigation. Reducing the pH of the effluent resulted in a greater than 100% improvement in physical plant growth and development compared to pH unadjusted effluent therefore the second null hypothesis; "*plants grown in brewery effluent at different pH values will have the same productivity*" was rejected (Multifactor ANOVA, $p<0.05$). The effluent-based nutrient solutions showed no significant differences compared to their same acid-treatment counterparts and therefore the third null hypothesis; "*plants grown in effluent with different forms of available nitrogen will have similar growth performance*" was not rejected (Multifactor ANOVA, $p>0.05$). The addition of acid resulted in excessive phosphate levels in the waste nutrient solutions. Further work should determine whether the correction of the effluent pH can be combined with specific mineral injection to improve plant performance, as well as reduce residual pollution. The plants were not effective at reducing COD levels in the effluent.

Chapter 3: Experiment 2 - Nitric acid versus phosphoric acid

3.1. Introduction

Anaerobically digested brewery effluent can be used as a hydroponic water and nutrient supply for *Solanum lycopersicon* "Moneymaker" tomatoes (Chapter 2). The N:P supply in the effluent is probably nitrogen deficient as the effluent plants were able to remove nearly all of the available nitrogen (as ammonium, nitrite and nitrate), while phosphate levels remained high (Chapter 2). The results confirmed the importance of adding phosphoric acid to reduce the pH of the effluent which significantly improved the growth performance of the effluent plants compared to the unaltered pH treatments. The plants grown in post-primary facultative pond (PFP) effluent with phosphoric acid matched or exceeded the performance of the plants grown in post-high rate algal pond (HRAP) effluent with phosphoric acid (Chapter 2). The results of experiment 1 (Chapter 2) showed no clear benefit from using the HRAP-treated effluent as a hydroponic nutrient source compared to using water drawn directly from the primary facultative pond.

Nitrogen deficiency can lead to reduced or abnormal plant growth, chlorosis or yellowing of leaves (Epstein & Bloom 2004, Freeman 2005, Roberto 2005). The effluent-based treatments grew significantly less than the inorganic-fertilizer treatments in all physical assessments (Chapter 2 - Section 2.3.2).The pH adjusted effluent plants showed some signs of leaf chlorosis, particularly in the older leaves, which can be a symptom of nitrogen deficiency (Epstein & Bloom 2004, Chapter 2 - Section 2.3.2). The results suggest that the effluent-based hydroponic nutrient solutions are nitrogen limited when compared to an inorganic-fertilizer.

The significant improvement in plant performance in the pH adjusted effluent solutions confirms the limiting effect of high pH (Tyson *et al*. 2007, Zhao *et al*. 2013). The need to

reduce the pH of the effluent, the high residual phosphate levels in the discarded irrigation solutions, and the potential nitrogen deficiency, warrant an investigation into alternative methods of reducing the effluent pH and possibly improving the N:P balance in the effluent. The use of nitric acid could improve the N:P ratio, as well as reduce the potential stress of an ammonium-rich solution, by changing the ratio of available nitrogen as ammonium, nitrite or nitrate in the effluent-based irrigation solutions (Strojny 1999, Lastra *et al*. 2009, Borgognone *et al*. 2012).

The intention in this second trial was to test whether using nitric acid would achieve a better balance between nutrient addition (as phosphorus or nitrogen) by increasing nitrogen supply and achieving the required pH reduction. This would enable the plants to grow normally and effectively to sequester nutrients from the effluent, thus reducing downstream nutrient management or pollution concerns. With the confirmation of the presence in the effluent of the necessary nutrients for plant growth, subsequent work needs to address the shortcomings and problems with effluent-based nutrition, plant stress factors, and nutrient removal. The second trial was the first attempt at optimizing brewery effluent application in hydroponic tomato production.

Aims and objectives

The main aim of this experiment was to test whether the addition of nitric acid instead of phosphoric acid could alleviate the nitrogen limitation suggested by the results of experiment 1 (Chapter 2). The use of brewery effluent should not contribute to downstream nutrient pollution levels through ineffective nutrient management. The addition of phosphoric acid in experiment 1 was successful in reducing the pH of the effluent and improving the performance of the plants but the plants could not consume enough phosphate to reduce the concentrations to below 30 $mg.L^{-1}$ while nitrogen, as ammonium, nitrite and nitrate, was

almost completely removed (Experiment 1, Chapter 2). The objectives of this experiment were to:

1. compare the growth, productivity and nutrient removal of tomato plants grown in anaerobically digested brewery effluent to plants grown in a control solution of municipal water and commercial inorganic-fertilizer; and,
2. compare the effects of using either phosphoric or nitric acid as a pH correction agent on plant development and productivity.

3.2. Methods and materials

3.2.1. Experimental system

The same experimental system as presented in Chapter 2 was used in the second trial (Chapter 2, Figure 2.1). The number of treatments in the experiment was reduced to four, with five replicates in each treatment so the entire system had 20 channels for the second trial. Each channel was configured in the same way, except that only three plants were grown per channel, instead of the five per channel in the first trial.

3.2.2. Experimental treatments

Only effluent from PFP was used in the second trial. The first experimental factor, nutrient solution, had two variables; control: municipal water and commercial inorganic-fertilizer and calcium nitrate and; anaerobically digested brewery effluent drawn from the primary facultative pond (post-PFP effluent). The control solution was comprised of commercially available Hygrotech® hydroponic inorganic-fertilizer and calcium nitrate mixed in a ratio of 1:0.8 and dissolved in municipal water to achieve an EC of 2000 µm (Hygrotech (Pty) Ltd., South Africa). See Chapter 2 - Section 2.2.4., for the composition of the fertilizer. Each of these treatments was subject to pH adjustment with either nitric or phosphoric acid to maintain a pH of between 6.0 and 6.5 as the second experimental factor (Table 3.1). Nitric

acid (55%) was chosen as a comparison to phosphoric acid (80%). This comparison tested whether the addition of acid or phosphate was the factor that contributed to the improvement in the performance of the acid adjusted effluent systems in experiment 1. The solutions were mixed to the correct conductivity before nitric or phosphoric acid was added to adjust pH.

Table 3.1 The multifactor experiment designed for the second trial.

	Acid	
Nutrient solution	Phosphoric acid	Nitric acid
Commercial fertilizer	Treatment 1	Treatment 2
Post-PFP effluent	Treatment 3	Treatment 4

Each treatment was replicated five times, with each replicate consisting of one independent recirculating hydroponic system (Chapter 2, Figure 2.1),

The following hypothesis was tested:

1: H_0= Plant development and productivity is not influenced by an interaction between nutrient solution and type of acid used to adjust the pH.

If there was no interaction between the nutrient source and the type of acid that was used to adjust the pH, then the following hypotheses were tested:

2: H_0= Plants grown in brewery effluent will have the same rate of physical development and fruit production as plants grown in municipal water and inorganic-fertilizer; and,

3: H_0= Plants grown in nutrient solutions with different inorganic acids will have the same rate of physical development and fruit production.

3.2.3. Data collection

Plant height and stem diameter were recorded at the start of the trial and once a week for 78 days (Chapter 2 - Section 2.3.2).

Chlorophyll concentration index data were collected once between every seven and 10 days throughout the trial, with one recording from the uppermost fully expanded leaf of each plant in the trial taken at each sampling session (Chapter 2 - Section 2.2.4.).

Fruit development was assessed by collecting, counting and weighing all the fruit from each plant at the end of the trial (Chapter 2 - Section 2.2.5.). Fruit development disorders including failed fruit and blossom-end rot (BER) were recorded as they occurred and at the end of the trial. Fruit failure rate was determined by comparing the number of failed fruit (combined BER-affected fruit and failed fruit-set sites) at the end of the trial.

$$Fruit\ failure\ rate = \left(\frac{Blossom\ end\ rot\ count + Failed\ fruit\ set}{Total\ number\ of\ fruit\ sites}\right) \times 100$$

Fruit samples were collected at the end of the trial and sent to a commercial analytical laboratory for elemental analysis (BemLab (Pty) Ltd., South Africa).

Daily visual observations were made of the plant stems, leaves and fruit when they appeared to identify any visual stress symptoms. Plant roots were examined every seven to ten days to assess root growth and to observe any growth disorders.

Air temperature was measured with an alcohol thermometer, placed in the shade between the channels on the hydroponic table with readings taken around midday periodically throughout the trial.

Each plant was pruned to a single stem which was supported by twine wrapped around the stem and tied to overhead wires (Figure 3.1). Side branches were removed as soon as they were identified.

Figure 3.1 The experimental system for experiment 2. Note; the overhead wires, plants trained around the suspended twine and only three plants per channel, hence the empty pots.

Nutrient solution pH and electrical conductivity (EC) were monitored with each nutrient replacement or acid adjustment (Chapter 2 - Section 2.2.5.). Dissolved oxygen was monitored periodically with an electronic meter (Oxyguard, Handy Polaris 1 portable DO meter, Los Angeles, United States of America). Spectrophotometric analyses of NO_3^-, NO_3^--N, NH_4^+, NH_4^+-N, PO_4, PO_4-P, NO_2^-, NO_2^--N, Cl^- and COD were conducted on fresh and discarded nutrient solutions in three replicates from each treatment in each replacement cycle (Chapter 2 - Section 2.2.5.). Water quality analyses were conducted using the standard reagents and methods supplied by Merck (Pty) Ltd., South Africa, for each of the selected parameters used

in experiment 1 (Chapter 2 - Section 2.2.3.) as well as chloride (product: 1.14897.0001, Merck, Darmstadt, Germany). Three replicates were sampled from each treatment as opposed to two samples in the earlier experiment.

3.2.4. Statistical analysis

Statistical analyses were conducted using the same methods and software as those in Chapter 2 (Section 2.2.7.). Multifactor analysis of variance (Multifactor ANOVA), one-way ANOVA were used to determine whether an interaction between factors occurred and to identify significant differences between treatment means ($p<0.05$).

3.3. Results

3.3.1. Water chemistry

There was a significant interaction in electrical conductivity of the nutrient solutions according to nutrient solution and type of acid used (Multifactor ANOVA, $F_{(1,16)}=277.63$, $p<0.00001$; Figure 3.2). The effluent systems had higher conductivity than the control treatments, with T4 having consistently higher conductivity than any of the other treatments throughout the trial (T4 EC: 3128 ± 44 µS). The nitric acid addition increased the conductivity of the solutions relative to the phosphoric acid additions (T1: 2045 ± 15 µS, T2: 2177 ± 18 µS, T3: 2460 ± 20 µS, Figure3.2).

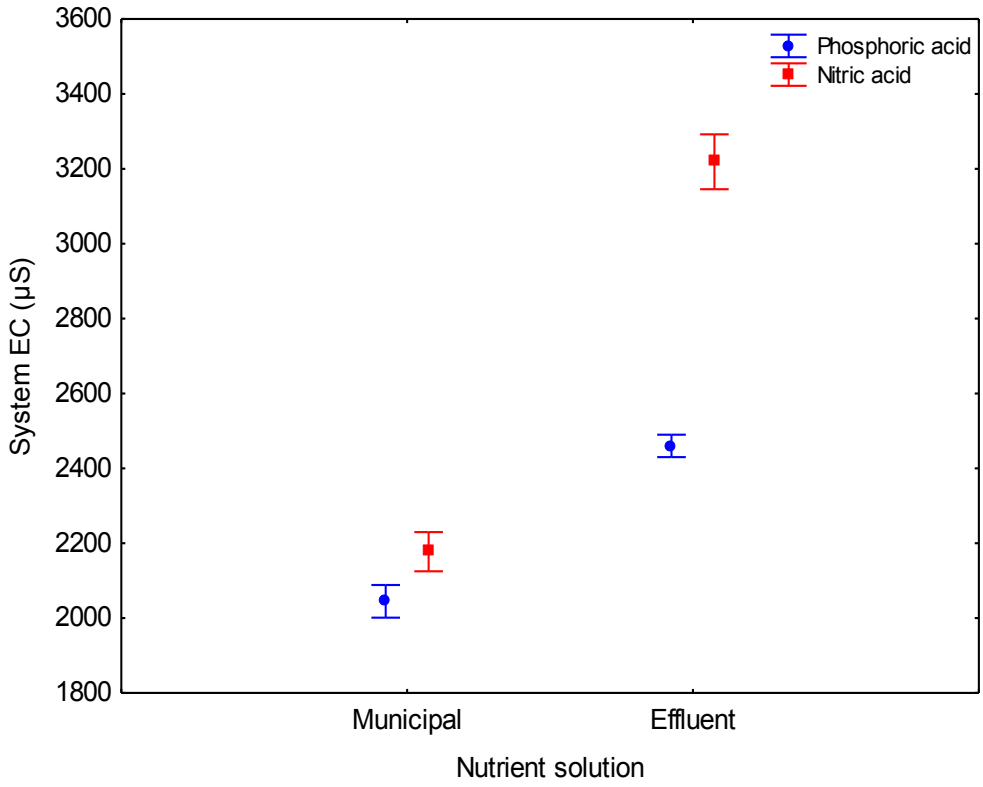

Figure 3.2 The mean electrical conductivity (EC) levels (± 95% confidence interval) of each over the course of the trial (Multifactor ANOVA, $F_{(1,16)}$=277.63, p<0.00001). The treatments are indicated as follows: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid.

Table 3.2 The mean, maximum and minimum solution pH recorded over the course of the trial. The treatments are indicated as follows: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid.

Treatment	pH		
	Mean	Maximum	Min
T1	6.33	7.50	5.74
T2	6.42	7.81	5.60
T3	6.75	8.38	6.34
T4	6.56	8.78	5.68

The mean pH values were similar when averaged across the trial. The variation of pH within the effluent systems was higher than the variation in the municipal systems (Table 3.3). The effluent systems had higher maximum values of 8.38 and 8.78 in T3 and T4 respectively (Table 3.3). Multiple pH adjustments were required in the effluent systems, with as much as 35 mL of phosphoric acid and 45 mL of nitric acid required to correct the pH of 30 L of

effluent. These corrections were carried out immediately when the nutrient solutions were replaced, and then pH was monitored until it stabilised over the following two to four days. After the initial pH adjustment, and excluding the pH values of the raw effluent, the pH in T3 and T4 systems reached a maximum of 7.71 and 8.40 respectively. The municipal water based nutrient solutions required less acid, around five mL of phosphoric, or eight mL of nitric acid, and almost always only required the single adjustment when the nutrient solution was replaced. The pH of the anaerobically digested effluent was not stable and this must be considered in the interpretation of these results.

