Advanced Integrated Treatment of Pig Slurry for Algal Biomass Production and Improved Pig Health

by

Megan Hawley
BSc. (hons)

Thesis
Submitted to Flinders University
for the degree of

Doctor of Philosophy

College of Science and Engineering

4th March 2019

CONTENTS

Summary i

DECLARATION iii

Acknowledgements iv

1. Introduction 1

1.1 Introduction 1
1.2 Australian Pork industry 2
1.3 Characteristics of piggery slurry 6
1.3.1 Health risks associated with wastewater reuse from piggeries 8
1.4 Pig manure management 8
1.4.1 Manure production 8
1.4.2 Current treatment strategies 11
1.5 Anaerobic digestion of piggery slurry 11
1.5.1 Factors that affect AD 13
1.6 Problems and Solutions associated with the reuse of pig slurry 14
1.6.1 Greenhouse gases 14
1.6.2 Suspended solids 15
1.6.3 Ammonia 16
1.7 Aerobic treatment 19
1.7.1 Carbon oxidation 20
1.7.2 Ammonification 20
1.7.3 Ammonia oxidation 21
1.8 Project objectives and scope 27
1.8.1 General objectives 27
1.8.2 Research Questions 27

2. General Materials and Methodology 28

2.1 Aerobic wastewater treatment system 28
2.1.1 System for the aerobic treatment of anaerobically pre-treated piggery slurry 28
2.2 Collection of anaerobically pre-treated slurry 29
2.3 Maintenance of a nitrifying activated sludge inoculums for the aerobic reactor 30
2.4 Aerobic wastewater treatment system – sampling and slurry analysis 36
2.4.1 Inlet ANPS 36
2.4.2 Aerobically treated pig slurry 36
2.5 Water quality analysis 36
2.5.1 Physical 36
2.5.2 Chemical analysis 37
2.6 Statistical analysis 40

3. Commissioning the laboratory scaled aerobic reactor vessel. 41

3.1 Introduction 41
3.2 Methods and materials 43
3.2.1 Experimental set up and operational configuration 43
3.2.2 Air saturation and re-aeration rates 46
3.2.3 Data analysis 47
3.3 Results of re-aeration tests 48
3.3.1 Tap water re-aeration 48
3.3.2 Anaerobic pig slurry re-aeration 52
3.4 Discussion 53

3.4.1 Tap water re-aeration....54
3.4.2 ANPS re-aeration....55
3.4.3 Future research....56
3.5 Conclusion....57

4. The effect changing aeration levels on suspended solids removal and ammonia oxidation in ANPS with a 5 and 10-day residence times....58

4.1 Introduction....58
4.2 Methods....61
4.2.1 Experimental set up and operational configuration....61
4.2.2 Sampling....63
4.2.3 Water Quality analysis....63
4.2.4 Statistical Analysis....63
4.3 Results....64
4.3.1 Trial conditions....64
4.3.2 Suspended solid removal....65
4.3.3 Effect of DO level and THRT on ammonium oxidation and nitrification....72
4.3.4 Carbon....83
4.4 Discussion....87
4.4.1 Operation performance....88
4.4.2 Effect of aeration level and residence time on suspended solid removal....89
4.4.3 Effect of aeration level and residence time on ammonia oxidation....91
4.4.4 Carbon....96
4.4.5 General observations and future research....97
4.5 Conclusion....98

5. Recycling aerated ANPS back through an aerobic reactor to drive ammonia oxidation and suspended solid removal; improving piggery slurry reuse quality....100

5.1 Introduction....100
5.2 Materials and methods....103
5.2.1 Equipment set up and operational configuration....103
5.2.2 Returned activated slurry (RAS) feedback – seeding the reactor....105
5.2.3 Sampling and Water quality analysis....106
5.2.4 Water Quality analysis....106
5.2.5 Statistical Analysis....106
5.3 Results....107
5.3.1 Performance of the reactor....107
5.3.2 Total Suspended solid....108
5.3.3 Effect of RAS feedback on ammonium oxidation and nitrification....115
5.3.4 Carbon....132
5.4 Discussion....137
5.4.1 Effect of RAS inclusion on suspended solid removal....137
5.4.2 Effect of RAS inclusion on ammonia oxidation....138
5.4.3 Effect of RAS inclusion on carbon mass balance....144
5.4.4 Future research....144
5.5 Conclusion....146

6. Algal Growth on aerated ANPS....147

6.1 Introduction....147
6.2 Methods....150
6.2.1 Algal growth experiments....150
6.2.2 Sampling....151
6.2.3 Water quality analyses....151
6.2.4 Statistical Analysis....152
6.3 Results....153
6.3.1 Algal growth....154
6.3.2 Removal of nutrients....159

6.4 Discussion....164
6.4.1 Algal growth....165
6.4.2 Suspended solids and Light....165
6.4.3 Nutrient removal....166
6.4.4 Future research....167
6.5 Conclusion....167

7. General Conclusions....168

8. Appendices....174

8.1 Appendix A – Tap water aeration plots....174
8.1.1 Re-aeration of tap water using equipment configuration 1 (DO probe top + air stones facing inwards)....174
8.1.2 Re-aeration of tap water using equipment configuration 2 (DO probe top + air stones facing out)....181
8.1.3 Re-aeration of tap water using equipment configuration 3 (DO probe bottom + air stones facing in)....183
8.1.4 Re-aeration of tap water using equipment configuration 4 (DO probe bottom + air stones facing out)....185
8.2 Appendix B – ANPS aeration plots....187
8.2.1 Re-aeration of ANPS using equipment configuration 1 (DO probe top + air stones facing inwards)....187

9. References....188

LIST OF TABLES

Table 1:1: Global populations of pigs (unit: million heads) for the period of 2001-2011. Adapted from (FAO, 2013). 3

Table 1:2: Average herd numbers for June 2009 per state. Adapted from: ABS Agricultural Commodities 2008-09, Available in: (Buchanan *et al.*, 2013). 5

Table 1:3: Pond effluent characteristics from conventional piggeries in Australia, according to the APL: National Environmental Guidelines for Indoor Piggeries (2018). Adapted from: (Tucker, 2018) 7

Table 1:4: Estimated outputs of pig slurry via PigBal 4, for a 1000 sow conventional piggery for each class of pigs according to the National Environmental Guidelines for Indoor Piggeries (NEGIP). Adapted from; (Tucker, 2018). 9

Table 1:5: The toxicity effect of NH_3^--N on anaerobic treatment. Source: (McCarty, 1964) 16

Table 3:1: The different equipment configurations assessed. 43

Table 3.2: Equipment configuration for the re-aeration of deoxygenated water per test in the model AWTS. DO probe was placed either 4-6 cm or 19-21 cm below waters (BW) surface and airflow direction either in towards or away from (out) the vessels centre. 47

Table 3.3: Oxygen transfer rates (k_La) obtained at different DO probe depths and air stone ring directions in 0% saturated water (tap) in order to characterise the modelled AWTS for optimal performance. 51

Table 3.4:Oxygen transfer rate (K_La) of ANPS in the modelled AWTS obtained when using the proposed "best case" configuration; air stones pointed "in" towards the centre of the vessel* and a DO probe depth 4-6 cm below the water surface (configuration 1). 52

Table 4:1: Operating conditions for each slurry trials 61

Table