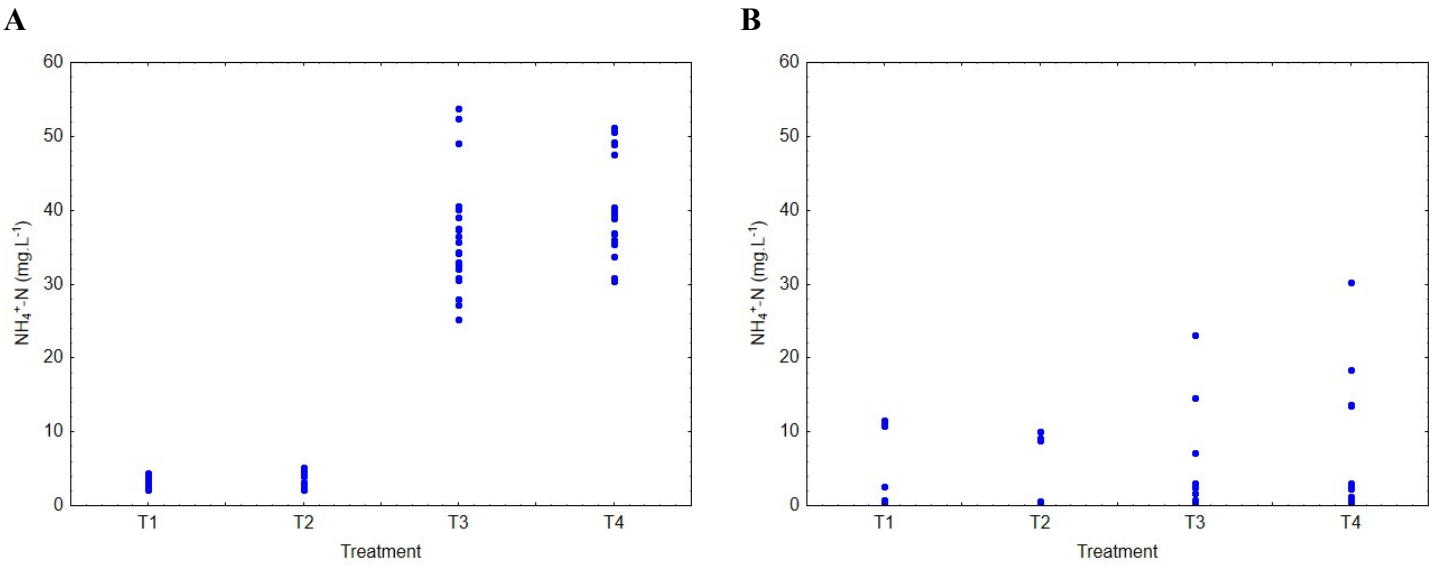

Figure 3.3 Ammonium (NH_4^+-N $mg.L^{-1}$) levels from fresh (A) and replaced irrigation solutions (B) (n=21 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid.

The municipal water and inorganic-fertilizer control treatments (T1 and T2) showed little variation between samples of fresh and replaced nutrient solutions for ammonium-nitrogen (Figure 3.3). The effluent systems had high starting concentrations of ammonium-nitrogen, which were generally reduced to very low concentrations after a few weeks in the trial (Figure 3.3).

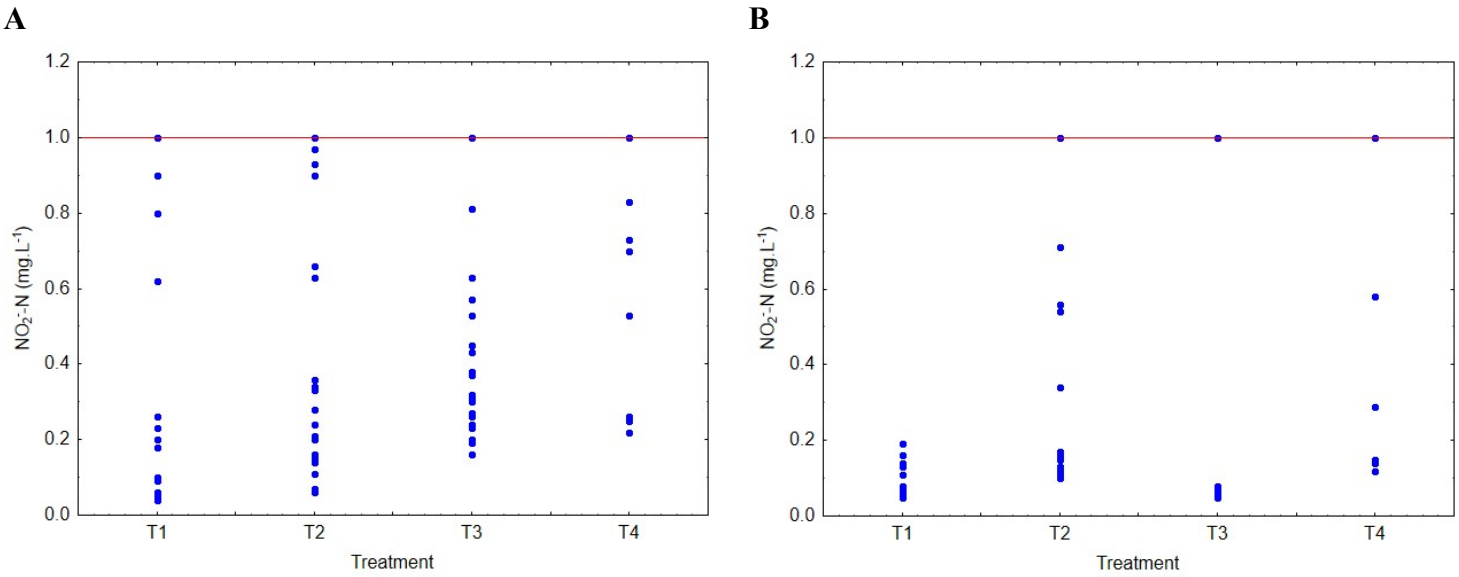

Figure 3.4 Nitrite-nitrogen (NO_2^--N mg.L^{-1}) levels from fresh (A) and replaced irrigation solutions (B) (n=21 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid. Red lines on graphs indicate the upper concentration limit of the test.

Nitrate-nitrogen levels had similar distributions in the fresh solutions, while the systems which received phosphoric acid showed very little residual nitrate-nitrogen in their waste solutions (Figure 3.4).

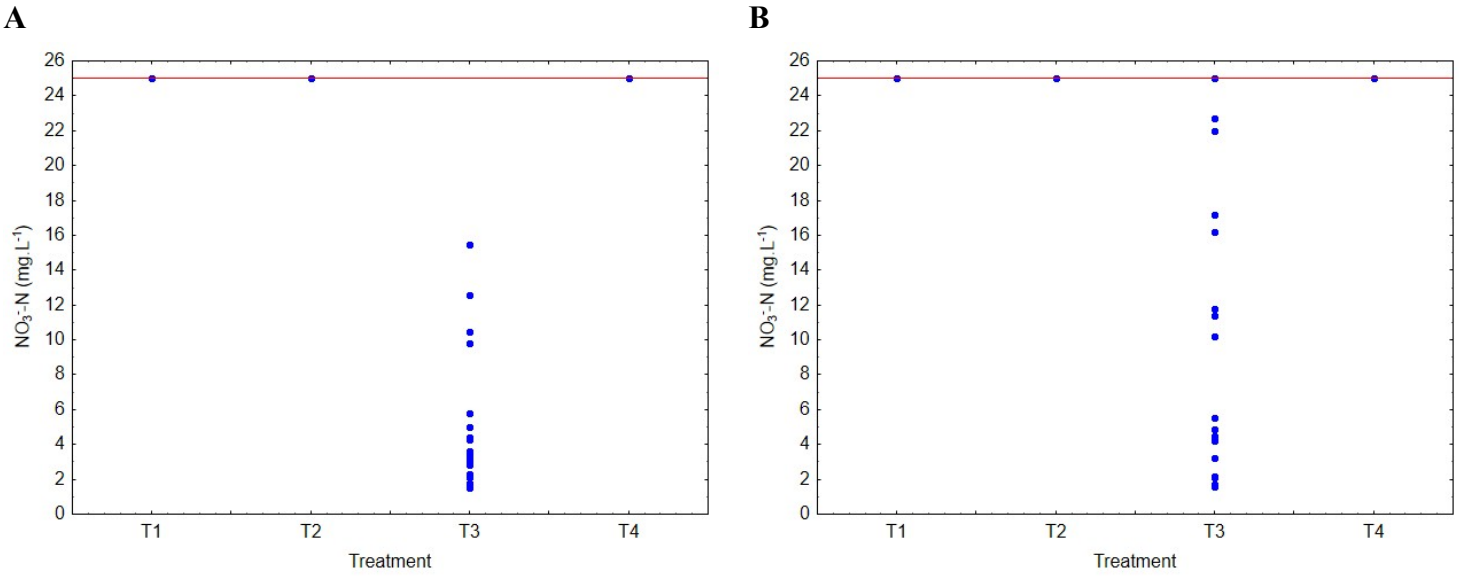

Figure 3.5 Nitrate-nitrogen (NO_3^--N mg.L^{-1}) levels from fresh (A) and replaced irrigation solutions (B) (n=21 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid. Red lines on graphs indicate the upper concentration limit of the test.

The treatments which received nitric acid (T2 and T4) had a wider range of residual nitrate-nitrogen in their replaced solutions than those which received phosphoric acid (T1 and T3).

Only T3 (post-PFP effluent and phosphoric acid) had nitrate levels within the test limit. This was to be expected given the volume of nitric acid added to the effluent in T4 and the inherent nitrogen load in the inorganic-fertilizer. The T3 system was able to remove most of the nitrate from the effluent once the plants had matured with the last three solution replacements with average values for the last three cycles of 5.2 $mg.L^{-1}$, 8.97 $mg.L^{-1}$ and 3.97 $mg.L^{-1}$ NO_3^--N on days 57, 64 and 75 respectively (Table 3.3).

Table 3.3 The final three solution nitrate-nitrogen (NO_3^--N $mg.L^{-1}$) concentrations in samples taken from three different Treatment 3 replicate systems.

T3 Sample	$NO3^-$-N $mg.L^{-1}$		
	Day 57	Day 64	Day 75
1	11.8	17.2	4.2
2	2.1	5.5	4.5
3	1.7	4.2	3.2

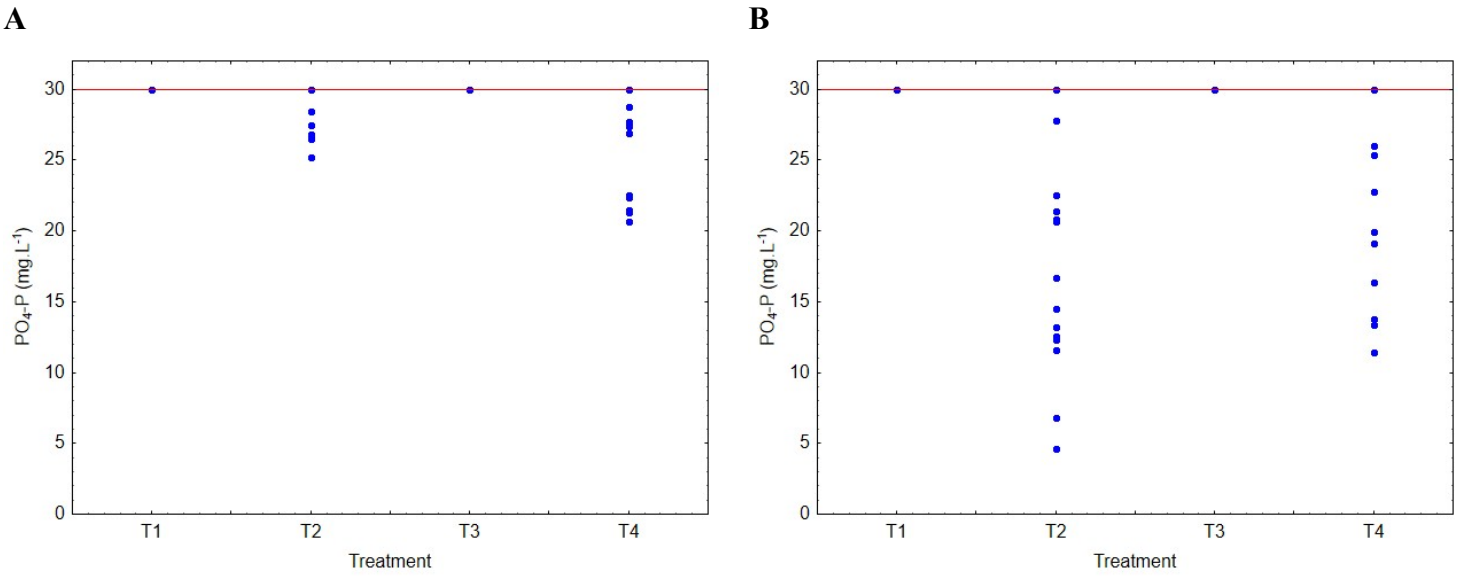

Figure 3.6 Phosphate-phosphorus (PO_4-P $mg.L^{-1}$) levels from fresh (A) and replaced irrigation solutions (B) (n=21 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid. Red lines on graphs indicate the upper concentration limit of the test.

Marginal reductions in PO_4-P were observed in the nitric acid adjusted treatments (T2 and T4). The phosphoric acid treatments had consistently high PO_4-P concentrations (Figure 3.6).

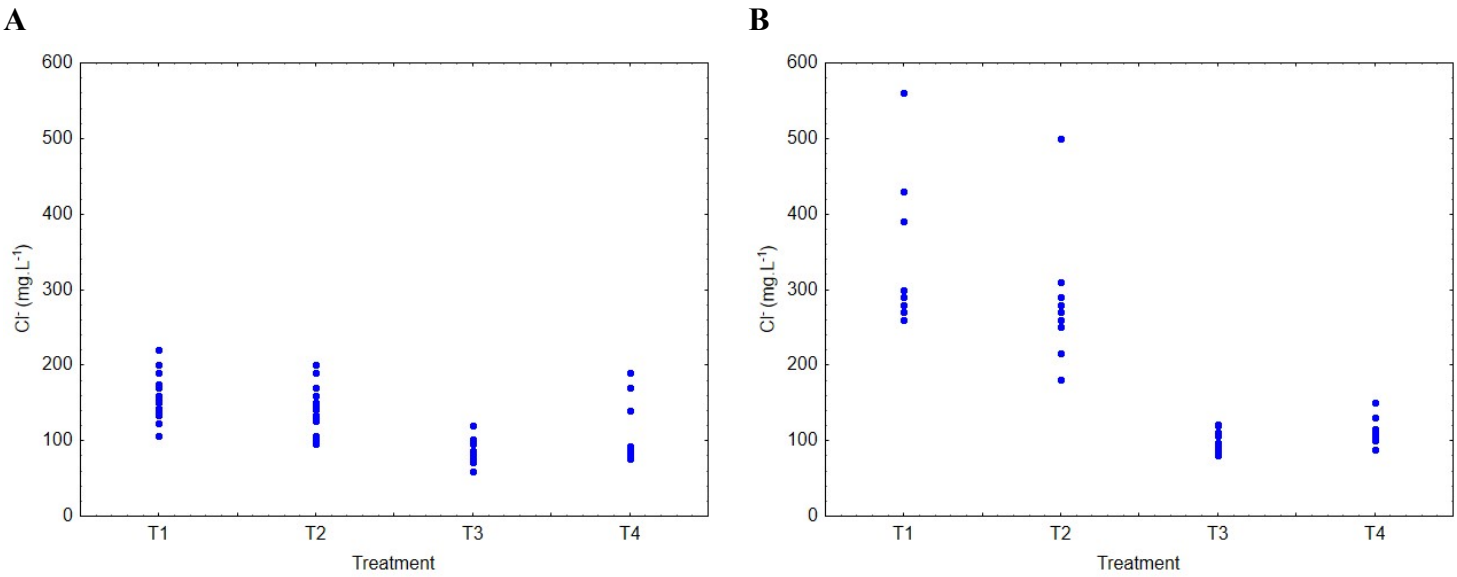

Figure 3.7 Chloride (Cl^- $mg.L^{-1}$) levels from fresh (A) and replaced irrigation solutions (B) (n=12 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid.

Chloride concentrations increased in the control treatments, irrespective of the acid used (T1 and T2). The effluent systems (T3 and T4) had stable chloride concentrations. The highest readings were recorded at the end of the trial when the plants were at their biggest and consuming the most water (Figure 3.7).

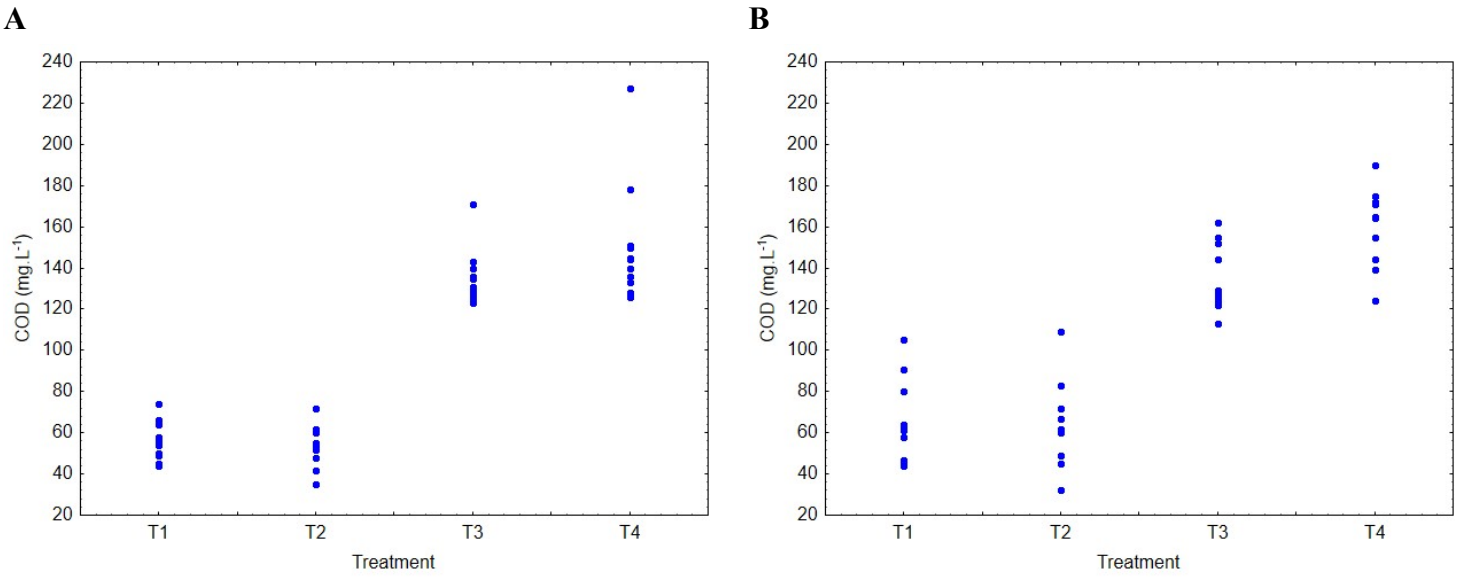

Figure 3.8 Filtered chemical oxygen demand (COD mg.L^{-1}) levels from fresh (A) and replaced irrigation solutions (B) (n=10 for each treatment). The treatment solutions indicated are: T1 and T2 – municipal water and inorganic-fertilizer, T3 and T4 – post-primary facultative pond effluent (post-PFP), T1 and T3 were subject to pH adjustment with phosphoric acid, while T2 and T4 received nitric acid.

Chemical oxygen demand did not change in any of the treatments (Figure 3.8).

Table 3.4 The mean (± standard error) dissolved oxygen saturation (%) measured in the nutrient solutions for each treatment across the trial and on day 78. The treatments comprised of municipal water and inorganic-fertilizer (municipal control; T1 and T2), or brewery effluent as post-primary facultative pond effluent (effluent; T3 and T4) for 78 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4).

	Dissolved O_2 (%)	
Treatment	Overall	Day 78
T1	87.7 ± 1.0	89.0 ± 0.6
T2	88.2 ± 1.2	90.0 ± 0.8
T3	80.1 ± 1.6	75.8 ± 3.6
T4	79.3 ± 1.9	71.2 ± 2.1

The effluent treatments appeared to have slightly lower dissolved oxygen concentrations (T3: 80.1 ± 1.6% and T4 79.3 ± 1.9%) than those in the municipal treatments (T1: 87.7 ± 1.0% and T2: 88.2 ± 1.2%). The final dissolved oxygen concentrations also appeared to be lower in the effluent systems than the overall mean concentration (Table 3.4).

3.3.2. Physical development of the plants

The stem diameter of the plants showed no interaction between factors nutrient solution and type of acid (Multifactor ANOVA $F_{(1,16)}$=0.36, p=0.55). There was no significant effect with type of acid used (ANOVA $F_{(1,16)}$=0.94, p=0.35). The nutrient solution grouped means differed significantly (ANOVA, $F_{(1,16)}$=172.83, p<0.00001; Figure 3.9). The municipal water control treatments (T1 and T2) had significantly thicker stems (13.4 ± 0.2 mm) than the effluent-fed plants (10.0 ± 0.1 mm) (Figure 3.9).

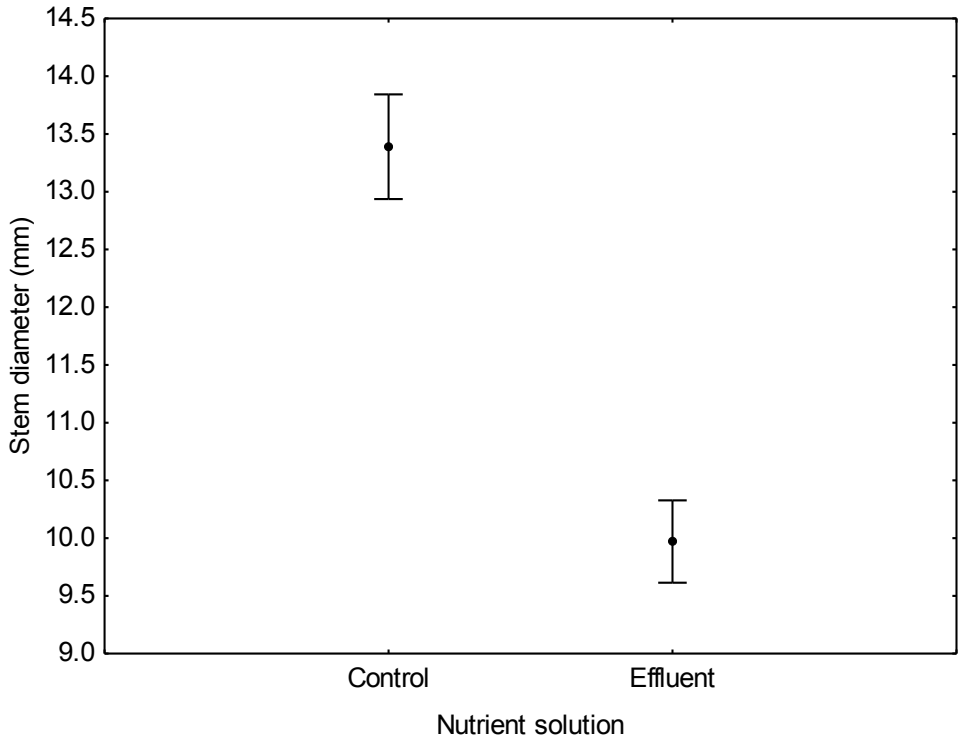

Figure 3.9 The mean (±95% confidence interval) stem diameter of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent drawn the post-primary facultative pond (post-PFP) for 78 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4) (ANOVA, $F_{(1,16)}$=172.83, p<0.00001). The results are grouped according to nutrient solution.

There was an interaction between water source and type of acid for plant height (Multifactor ANOVA, $F_{(1,16)}$=9.16, p=0.008; Figure 3.10). The municipal control systems produced plants that were similar in height compared to each other (T1: 1910 ± 53.12 mm; T2: 1873 ± 51.87 mm), but significantly taller than either of the effluent treatments (Figure 3.10). There was an

interaction between the water sources and acids as the phosphoric acid adjusted effluent treatment (T3: 1573.33 ± 50.45 mm) grew significantly taller than the nitric acid treatment (Figure 3.10).

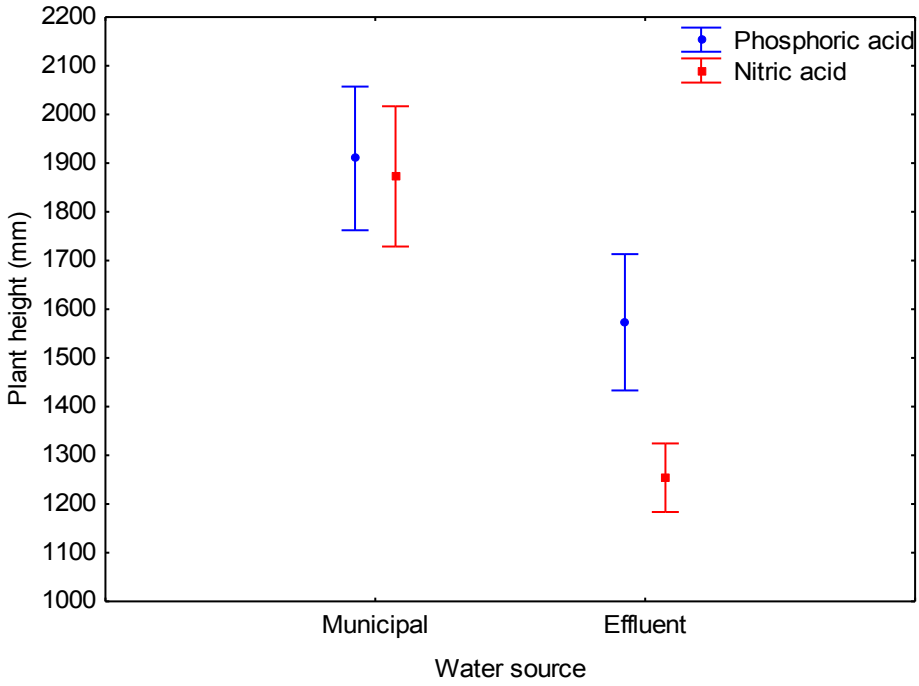

Figure 3.10 The mean (±95% confidence interval) plant height of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (Control), or brewery effluent drawn the post-primary facultative pond (post-PFP) for 78 days (Multifactor ANOVA, $F_{(1,16)}$=9.16, p=0.008). Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4).

There was no significant interaction between nutrient solution and type of acid used when comparing the results of the number of fruit developed per plant (Multifactor ANOVA, $F_{(1,16)}$=0.08, p=0.78). There was no significant difference in the number of fruit produced per plant between acid treatment groups with 30 ± 3 fruit per plant (ANOVA, $F_{(1,16)}$=1.05, p=0.18). However the nutrient solutions resulted in a significant effect on number of fruit produced per plant (ANOVA, $F_{(1,16)}$=51.11, p<0.00001; Figure 3.11). The municipal water and inorganic-fertilizer systems (T1 and T2) produced significantly greater numbers of fruit per plant (42 ± 3 fruit per plant) than the effluent plants (T3 and T4; 18 ± 1 fruit per plant) after 78 days (Figure 3.11).

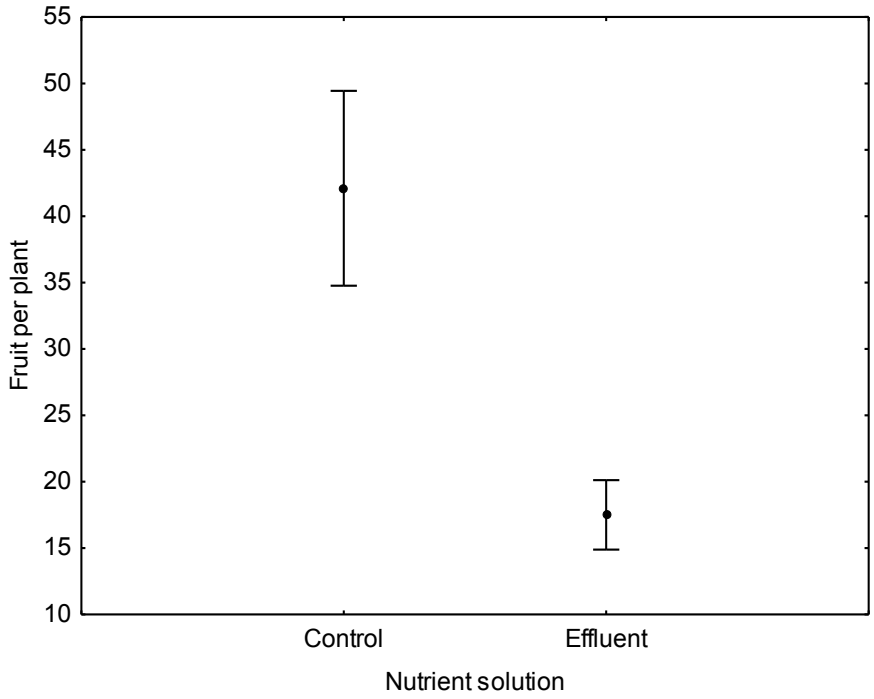

Figure 3.11 The mean (±95% confidence interval) number of fruit produced per Moneymaker tomato plant subject to treatments of municipal water and inorganic-fertilizer (municipal control; T1 and T2), or brewery effluent as post-primary facultative pond effluent (effluent; T3 and T4) for 78 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4) (ANOVA, $F_{(1,16)}$=51.11, p<0.00001). Treatments are grouped according to nutrient solution.

There was no significant interaction between nutrient solution and type of acid used for the effect on fruit mass (Multifactor ANOVA, $F_{(1,16)}$=2.99, p=0.10). Nutrient solution had a significant effect with effluent-grown fruit (23.51 ± 2.30 g) weighing significantly less than the fruit grown in the municipal water and inorganic-fertilizer (43.06 ± 1.28 g) (ANOVA, $F_{(1,16)}$=65.68, p<0.00001; Figure 3.12). The acid treatments had no effect on fruit size (phosphoric acid: 31.41 ± 3.06 g and nitric acid: 35.16 ± 4.24, ANOVA, $F_{(1,16)}$=2.41, p=0.14). In summary, the effluent-grown plants produced fewer, smaller fruit and did not grow as large as the inorganic-fertilizer plants.

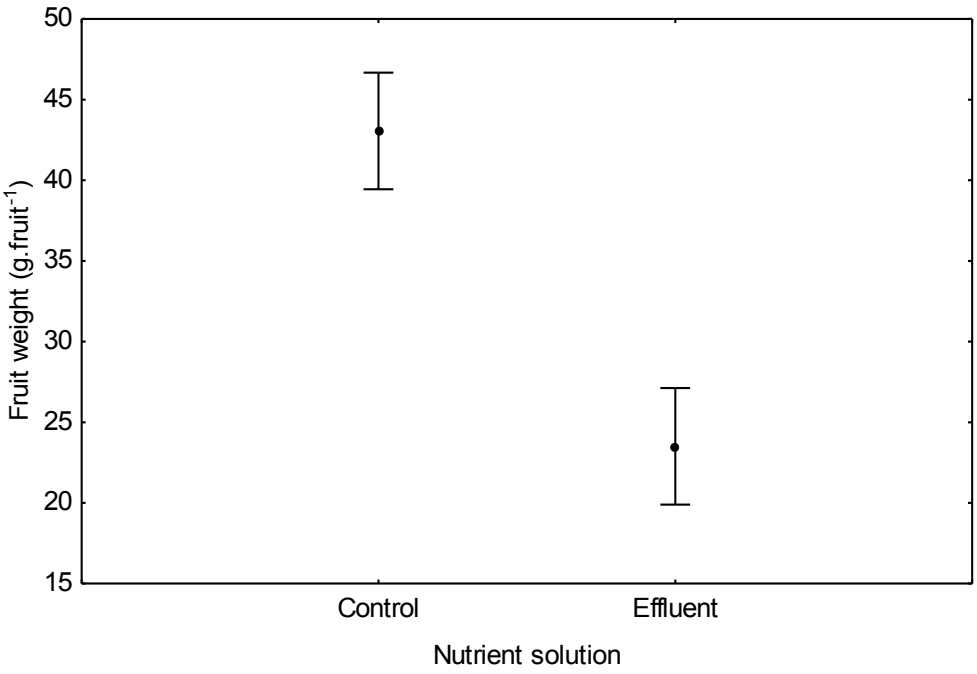

Figure 3.12 The mean (±95% confidence interval) individual fruit weight (g) produced by Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (municipal control; T1 and T2), or brewery effluent as post-primary facultative pond effluent (effluent; T3 and T4) for 78 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4) (ANOVA, $F_{(1,16)}$=65.68, p<0.00001). Treatments are grouped according to nutrient solution.

Most plants in the inorganic-fertilizer systems did not experience fruit failure (Figure 3.13). Only two of the inorganic-fertilizer systems experienced fruit failure with one system treatment 2 replicate 1, having a fruit failure rate of 2.3% (Figure 3.13). Treatment 2 replicate 3 had a fruit failure rate of 0.8%. A large proportion of the plants in the effluent treatment systems experienced fruit failure (Figure 3.13). Treatment 3 had an average failure rate of 22.92% and treatment 4 an average rate of 9.55% (Figure 3.13).

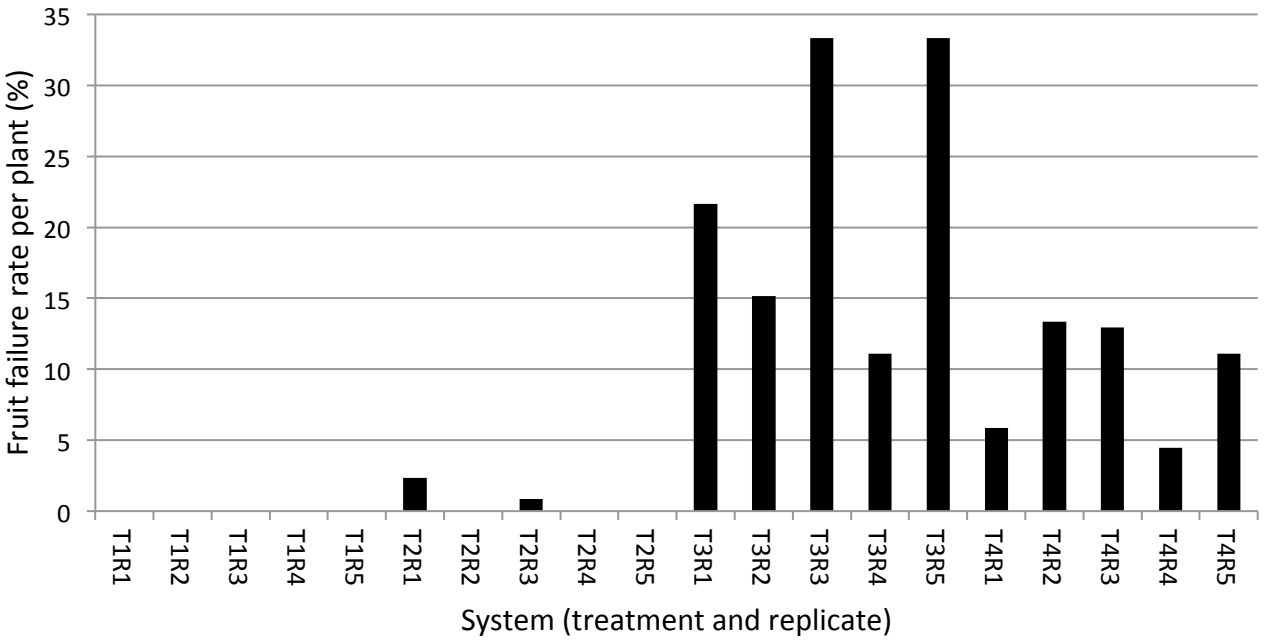

Figure 3.13 The percentage of fruit failure by Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (municipal control; T1 and T2), or brewery effluent as post-primary facultative pond effluent (effluent; T3 and T4) for 78 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4). Results are grouped by replicate.

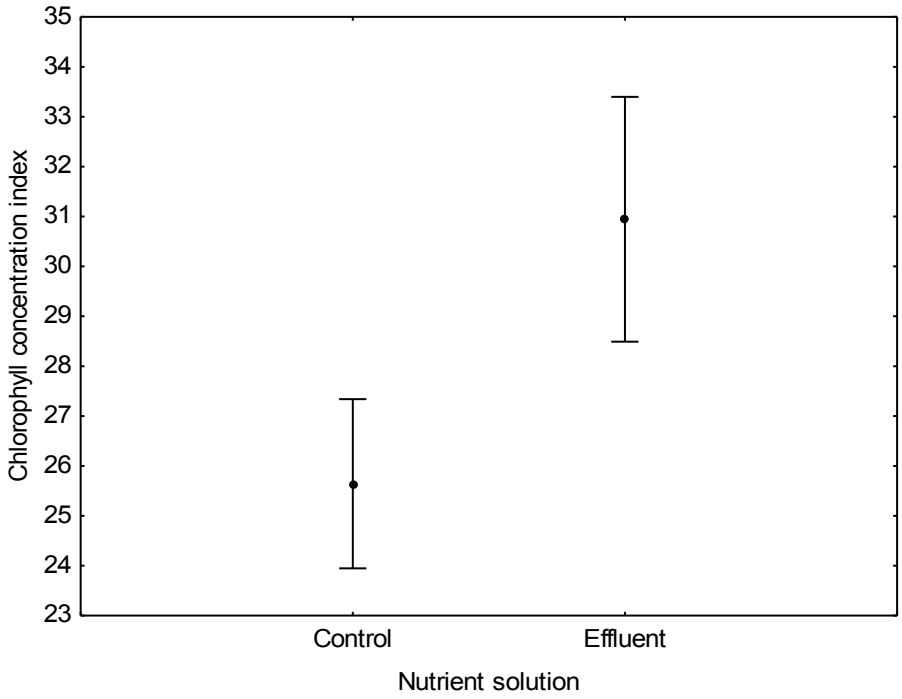

Figure 3.14 The mean (±95% confidence interval) chlorophyll concentration index (CCI) of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (municipal control; T1 and T2), or brewery effluent as post-primary facultative pond effluent (effluent; T3 and T4) measured after 70 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4) (ANOVA, $F_{(1,16)}$=17.62, p=0.00068). Treatments are grouped according to nutrient solution.

No significant interactions between the nutrient solutions and type of acid used were observed for chlorophyll concentration index (CCI) (Multifactor ANOVA, $F_{(1,16)}$=0.00, p=0.98). A comparison between the nutrient solutions, irrespective of the type of acid used showed that the effluent plants had significantly higher chlorophyll concentration values (effluent CCI: 30.94 ± 1.08) than those grown in municipal water and inorganic-fertilizer (25.64 ± 0.75) (ANOVA, $F_{(1,16)}$=17.62, p=0.00068; Figure 3.14). There was no significant difference between the CCI values of the acid treatments (ANOVA, $F_{(1,16)}$=3.40, p=0.084).

Effluent plants had consistently higher foliar CCI than those recorded from the municipal water and inorganic-fertilizer grown plants (Repeated measures ANOVA, $F_{(16,256)}$=4.56, p<0.00001; Figure 3.15).

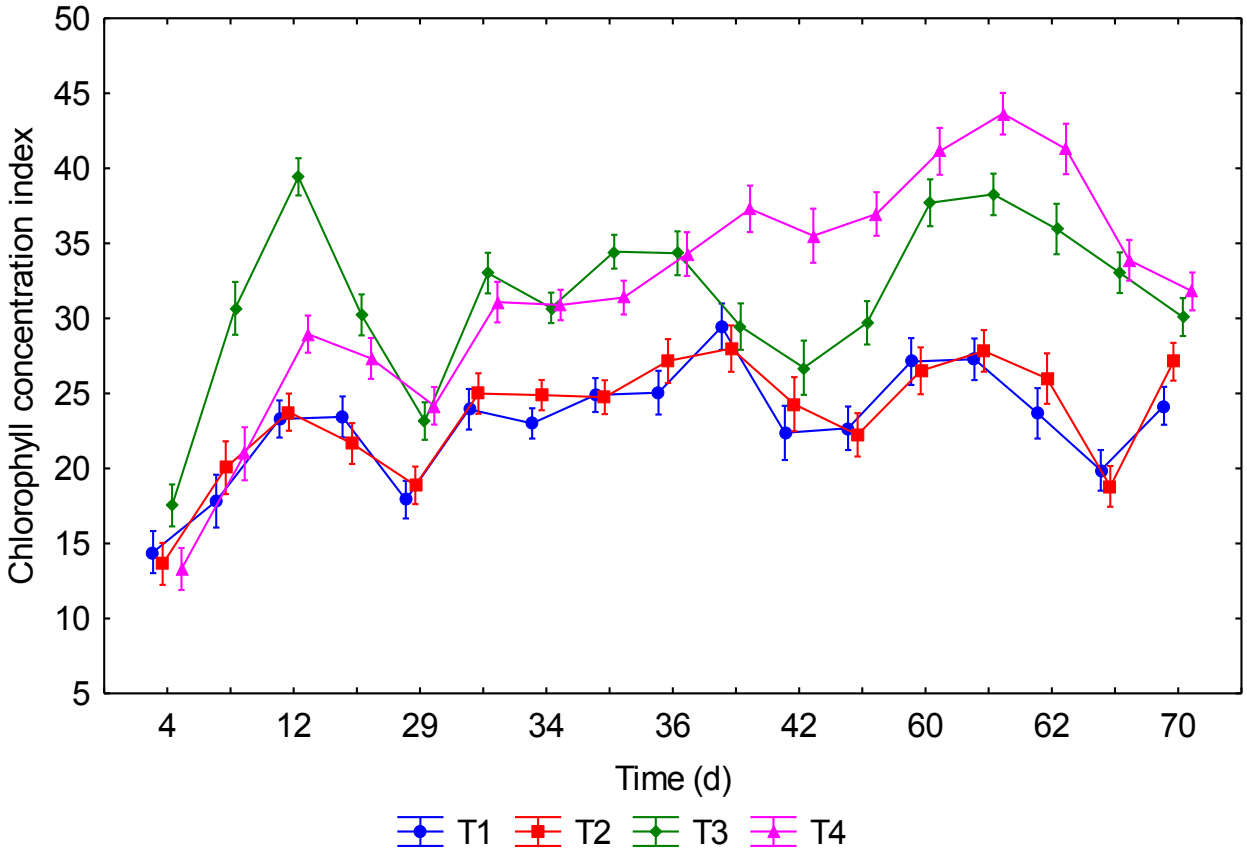

Figure 3.15 The mean (± standard error) chlorophyll concentration index (CCI) of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (municipal control; T1 and T2), or brewery effluent as post-primary facultative pond effluent (effluent; T3 and T4) measured after 70 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4) (Repeated measures ANOVA, $F_{(16,256)}$=4.56, p<0.00001).

The nitrogen concentrations in the T4 fruit was higher compared to the other treatments; inorganic-fertilizer fruit (T1 and T2), and the post-PFP effluent and phosphoric acid (Table 3.5). The sodium concentrations in both effluent systems were higher than the municipal controls (Table 3.5). The sodium levels, compared between acid treatments, are eight and ten times higher in the effluent systems compared to their respective acid counterparts in the municipal control systems (Table 3.5).

Table 3.5 The fruit tissue chemical analysis of Moneymaker tomato plants subject to treatments of municipal water and inorganic-fertilizer (municipal control; T1 and T2), or brewery effluent as post-primary facultative pond effluent (effluent; T3 and T4) measured after 70 days. Treatments were subject to pH correction with phosphoric acid (T1 and T3) or nitric acid (T2 and T4). These are the raw data of the two samples per treatment that were tested.

		N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B	
		mg.100g^{-1} fresh mass					mg.kg^{-1} fresh mass						Water %
T1	Sample 1	168.00	25.95	184.00	9.50	8.20	55.20	0.60	3.10	0.30	1.20	0.30	93.30
	Sample 2	156.00	26.16	184.00	8.40	8.10	47.00	0.50	2.30	0.20	1.10	0.30	93.49
T2	Sample 1	164.00	21.20	170.00	6.50	7.10	53.60	0.60	1.80	0.20	1.10	0.20	94.17
	Sample 2	161.00	21.91	168.00	6.50	7.40	64.70	0.60	1.80	0.20	1.20	0.20	94.10
T3	Sample 1	193.00	35.35	121.00	3.60	6.50	404.00	0.50	2.20	0.10	0.90	0.20	93.49
	Sample 2	212.00	38.44	124.00	3.90	7.80	395.70	0.70	3.00	0.10	1.10	0.40	92.95
T4	Sample 1	266.00	33.07	106.00	4.40	7.80	564.70	0.70	4.40	0.20	1.50	0.30	91.55
	Sample 2	266.00	32.65	104.00	4.00	7.80	554.60	0.70	4.00	0.20	1.50	0.20	91.66

3.3.3. Visual stress symptoms

The effluent-grown plants in treatments 3 (phosphoric acid) and 4 (nitric acid) displayed a variety of visual stress symptoms. Similar to the results of experiment 1, blossom-end rot affected many of the fruit grown in the effluent systems (Figures 3.13 and 3.16).

Figure 3.16 Fruit affected by blossom-end rot (BER). These fruit were grown in both effluent treatments (treatment 3 with phosphoric, and treatment 4 with nitric acid adjustment). The images on the left are from treatment 3 and the images on the right from treatment 4. The images on the left show the early stages of BER while the images on the right show the advanced stages with the fruit ripening.

Figure 3.17 Leaf-tip yellowing and tissue necrosis seen on a plant grown in post-primary facultative pond effluent with pH adjustment with nitric acid (Treatment 4).

Figure 3.18 Leaf-tip yellowing and tissue necrosis on effluent-grown plants. Treatment 1 is shown on top (municipal water and inorganic-fertilizer with phosphoric acid pH adjustment), Treatment 3 is shown on the left (effluent with nitric acid pH adjustment) and treatment 4 on the right (effluent with phosphoric acid pH adjustment).

Slight leaf discolouration and leaf-tip necrosis were observed in plants from both effluent treatments (Figures 3.17 and 3.18). Partial root-rot and root discolouration were observed in a few plants in the effluent treatments early on in the trial but the plants recovered and continued to develop (Figure 3.16). There was no evidence of leaf or root discolouration or necrosis in the municipal water and inorganic-fertilizer treatments (T1 and T2).

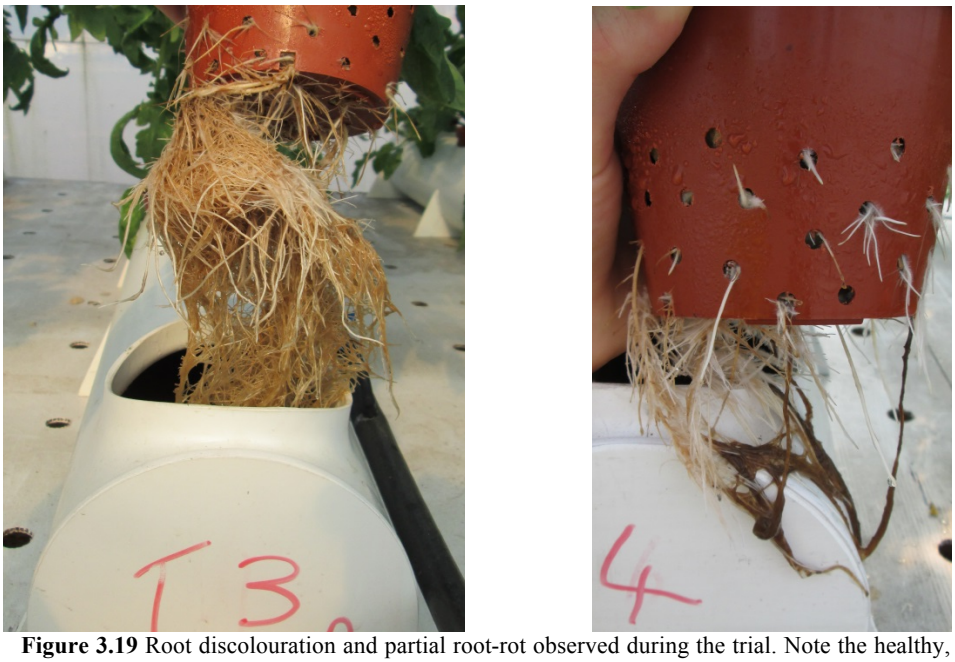

Figure 3.19 Root discolouration and partial root-rot observed during the trial. Note the healthy, bright white roots which are indicative of healthy roots. The plants recovered and continued to grow. The treatments shown are treatment 3 on the left (effluent with phosphoric acid pH adjustment) and treatment 4 on the right (effluent with nitric acid pH adjustment).

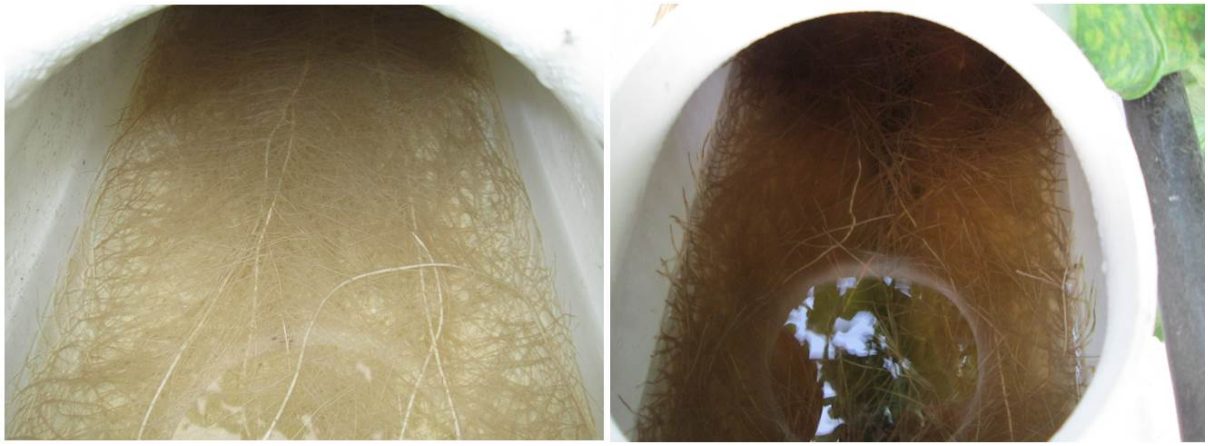

Figure 3.20 A visual comparison of the roots growing in a municipal water system (left) and an effluent system (right).

Despite the root stress observed, the plants seemed to recover well and did develop substantial root systems in both the municipal and effluent treatments (Figure 3.20). The darker colour in the effluent systems is the result of the staining effect of the effluent and not a stress symptom.

Figure 3.21 Symptoms of heat stress and tissue damage on a municipal water and inorganic-fertilizer treatment on the left, and an effluent treatment on the right.

Figure 3.22 Wilting plants in the effluent treatments at the end of the trial.

Some plants, across all the treatments, showed symptoms of heat stress, particularly on growing tissue or near the extremities of the plant (Figure 3.21). The impact was not deemed significant as typically it was only a small area of a single stem or leaf. It was however indicative of the effect of the heat in the greenhouse tunnel. Air temperature in the greenhouse tunnel ranged from 16 to 41 °C with a mean of 26 ± 5 °C (n=38).

Some plants began to wilt towards the end of the trial. Many plants from T3 and T4 systems were severely affected and the trial was terminated as a result of this (Figure 3.22). When the plants were examined during the final stages of the trial, it was found that many of the plants were affected by severe root rot (Figure 3.23). The occurrence of root necrosis was limited to effluent-fed plants and affected systems from both acid treatments. Despite the apparent severity of the necrosis, new, bright white root shoots were observed among the rotten material, similar to what had happened earlier in the trial (Figures 3.19 and 3.23).

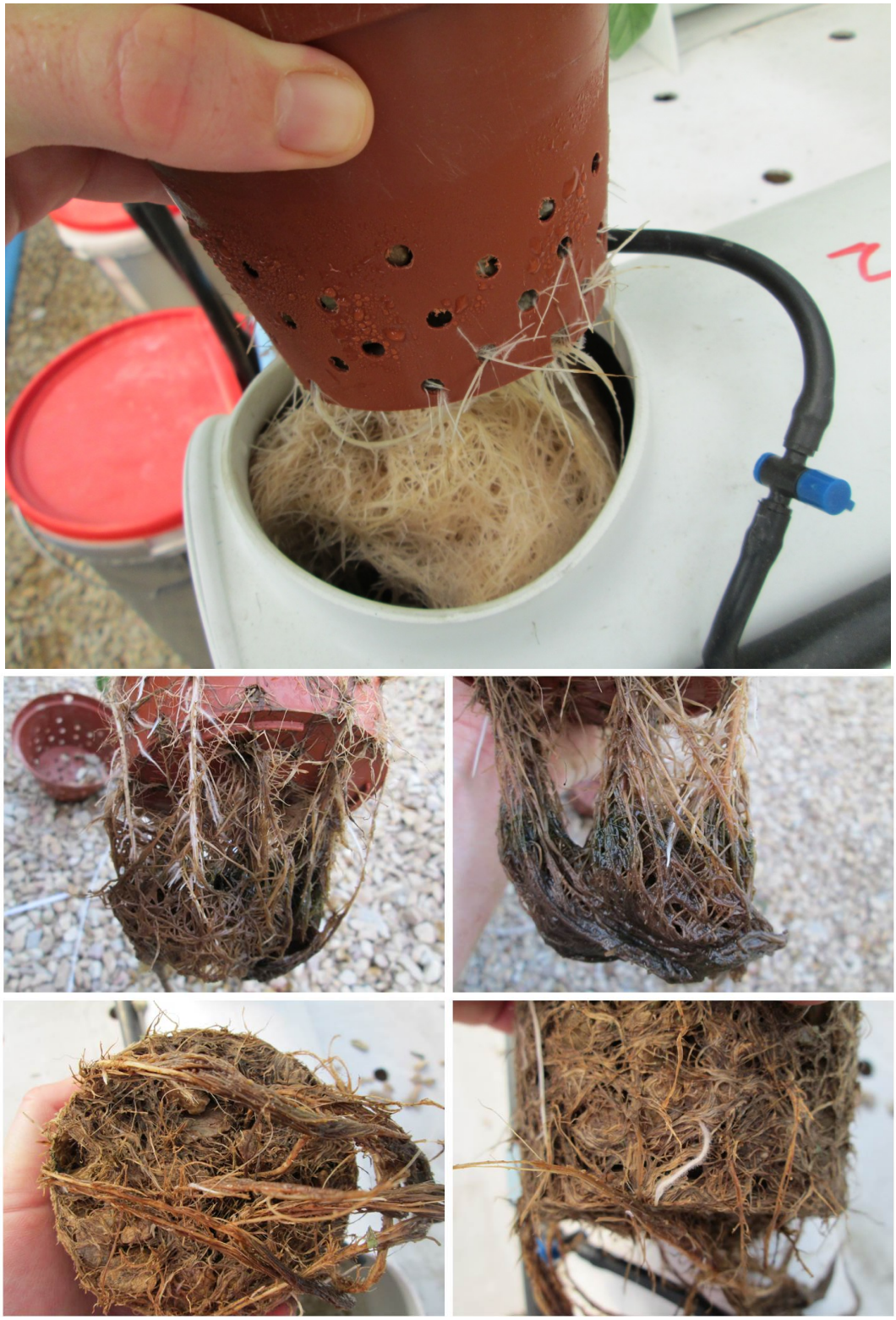

Figure 3.23 The top picture shows part of the root mass of a plant from the inorganic-fertilizer treatments. The density, size and white colour of the root system indicate healthy roots. The middle and bottom rows show the rotten root mass found in the effluent treatments after the termination of the trial. Note the white root shoots indicating new root growth.

3.4. Discussion

3.4.1. Plant stress

The greenhouse in which the plants were grown may have been a contributing factor to plant stress (Figure 3.21). The tunnel had large doors on either end which could be opened to allow more air to move through the tunnel. Even with these doors open, the temperature in the tunnel could reach 35 °C, the point at which heat stress in tomato plants has been demonstrated to occur (Rivero *et al*. 2001). The temperature in the tunnel was sometimes as high as 40 °C. May 1st was the hottest day on record during the trial, with a maximum external air temperature of 34 °C (Weather Underground 2013). This temperature was recorded at the Port Elizabeth airport weather station, not inside the greenhouse where the temperature exceeded 40 °C. All the plants were grown in the same environment, and the purpose and conclusions of the trial were not compromised. It has been shown that tomato plants can tolerate fluctuating temperatures, within certain limits and extremes, with no overall effect on growth or yield within a range of above 14 °C and less than 26 °C (Adams *et al*. 2001). However outside of that range, fruit development and yield is negatively affected (Adams *et al*. 2001). Temperature is a factor that should be considered in future trials, although it is probably not necessary to design complete environmental regulation. Avoiding the extremes currently experienced in the greenhouse would probably suffice.

Heat stress, along with high salinity and ammonium nutrition has also been linked with blossom-end rot occurrence (Saure 2001, Magán *et al*. 2008). Ion competition and nutrient stress contribution to plant development disorders was discussed in Chapter 2. In experiment 2, blossom-end rot occurrence, or fruit failure (combined BER and flower drop), was more prevalent in the effluent systems than the control systems, regardless of which acid was used. This suggests that the effluent was an aggravating factor in fruit failure but does not clearly

identify the cause. Hartman *et al*. (1986) recorded an increase in BER occurrence and a decrease in fruit size with increases in the proportion of ammonium fed to plants. However, they also observed no differences in the concentration of calcium in the fruit grown in the various ammonium concentrations, suggesting other synergistic variables affecting the incidence of BER (Hartman *et al*. 1986). The chloride tests suggested that the effluent contains no more, and frequently less chloride than the municipal systems, but the tissue analysis showed the presence of high concentrations of sodium. Sodium is probably a more influential factor than chloride in total salinity in the effluent. Increased salinity was shown to reduce fruit number, weight and increase BER (Magán *et al*. 2008). It is most likely that a combination of heat stress, ammonium-induced cation deficiency, and salt stress caused mainly by sodium concentrations contributed to overall fruit failure (Saure 2001, Britto & Kronzucker 2002, Magán *et al*. 2008).

Plant stress also occurred as root necrosis in both effluent treatments regardless of which acid was used. *Pythium* root rot in hydroponic crops is mainly caused by pathogenic species of the genus *Pythium* including *Pythium aphanidermatum*, *Pythium ultimatum*, *Pythium intermedium* and *Pythium irregular* among others (Sutton *et al*. 2006). These principal species are common in the environment and can be introduced into hydroponic systems via airborne dust, contaminated tools, clothing or plants, or in water used for nutrient solution preparation (Sutton *et al*. 2006). The greenhouse in which these experiments were conducted is not bio-secure or sterile and the infections could have originated from any of the aforementioned sources. The primary facultative pond is a breeding ground for micro-flora and fauna and could well have been the source of the pathogens, given the nearly ubiquitous occurrence of the infections in the effluent systems at the end of the trial. The presence of *Pythium* pathogens alone does not always result in root necrosis (Sutton *et al*. 2006). Environmental factors such as solution temperature, presence of phenolic compounds and

dissolved oxygen concentrations have also been found to increase the susceptibility of hydroponic crops to root rot (Chérif *et al*. 1997, Sutton *et al*. 2006).

While the dissolved oxygen levels were lower in the effluent systems than those in the municipal systems, the conditions were not hypoxic (<30% saturation) so it is difficult to ascertain whether the oxygen levels were responsible for, or contributed to root rot. The lower oxygen levels could be indicative of higher microbial metabolic activity within the effluent nutrient solution which is known to reduce saturated oxygen concentration (Soffer *et al*. 1991). This is possible as the effluent solutions were "seeded" with microbes drawn from in the facultative pond along with the effluent. The microbial load in the nutrient solutions was not quantified so a definitive conclusion cannot be made here. There is an opportunity for further research to determine the influence of the microbial load. The lower oxygen concentrations were possibly a contributing factor to the outbreak of *Pythium* root rot, but additional nutrient and temperature stresses probably contributed to the susceptibility of the effluent plants.

Leaf-tip yellowing and necrosis were observed but, without foliar elemental analysis, the cause of this condition cannot be confirmed. The symptoms occurred in both effluent treatments. Leaf chlorosis can be symptomatic of a wide range of nutrient stresses including potassium, magnesium, iron or manganese (Freeman 2005, Roberto 2005). Further work is required to identify the specific nutrient stress that contributed to the leaf chlorosis and necrosis observed in this study.

The concentration of sodium in the fruit tissue of the effluent plants was higher than in the control treatments. This was similar to the results of Experiment 1 (Chapter 2), in which the effluent-grown fruit had higher concentrations of sodium than the control plants. Sodium stress and secondary induced stresses can restrict plant growth and increase the incidence of

blossom-end rot (Grattan & Grieve 1999, Magán *et al*. 2008, Chapter 2). The combined effects of ammonium, sodium, water, heat and other as yet unidentified stresses may have contributed to the outbreaks of *Pythium* root rot and the incidence of BER.

Using brewery effluent as a hydroponic nutrient solution presents a variety of physical, chemical or nutritional challenges which negatively affect the plants. The causes of the observed stress symptoms require further investigation and redress if the effluent is to be used effectively as a nutrient and water resource. In the case of outbreaks such as *Pythium* root rot, ultraviolet disinfection of the irrigation solution has been shown to remove pathogens and could potentially be employed in this situation (Zhang & Tu 2000). The key is to identify the major contributing cause of a given disorder.

3.4.2. Water quality and nutrient removal

There were problems with managing the effluent alkalinity, regardless of which acid was used. The effluent systems required large and repeated pH corrections with acid. This fluctuation demonstrates the gas-stripping effect observed during the aeration of anaerobic digester effluent (Musvoto *et al*. 2000a). The pH management of the effluent compromises the nutrient removal potential of the plants because the amount of acid required to adjust the pH creates extremely high concentrations of the compound associated with the acid. In addition, the fluctuations in pH affect the bioavailability and hence inhibit the removal of nutrients (Lucas & Davis 1961). This is shown in the water quality data for the replaced effluent solutions. The phosphoric acid effluent treatment was able to remove ammonium, nitrate and nitrite, but phosphate levels were above the range of the test. The opposite was noted in the nitric acid systems which were able to consume some of the effluent phosphate, although complete removal was not achieved, but nitrate readings were above the range of the test. This trial did not demonstrate effective nutrient removal potential. The management of post-anaerobic digestion alkalinity is a major challenge to using brewery effluent as a

nutrient source for plants, or for using plants to remove nutrients from brewery effluent. Further research must find a solution that addresses the pH adjustment and nutritional needs of the plants, and the required nutrient removal of the wastewater treatment.

Chemical oxygen demand is used as a measure of wastewater quality and organic load at the brewery (Mabuza *pers. comm.* 2012). The data collected in Experiment 2 show that a hydroponic system such as the one used in this trial cannot reduce the concentration of chemically oxidisable compounds in the effluent. Some biological treatments using various plants in constructed wetlands have been successful in reducing the COD of different effluents (Kivaisi 2001, Konnerup *et al*. 2009, Taylor *et al*. 2011). The main mechanisms for nutrient, suspended solids removal and COD/BOD reduction in constructed wetlands are sedimentation, filtration, adsorption, plant uptake and microbial metabolism (Lee *et al*. 2004, El-Khateeb *et al*. 2009, Ong *et al*. 2009, Fan *et al*. 2013). The experimental system design specifically included a substrate-free root zone so as to eliminate as many of the substrate-related processes as possible and focus on the plant-effluent relationships. The minimal amount of gravel in each system restricted the substrate-bound microbial growth and therefore its effect on effluent quality, which allows the focus to remain on the effects of the plants (Taylor *et al*. 2011). The minimal amount, and relatively coarse structure of the gravel used in these experiments also minimizes the active surface area of the substrate, and therefore its filtration or adsorption potential (Bigambo & Mayo 2005). By the time the effluent had passed through the equalization tank, neutralization tank, anaerobic digester and facultative pond, most of the settleable suspended solids were removed from the effluent. This largely eliminates the actions of filtration, sedimentation, substrate-microbial action, and adsorption which is possibly why the COD removal is relatively poor in this trial. There are other potential causes for these residual COD levels related to anaerobic digestion which are dealt with in Chapter 4. Plant nutrient uptake and COD reduction are largely incompatible

processes when isolated as they were in this trial, particularly after anaerobic pre-treatment of the effluent. They can function well as part of a broader symbiotic wetland system, particularly by providing support for microbial biofilm development, but they have little direct effect on one another (Osem *et al*. 2007, Ding *et al*. 2011). The intermediate physical, chemical and microbiological processes, largely related to the substrate material, could be the missing link in COD reduction (Ding *et al*. 2011).

Another contributor to the residual COD in the final effluent could be the anaerobic digestion process itself. Inert COD, either as incoming unbiodegradeable COD or inert microbial products from the digestion process form a fraction of the total chemically oxidisable COD in anaerobically digested effluent (Kaspagil Ince *et al*. 2000). This may partially account for the inability to reduce the COD of the effluent in the hydroponic systems.

3.4.3. Plant and fruit development

This trial confirmed that Moneymaker tomato plants can be grown in brewery effluent. The effluent however, does not contain the optimum balance of available nutrients as demonstrated by a comparison to the performance of the municipal water and inorganic-inorganic-fertilizer plants, and the stress symptoms observed during the trial. The chemistry of the effluent, the nutrient load, and particularly the alkalinity, are inhibiting factors in plant growth and development.

The effluent grown plants, regardless of which acid was used, developed similarly to each other, but significantly poorer than the control plants when stem diameter, fruit per plant and average fruit weight were measured. The only difference in plant development between the effluent systems was plant height. The T3 (phosphoric) acid effluent plants grew taller than the nitric acid (T4) effluent plants. Plants with more available nitrogen should have more vigorous physical development (Freeman 2005, Roberto 2005). Restricted access to other

nutrients through pH fluctuations or ionic competition may have reduced the plant development here.

One of the inhibiting effects on plant growth may have been the fluctuating, and higher maximum pH of the effluent systems compared to the municipal systems. The effluent systems required large and repeated corrections with their respective acids.). The primary cause was probably the aeration of the anaerobic effluent which caused a loss of acidity and an increase in pH (Musvoto *et al*. 2000a - Section 3.4.2.). The fluctuations in the pH of the effluent systems were large, with T3 (phosphoric acid) and T4 (nitric acid) having maximum pH values of 8.38 and 8.78 respectively, and maximum post-correction (i.e. maximum pH values after at least one acid correction to pH 6.5) pH of 7.71 (T3) and 8.40 (T4). The bioavailability of nutrients has been shown to vary with key nutrient restrictions beginning to occur with pH levels above 7.5 (Lucas & Davis 1961). The pH fluctuations may have contributed to plant stress by restricting the availability of certain nutrients (Lucas & Davis 1961). It should be noted that from these observations, the nitric acid effluent systems (T4) had wider fluctuations of the solution pH than the phosphoric acid corrected systems. These fluctuations may have shocked the plants or cause intermittent nutrient stress (Lucas & Davis 1961). This intermittent stress and the difference in fluctuations between the acid treatments could explain why the effluent treatment with the higher nitrogen content (T4), produced shorter plants than the phosphoric acid effluent treatment (T3).

The effluent plants had similar chlorophyll concentration index (CCI) values to one another when compared over the course of the trial, or their final values, irrespective of which acid was used. The municipal plants had significantly lower CCI values than the effluent plants, independent of which acid was used. The CCI data followed a similar profile to the results of experiment 1. The high ammonium concentrations in the brewery effluent are influenced by the foliar chlorophyll concentration values in Treatment 3 and 4 (Horchani *et al*. 2010,

Chapter 2: Section 2.4.4.). The similarity of CCI results between the nitric acid (T3) and phosphoric acid (T4) effluent systems suggest that the effluent plants were suffering from alternative, or broader stresses that solely nitrogen deficiency. While the T4 systems were able to remove or consume nearly all of the available nitrogen in the last few weeks of the trial, the T3 systems had abundant nitrate in the waste solutions over the same period. Despite the difference in available nitrogen, the effluent plants had a similar CCI. This only explains the effect of ammonium on CCI levels, not on plant development. The restricted development of the effluent plants compared to the inorganic-fertilizer plants is probably the result of other nutrient stresses, among them a nitrogen deficiency.

Nutrient accumulation variance in the fruit tissue is difficult to assess because of the small sample size. One difference that can be seen is the sodium concentration in the fruit tissue. The effluent plants, consistent with the results of the acid adjusted fruit in experiment 1, had very high concentrations of sodium in their fruit. Sodium and chloride content in tomato leaves increased as root substrate salt concentrations increased (Pasternak *et al*. 1986). Sodium stress was shown to increase calcium stress which can increase the incidence of blossom-end rot (Chapter 2: Section 2.4.2.).

3.5. Conclusions

There is some potential to produce tomato fruit using pH adjusted brewery effluent as a nutrient and nutrient solution. The effluent does contain sufficient nutrients to allow for the growth, development and fruiting of “Moneymaker” tomato plants however there are multiple stress factors inhibiting the growth and development of the plants. The alkalinity of the effluent contributed to plant stress and reduced plant growth and yield. The balance of nutrient supply and removal is unresolved as nitrogen supplementation with nitric acid provided no benefit to the crops as compared to phosphoric acid across various measurements

and in both nutrient solutions. Therefore the first null hypothesis; *"plant development and productivity is not influenced by an interaction between nutrient solution and type of acid used to adjust the pH*" was not rejected (Multifactor ANOVA, $p>0.5$). The nutrient solution factor was shown to be a significant factor across various assessments and therefore the second null hypothesis; "*plants grown in brewery effluent will have the same rate of physical development and fruit production as plants grown in municipal water and inorganic-fertilizer*" was rejected (Multifactor ANOVA, $p<0.05$). The final null hypothesis; "*plants grown in nutrient solutions with different inorganic acids will have the same rate of physical development and fruit production*" was not rejected as the different acids had no significant effect on the plants within the nutrient solutions (Multifactor ANOVA, $p>0.5$). The effluent plants may still be nitrogen limited, but it is only one of many potential stresses affecting plant development. Sodium toxicity, general salt stress, magnesium deficiency and ammonium-rich nutrition should all be considered in future work. A broader perspective of the sources and flows of the essential and stress-inducing elements through the brewery effluent cycle is required to address the *end-of-pipe* nutrient, stress, and alkalinity problems identified in this experiment.

Chapter 4: Discussion

The two experiments presented in this thesis demonstrated the nutrient and water potential in anaerobically digested brewery effluent. The downstream nutrient and water potential of the effluent is subject to the operation of the anaerobic digester (AD). This discussion includes the function of the AD and the implications on effluent chemistry with particular focus on the weak carbon and nitrogen acid-base systems. It then moves to the effluent-plant dynamics and considers the main difficulties with hydroponic plant production, nutrient dynamics and water reuse. It then considers brewery effluent and its potential as an alternative water resource. Finally, proposals are made for potential future work that may mitigate some of the previously identified problems, and enhance the potential for nutrient and energy recovery from this system.

4.1. Anaerobic digestion and downstream physico-chemical implications

Anaerobic digestion is the keystone of this project. Any downstream developments rely on, and are entirely subject to, the performance of the AD and the associated implications for the chemical and physical dynamics of the waste stream. Some important factors are the available nitrogen and phosphorus, alkalinity, sodium content/ salinity, and organic load in the effluent stream. All of these determine the quality of the effluent and its potential for reuse. The anaerobic digester's primary function of breaking down organic waste has potentially valuable by-products; biogas, mineralized nutrients, and water (Álvarez *et* al. 2008, El-Khateeb *et* al. 2009, Rao *et al*. 2010, Tauseef *et al*. 2013). These products should be considered as resources and not waste, which is the essence of this research.

These three factors are inseparable, and must be considered in relation to one another. The raw effluent and its organic load is the source of nutrients for hydroponics. However the process that is required to release that nutrient potential is first and foremost a biological

treatment to reduce the organic load of the effluent as per the needs of the brewery and requirements of the local municipality.

The first resource is biogas, which is currently flared, not recovered, but there could be potential energy value for the brewery through heat or electricity generation. The second is the mineralized products of the digestion process, which are essential for and have been demonstrated to support plant growth (Chapters 2 and 3). The third is the water resource itself, which was discussed above. The AD, its influent feedstock, and the operational goals have additional, sometimes overlooked consequences for further effluent treatment or reuse, especially in a hydroponic system. Of particular concern are the effects on the carbon and nitrogen weak acid-base systems and how these systems influence the effluent stream.

4.1.1. The carbon/nitrogen acid-base systems

A key consequence of the anaerobic digestion of organic waste is the generation of CO_2 from incoming biodegradable organic carbon (Batstone *et al*. 2002). The anaerobic digester acts as a closed system when considering the dissolution of gasses and gaseous partial pressure (van Rensburg *et al.* 2003). A fraction of the CO_2 generated in the AD dissolves in the liquor and generates carbonic acid and carbonate alkalinity (van Rensburg *et al.* 2003). This has the effect of increasing both the alkalinity and acidity of the liquor (Figure 4.1). In order to maintain a stable pH, suitable for the sensitive anaerobic methanogens, the incoming brewery effluent pH (average 5.48) is neutralized with sodium hydroxide (Whittington *pers. comm.* 2013). This increase in alkalinity buffers the net acidifying effects of anaerobic digestion and carbonic acid generation.

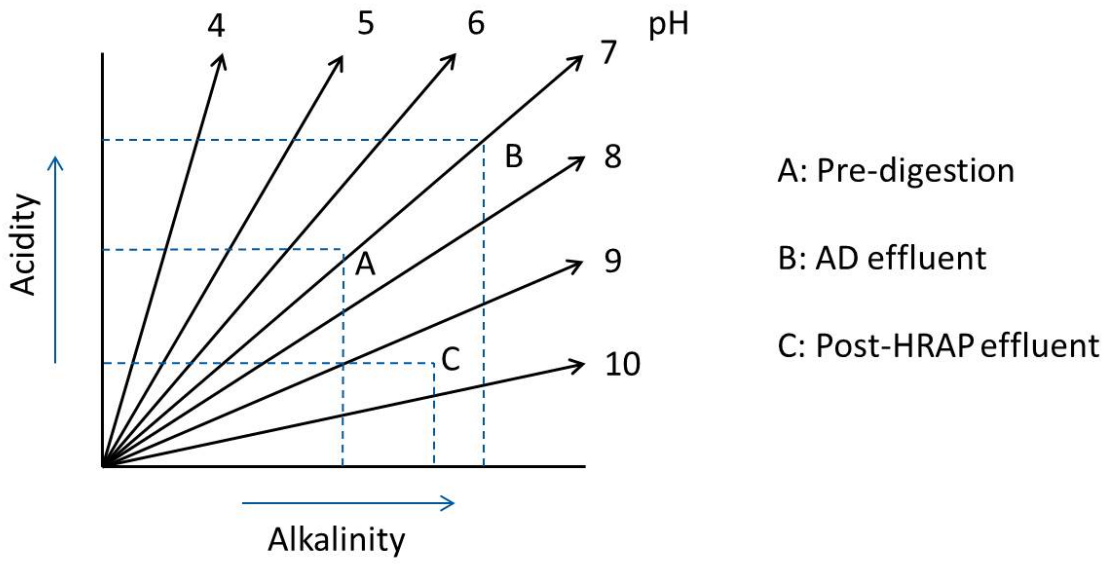

Figure 4.1 Acidity and alkalinity variations through the treatment system (Rensburg *et al.* 2003, Ekama *pers. comm*. 2013). A indicates raw effluent pH, B indicates the pH of the effluent post- anaerobic digestion (AD), and C indicates the final pH after treatment in the high rate algal (HRAP) ponds.

When the AD effluent is exposed to normal atmospheric gaseous partial pressure, and aerated as in the case of the algal ponds or hydroponic systems, the volatile CO_2 present as carbonic acid is stripped from the effluent which causes a loss of acidity (Musvoto *et al*. 2000a, van Rensburg *et al.* 2003; Figure 4.1). The carbonate alkalinity however, is stable and remains in the system so the acid-alkalinity balance of the effluent changes and the pH increases (van Rensburg *et al.* 2003). The resulting high alkalinity of the effluent requires a large amount of acid to achieve the desired pH for optimal nutrient availability for plants. Unfortunately, the addition of large amounts of phosphoric acid creates a problem with residual phosphate pollution as the plants cannot consume enough phosphate from the effluent systems (Chapter 2: Figure 2.10).

Anaerobic digestion theory suggests that the digester's internal pH could benefit from an increase in the nitrogen input to the anaerobic digester (Gallert *et al.* 1998, Hafner & Bisogni 2009). A higher protein input would increase the generation of ammonia which would in turn increase the production of ammonium bicarbonate, increasing the alkalinity of the substrate.

While there are multiple interactions and speciation of both inorganic nitrogen and carbon, the alkalinity-generating species are the key factors in this scenario (Hafner & Bisogni 2009). This may potentially reduce the reliance on sodium hydroxide as a pH buffering agent in the case of this brewery. The increase in ammonium would also provide a potential hydroponic system with more available nitrogen for the plants to consume, as well as easing the pH management challenges by reducing the input of sodium hydroxide in the raw effluent and therefore total alkalinity in the final effluent. The daily organic load on the digester would also increase, however this could also serve as an opportunity if energy recovery from the digester biogas were explored. High ammonia generation can lead to ammonia toxicity in the digester so the optimal feed ratios would have to be determined to avoid compromising the digester (Gallert *el al*. 1998, Hafner & Bisogni 2009).

An additional opportunity for reducing the reliance on sodium hydroxide added alkalinity is the degassing and open pipe recycling of AD effluent back into the raw brewery influent (Whittington *pers. comm*. 2013). The same effect of CO_2 stripping will reduce the carbonic acid acidity from the digested effluent, while returning the carbonate and bicarbonate alkalinity generated in the AD back into the raw effluent, increasing the influent alkalinity and potentially reducing the reliance on sodium hydroxide (Whittington *pers. comm*. 2013).

4.1.2. Brewery effluent as an energy source: Methane production potential

The digestion of organic waste which releases the mineralized nutrients that the hydroponic plants rely on also generates methane (Tauseef *et al*. 2013). The results should not be considered exclusive or independent from each other. The composition and proportions of the organic macromolecules and feedstock fed into the digester as $C_xH_yO_zN_aP_{b...}$ determine the final proportions of oxidized or reduced carbon, nitrogen and phosphorus in the gas and effluent streams, the former holding the energy potential and the latter the nutritional potential (Angelikadi & Sanders 2004). The three main benefits of anaerobic digestion are

recognised as: low energy inputs, low sludge production, and the opportunity for energy recovery from the methane generated (Tauseef *et al*. 2013). This research has demonstrated potential for a fourth: nutrient recovery through hydroponic crop production (Chapters 2 and 3). If the nutrient generation is to be addressed fully, the opportunity for methane energy recovery should also be considered as another product of the same metabolic cascade which produces the hydroponic nutrients. The biogas energy value of the AD is arguably the most significant area of interest for the brewery, given their position on recycling water into the brewing process, and therefore should be considered as part of the broader economic case for developing the brewery effluent system (Mabuza *pers*. *comm*. 2012).

A number of factors can affect the metabolism of the organisms and thus the production of nutrients and biogas including; temperature, pH, inhibiting compounds and the biodegradability of the effluent (Sialve *et al*. 2009). The volume of effluent fed through the system will cause the hydraulic retention time to vary which will result in a more or less complete digestion of the volatile organic carbon compounds in the effluent (Salminen & Rintala 2002, Kim *et al*. 2006, Laubscher *pers. comm*. 2012). The intended purpose of the anaerobic digestion system will dictate the required level of efficiency. By their nature, water treatment plants focus on water quality objectives and not methane production or complete biodegradation (Laubscher *pers. comm*. 2012). Therefore the emphasis on the economic efficiency of the water treatment may result in incomplete digestion of the organic material, and nutrient release and methane production below the theoretical potential of the effluent (Laubscher *pers. comm*. 2012). Anaerobic digestion can be inhibited by the availability of nutrients in the effluent, temperatures or pH levels outside of the optimum range, the presence of certain toxic compounds, and concentrations of volatile fatty acids or ammonia (Kryvoruchko *et al*. 2009). Manipulation of the conditions of the effluent stream and AD system allows for the optimisation of the microbial activity and optimum digestion potential

of a given AD (Kim *et al*. 2006). Methane production and nutrient release are related to COD reduction, thus well-functioning AD treatment plants will also achieve one result as a consequence of pursuing anther. An AD producing large amounts of nutrient rich effluent will also be producing large amounts of biogas.

Support for the development of the post-AD effluent stream will require demonstrating some potential value for the brewery. The brewery is reluctant to recycle wastewater for reuse as a brewing ingredient because of the potentially negative market perception of a beer brewed with recycled water (Mabuza *pers. comm*. 2012). Other potential value in the waste stream has to be demonstrated to garner support and investment from the brewery. Aside from reducing their wastewater treatment costs, the other economic opportunity for the brewery is to recover the energy from the methane generated in the AD as an energy source for use in the brewery. A consequence of the recommendation to increase the nitrogen (mainly as protein) content of the raw effluent, to increase available nitrogen content in the final effluent and reduce total alkalinity for hydroponic use, would also be to increase the total organic load on the digester. This would also increase the daily biogas production of the AD and thus the opportunity for energy recovery (Tauseef *et al*. 2013). There is a direct relationship between the management of the AD, the final chemical and nutritional profile of the effluent, and the methane generated by the AD. An argument for increasing the organic load on the AD to improve final effluent quality can begin by estimating the value of the AD biogas resource and demonstrating the current value of the resource to the brewery. Biogas production could be considered a proxy for nutrient release in the post-AD effluent stream, particularly when organic load is increased or the proportions of C, H, O, N and P in the feedstock change.

Estimating and monitoring the generation of biogas, combined with the current practice of regular COD testing, provides a representation of the effectiveness of the digestion process and therefore nutrient output, as well as supplements the economic case for developing the

brewery effluent stream. The methods and detailed results of this assessment are included in Appendix I. The energy cost per HL of beer produced varies between 100 and 200 MJ, depending on brewery design and operation (Fakoya & van der Poll 2013). The SAB Ibhayi brewery currently operates at a ratio of 108 MJ per HL, with an annual production of about 1.8 million HL of beer (Mabuza *pers. comm.* 2013). The current biogas energy value is the equivalent of around 7% of the production volume of the brewery, or 345 HL per day, an annual equivalent production value of 12 600 000 L of beer. Combined with the potential to reduce the wastewater management costs of the brewery through hydroponic production and the true value begins to show potential.

The suggested increase in the protein fraction fed into the AD to balance effluent pH and increase final effluent nitrogen levels will increase the daily organic load and methane generation of the AD. It follows that there will also be more energy available for reuse in the brewery, potentially increasing the available biogas energy value above the current 7% of demand. This would be the main economic opportunity for the brewery and arguably the greatest opportunity to present the development of the brewery effluent system as economically rewarding for the brewery. To use COD reduction and biogas production as a measure of the efficiency of the AD, the proportions of biodegradable and unbiodegradeable COD must be determined for the mixed effluent (Simate *et al*. 2011). This brewery-abattoir blend solution also provides the opportunity for improving the final effluent chemical profile as a hydroponic nutrient solution through reduced final alkalinity and pH, and increased nitrogen output. A combination of reduced wastewater municipal discharge costs, through the hydroponic or wetland development, the crop value of a productive hydroponic system, and energy recovery from the AD begins to shape the economic and cost-reduction potential for the development of the brewery effluent system.

4.2. Brewery effluent as a nutrient source for hydroponic crop production

These experiments demonstrated that anaerobically digested brewery effluent is a complete, but not optimum, source of macro- and micronutrients for plant growth. The ions in the effluent, particularly sodium and ammonium, have been identified as potentially problematic (Chapter 2 and Chapter 3).

This brewery effluent, among other complications, is nitrogen deficient while there is excessive phosphate. When the anaerobic digester is considered, the macro-molecule sources of nitrogen and phosphate will primarily be proteins and nucleic acids, and yeast and lipids respectively (Brito *et al*. 2007). There are also inorganic sources of phosphate mainly from cleaning processes (Brito *et al*. 2007). The inference then is that the raw effluent is protein (i.e. nitrogen) deficient, which will reduce plant productivity.

In the brewing process, the generation and disposal of waste known as the hot break or trub is potentially important. The hot break is a sludge waste formed by the coagulation of suspended colloidal proteins during the rolling boil that follows the addition of hops to the wort (Miedaner 1986). This protein sludge is mixed with the spent grains and disposed of as solid waste or sold as livestock feed (Fillaudeau *et al*. 2006). It would be useful to conduct a mass balance of the nitrogen flow through the brewing process to estimate the effect this protein loss could be having on the final effluent nitrogen content. The final nitrogen content, among other factors, will affect plant productivity.

The protein fraction in the raw brewery effluent has a dual effect as a source of alkalinity in the anaerobic digester and a source of nitrogen for downstream use (Section 4.1.). The findings that the post-AD brewery effluent is nitrogen deficient, and requires an impractical and excessive injection of mineral acid(s) to correct the pH of the effluent to make it suitable to crop production suggests that future work should consider other methods of alkalinity

management and increasing available nitrogen. Instead of concentrating on 'end-of-pipe' corrections to the effluent chemistry, consideration of the whole cycle from brewing, with trub related protein loss, to the management of the raw effluent and digester environment, is needed. This will extend the opportunity to manipulate the management of the effluent system as a whole in order to improve downstream nutrient availability for crop production.

One potential intervention could be the co-digestion of a protein rich effluent such as fish processing waste or abattoir run-off (Ekama *pers. comm*. 2013). Abattoir effluent may be a particularly promising co-substrate for brewery effluent digestion because of the higher nitrogen levels and higher pH in abattoir waste (Table 4.1).

Table 4.1 Some characteristics of raw abattoir wastewater (± standard deviation).

Study:	Gannoun *et al*. 2009	Keskes *et al*. 2012	McCabe *et al*. 2013
Parameter			
pH	6.8 - 7.4	7.3 ± 0.5	7.9 ± 0.3
COD ($mg.L^{-1}$)	5800 - 6800	2004 ± 240	7051 ± 2841
Total-N ($mg.L^{-1}$)	530 - 810	550 ± 115	459.5 ± 118.9

One effect of blending the two raw effluent streams would be the buffering of the low brewery effluent pH, which has an average equalizer tank inflow at this brewery of pH 5.5, with the higher abattoir waste pH (Talbot & Talbot 2012). The effect may be small but it would contribute to reducing the reliance on sodium hydroxide buffering which may reduce final alkalinity and sodium concentrations in the effluent. The digester itself could benefit from the nitrogen input and the generation of ammonium alkalinity in the effluent. All of these effects could improve nitrogen supply, reduce sodium stress, and reduce the volume of acid required to correct the pH of the effluent, all of which would improve the performance of a crop producing system.

Introducing abattoir waste would potentially introduce complications for waste processing such as fats, suspended solids, and potentially pathogens, in the effluent stream which may have an effect on the operation of the digester or the downstream contamination of crops (Gannoun *et al*. 2009). These are common problems which can be addressed with existing technologies. The fats, oils and greases (FOGs) can contribute up to 70% of the total BOD of abattoir waste. The use of screening, solids removal or dissolved air flotation as a pre-treatment to remove FOGs would then also reduce the organic load on the digester (Gannoun *et al*. 2009, McCabe *et al*. 2013). While this would reduce the methane generation potential of the digester, the efficiency and stability of the active biomass would be more easily maintained. High loads of suspended solids and fats in high-rate digesters can reduce methanogenic efficiency and lead to biomass washout (Gannoun *et al*. 2009). Screening for FOGs and suspended solids may be required, but the effects of the neutral pH and soluble COD would still benefit the digester. The combined effects of reduced sodium hydroxide use (lower final alkalinity and sodium concentration), lower alkalinity, and higher nitrogen output would make the final effluent more suitable for hydroponic crop production.

The volume of effluent would increase if the two streams were combined, as well as the daily COD load on the digester. This would make this proposal difficult to accommodate in existing anaerobic digesters which have been designed with specific loading and hydraulic retention time capacities. Another condition is the proximity of the abattoir to the brewery and the ease or expense of transporting the effluents to the same facility, something which is unlikely to be feasible in existing breweries. However this may provide opportunities for new plants or developments to identify favourable effluents for codigestion in the design stage of the facility. The increased COD load would result in a higher biogas output meaning more energy available for recovery and reuse (Tauseef *et al*. 2013). The scaling effects of greater biogas energy availability would also reduce the return on investment risk for biogas energy

recovery. A wider, bold approach to effluent management could address many of the challenges identified with the end-of-pipe management of brewery effluent. It could also offer the wastewater management company or the brewery an opportunity to harness the energy and nutrient potential from two waste streams.

The principal value of the brewery effluent may not be the nutrient load but its value as a water resource. While it does possess some nutritional potential, its nutrient value is restricted by the limited concentrations of some nutrients (e.g. nitrogen) and the presence of other potential problems and stresses such as the high pH or the presence of salts. Aside from these nutritional problems, there is still value in considering the water resource value of brewery effluent.

4.2.1. Salts

Sodium was found in plant tissues in both trials in higher concentrations than the control treatments. The potential actions of sodium were discussed in Chapter 2 but the complexity of the ion processes makes it very difficult to identify what effects sodium was having in these experiments. Salt concentrations and toxicities need attention in future work. One factor that is repeated in the literature is the cultivar specific tolerance to various stresses including salt stress and ammonium rich nutrition (Cuartero & Fernández-Muñoz 1999, Lastra *et al*. 2009). An alternative approach to manipulating the chemistry of the effluent would be to test the performance of a variety of different crops and cultivars to determine their individual suitability for slightly saline, ammonium rich, effluent nutrition. Identifying the most compatible cultivar would minimize the need for complicated effluent manipulation in a large scale hydroponic or wetland facility.

4.3. Brewery effluent as a water resource in hydroponic crop production

Given the large volume of water used and discharged by breweries (1.8 billion HL beer production in 2010 with an average industry ratio of beer production to water consumption between 1:4-6; Fillaudeau *et al*. 2006, Ascher 2012), the water value of the effluent stream alone should be explored, especially in areas under or at risk of water stress. The current water use model consisting of a single user, effluent treatment, and then disposal is not maximising the resource potential of the water. The key is to find a solution that poses no business risk to the brewery, neither in operation nor cost.

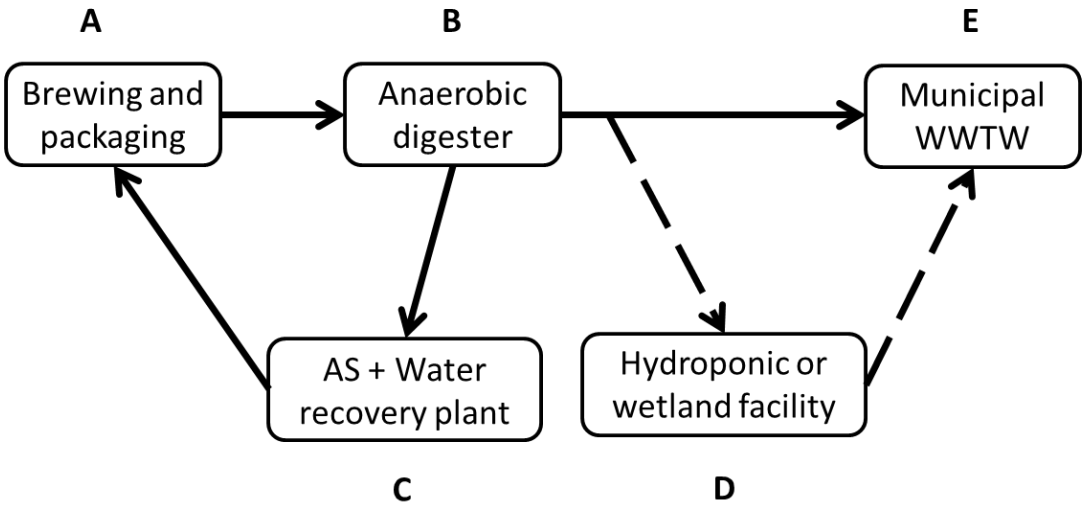

Figure 4.2 Potential placement of a hydroponic or wetland nutrient and water recovery system (D) as a parallel discharge point to the municipal wastewater treatment works (WWTW) (E). A, B and C (AS - activated sludge, and water recovery plant) represent the current brewing, packaging and wastewater processing facilities on-site at the brewery.

One potential point of resource recovery is between the brewery anaerobic digester and the municipal wastewater treatment works (WWTW) (Figure 4.2). This follows the 'ecosystem approach' of managing water and nutrients by reducing waste and maximizing sustainability and resource potential through the recovery of nutrients and water (Todd *et al*. 2003, Nhapi & Gijzen 2005, Harrington & McInnes 2009). This would allow the project to draw as much effluent as it can from the AD outflow without being relied on to process the total effluent flow. The facility could then process the volume of water required for its needs. The low

hydraulic loading rate of constructed wetlands is a limiting factor in their design and operation, however given enough space, large volumes of water can be treated (Abou-Elela *et al*. 2013). Further work could investigate what the most efficient depth, hydraulic loading rate, organic loading rate, aspect ratio, substrate size and material would be for optimising the capacity of a crop-producing wetland treating brewery effluent (García *et al*. 2005).

Any effluent drawn in to the hydroponic or wetland facility would reduce the brewery's discharge volume and therefore its municipal treatment costs. The facility would then be responsible for its own effluent treatment and disposal with no risk to the brewery and no requirement for the brewery to alter its current brewing or waste management practices in any way. This placement gives the hydroponic project the opportunity to use as much effluent as possible, without being relied upon as a water treatment facility, protecting both the brewery and the environment from unnecessary risks.

Depending on the success of future work and the demand for water in the area, the brewery effluent could become a valuable water resource in downstream industries. This does not exclude the possibility of harnessing the nutrient value with crops. That step could be an intermediate operation to produce a valuable crop and polish the effluent for reuse in a tertiary application or release into the environment (Kivaisi 2001, Todd *et al*. 2003). The productive potential of a wetland would also have numerous social and environmental benefits, making a positive contribution to the food-energy-water nexus by producing a low-energy, local product, with reclaimed water (Gulati *et al*. 2013). Brewery effluent is a prime opportunity to demonstrate the value of integrated water, energy and agricultural management (Todd *et al*. 2003, Nhapi & Gijzen 2005).

4.4. Limitations

The time required for each water chemistry test meant that it was not possible to determine individual dilution ratios for each test, each treatment, for both fresh and replaced solutions, on each sampling date and for each replicate sampled. Therefore specific concentrations of nutrients could not be determined. Further work should be done focussing on only the PFP effluent and its specific chemical profile and the plants' specific removal rates of nitrogen and phosphorus from the effluent.

4.5. Recommendations for future work

Three areas need to be addressed: effluent alkalinity, sodium concentrations, and effective pH adjustment without compromising nutrient removal or plant performance. The nutritional potential of the effluent is difficult to isolate as it is bound to many other processes and factors (composition of raw waste, complex ion balances and process, plant-effluent compatibility, the performance of anaerobic digester and pH). Each of these factors needs to be addressed directly, with projects focussing on identifying and alleviating stresses or nutrient deficiencies. Addressing nitrogen (or any other nutrient) limitation must take place alongside, and collaborate with, efforts to reduce sodium stress, alkalinity, ammonium stress, or any other identified limiting factor.

Experiments should test the manipulation of anaerobic digester operation and raw effluent composition to recycle carbonate alkalinity into the raw effluent and increase the protein fraction to produce ammonium alkalinity so as to reduce reliance on sodium hydroxide. This may reduce final effluent sodium concentrations, alkalinity, and increase available nitrogen in the final effluent.

Future work should test alternative methods of pH manipulation, blends of nitric, hydrochloric, phosphoric, sulphuric or other acids to achieve pH adjustment and boost

nutrient levels but not beyond the nutrient consumption ability of the plants. Alternative nutrient supplementation through specific nutrient or fertilizer additions could also be investigated. The performance of a variety of crops and cultivars should also me tested to determine the most effluent compatible plant.

4.6. Conclusion

Brewery effluent contains sufficient essential elements and nutritional value necessary for partially successful Moneymaker tomato vegetative growth, flowering and fruit development. However, these nutrients were not always present or available in ideal concentrations or proportions. This nutrient imbalance or stress resulted in restricted vegetative growth and fruit development disorders. The high pH of the anaerobically digested brewery effluent restricted the availability of nutrients. Lowering the pH of brewery effluent with phosphoric acid resulted in significantly increased plant development and fruit yield during the trial period for effluent drawn from either the primary facultative pond or after the high rate algal ponds. The chemical profile of the effluent also requires further investigation. The presence of sodium, chloride and ammonium could be contributing to or alleviating a variety of plant stresses or disorders.

The physical development of the plants and the chemical analyses of the leaf and fruit tissues confirmed the presence of essential elements in the brewery effluent stream. It is important not to conflate the presence of these nutrients in the effluent with the effluent's suitability for hydroponic Moneymaker tomato farming. Pairing the most suitable crop with the effluent should be considered a key part of further research. A fibre or biomass crop may be a more appropriate selection in this case. The sensitivity of fruiting crops to nutrient and environmental stresses makes the task of managing the effluent, nutrient supply, and plant

development complicated. A fibre or biomass crop also reduces pathogen or contamination risks associated with food crops.

The high alkalinity of the effluent required large amounts of phosphoric acid or nitric acid to reduce the pH to suitable levels. Apart from increasing operating costs for any potential venture, this injection of acid can cause residual pollutant problems as shown by the off-the-scale phosphate and nitrate concentrations in the replaced acid corrected effluent solutions in both experiments. Furthermore, the addition of this phosphorus or nitrogen as a nutrient did not have any significant effect on the growth or development of the plant. The effluent plants did not develop as well as the inorganic-fertilizer plants and despite the leaf tissue of the T4 plants in Experiment 1 having elevated phosphorus concentrations; this did not translate into improved plant or fruit development. This suggests that further research should consider other acids or interventions that will achieve the required pH reduction without contributing to downstream nutrient pollution.

Despite the ammonium toxicity warnings present in the literature, the post-primary facultative pond effluent plants were the best performing effluent treatment in Experiment 1 which is why the post-algal ponds effluent was excluded for Experiment 2. Without the need to treat the effluent in the algal ponds, the proposed hydroponic system would be cheaper and easier to build and maintain. Sodium concentrations in the effluent plant tissue samples from both trials suggest that the level of sodium in the effluent is a problem that needs attention. Given that the main source of sodium is the pH buffering of the raw influent in the anaerobic digester, exploring alternative operating procedures or digester management could reduce the overall sodium concentration, as well as the total alkalinity of the final effluent, potentially improving the performance of downstream crops.

Responses to sodium stress and ammonium preference have been demonstrated as cultivar specific characteristics of plants. Identifying the most suitable crop that has the necessary tolerances would simplify the task of effluent manipulation and trials should be conducted on a variety of species and cultivars to that end.

The combination of high-protein waste co-digestion with brewery effluent, and the recycling of degassed anaerobic effluent into the raw influent stream, could contribute to the effluent and digester pH management and reduce the total alkalinity and sodium concentrations. The effects on downstream nitrogen and phosphorus ratios and availability for crop nutrition should also be assessed as the protein input should improve nitrogen output. Finally there is the opportunity for energy recovery in a combined waste treatment system with a higher protein and total organic load.

There is an opportunity to develop brewery effluent drawn directly from the anaerobic digester as an alternative water resource for use in hydroponic crop production. The challenge that remains is two-fold; finding the most suitable plant, and developing a pH reduction/ nutrient addition technique to boost crop performance that will not create downstream pollution problems.

There is another opportunity for a wider scale project but it is necessary to rethink the current model for brewery effluent, and water resource management. For optimum downstream nutrient and water reuse, the model and scope of investigation must include the raw effluent composition and management, the operation and management of the anaerobic digester, as well as the needs of the downstream recovery system as algae, or hydroponics. The concept of a single-use, treatment and discharge system will not be able to address the water resources needs of society in the future. Here is an opportunity to be the first movers in a largely unexplored field.

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Appendix I

Biogas energy assessment at SAB Ibhayi

The first method of estimating the methane generation potential at the brewery is based on extrapolating limited gas composition analysis data, and combined biogas volumes monitored at the anaerobic digester. The volume of biogas produced by the AD is constantly monitored by the operating engineering company, although the chemical composition of the gas is not. The average volume of gas produced over the period January 2010 to March 2012 was 1560 Nm^3 per day at an average rate of 64.65 Nm^3/h (Talbot & Talbot 2012). In 2008 a third independent engineering company conducted an analysis of the biogas produced in the AD system at the brewery. Six samples of gas were collected from the gas duct leading to the flare stack on the 30th of October 2008 (Table 1).

Table 1 Composition of gas samples taken from an anaerobic digester used to treat brewery effluent (C & M Consulting Engineers 2008).

		13h33 Test 1	13h35 Test 2	13h37 Test 3	13h45 Test 4	13h48 Test 5	14h50 Test 6
CO_2	%	24.19	24.13	14.51	24.11	24.51	21.97
CH_4	%	72.80	72.94	42.00	72.97	72.04	64.74
O_2	%	1.12	1.16	9.74	1.14	1.19	3.27
N_2	%	1.88	1.77	33.75	1.78	2.25	10.02

Five samples were taken on the 25th of November 2008 to determine the concentration of H_2S in the AD gas. The H_2S concentrations in the samples were recorded as follows: 455, 922, 527, 586 and 30 $mg.m^{-3}$ (C & M Consulting Engineers 2008).

It was stated in the engineering report that the results from the third and fifth test (Table 1) "should be interpreted with care" (C & M Consulting Engineers 2008). The sample containers might have leaked and the samples been contaminated which would explain the

variation in those results. As such, the mean composition profile was determined, excluding tests three and five (Table 2).

Table 2 Mean composition of the gas produced by an anaerobic digester that was used to treat brewery effluent (C & M Consulting Engineers 2008).

Compound	Average Concentration
CO_2	23.78%
CH_4	71.28%
O_2	1.57%
N_2	3.54%
Average H_2S Concentration	
H_2S	633.5 $mg.m^{-3}$

The estimated volume of gasses produced by the anaerobic digester of the brewery were extrapolated using Table 2 and the records that were made available by the brewery and the independent consultants, and the results are presented in Table 3.

Table 3 Estimated volume of the different gasses produced by an anaerobic digester that is used to treat brewery effluent.

Compound	Rate of production ($Nm^3.h^{-1}$)	Rate of production ($Nm^3.d^{-1}$)
CO_2	15.37	371.11
CH_4	46.08	1112.35
O_2	1.01	24.44
N_2	2.29	5.24

These figures are a rough estimate only, since the process of biogas production can be limited or fluctuate according to a number of factors, as described earlier.

Angelidaki & Sanders (2004) describe an alternative method for estimating the methane potential of waste based on chemical oxygen demand (COD). The method was used to estimate the amount of methane generated in an AD used to treat brewery effluent, using COD measurements of the effluent entering and leaving the AD (Angelidaki & Sanders 2004). From the independent engineer's data it was established that the average reduction in

COD across the AD was 2.53 $g.L^{-1}$ of effluent (Talbot & Talbot 2012). The following equation was used to estimate the methane production from the previous two years COD reduction data (Angelidaki & Sanders 2004):

$$B\mathrm{o,th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) 22.4}{\left(n + \frac{a}{4} - \frac{b}{2}\right) 32} \left(STP \frac{l\mathrm{CH_4}}{g\text{–}COD}\right)$$

Where $B_{\mathrm{o.th}}$ refers to Buswell's equation of potential methane yield as used by Angelikadi & Sanders (2004), *n* stands for the number of carbon atoms, *a* stands for the number of hydrogen atoms and *b* stands for the number of oxygen atoms in the material being digested. Angelikadi & Sanders (2004:125) show the "theoretical characteristics of typical substrate components" which include carbohydrates, protein, lipids, ethanol, acetate and propionate and the potential methane yield for each substrate. Standard Temperature and Pressure (STP; 0 °C and one atmosphere) which is the unit used to compare gas densities and volumes, where $l\mathrm{CH_4}$ stands for litres of methane produced and *g*-COD stands for the reduction of grams of chemical oxygen demand (COD) in the effluent after treatment in the AD. The formula provides us with a theoretical estimation of the litres of methane gas, at the standard temperature and pressure, produced per gram of chemical oxygen demand consumed in the AD.

We thus estimate a methane yield of 0.35 STP l/g-COD. Based on this we can estimate the average daily yield of methane:

$$STP \frac{0.35\ l\mathrm{CH_4}}{g - COD} \times l/d$$

$$= \frac{l\mathrm{CH_4}}{\mathrm{gCOD}} \times 2.53\mathrm{gCOD}/l\ \text{effluent}$$

$$= \frac{0.0886\ l\mathrm{CH_4}}{1{,}191{,}000}$$

$$= 1{,}054{,}630\ l\mathrm{CH_4}.\mathrm{day}^{-1}$$

Therefore the average daily yield of methane, using the COD data and the method by Angelidaki & Sanders 2004), was approximately 1,054.63m³.d^{-1}. Both the biogas and COD based analysis show that there is a daily average of over 1000 m³ methane in the volume of biogas generated by the AD in the brewery. Based on an energy conversion value of 6.0-6.5 kW.m^{-3} (kilowatts per cubic metre of biogas) we can estimate that the available energy potential of the anaerobic digester at SAB Ibhayi is around or above 9000 kW per day (Rao *et al*. 2010).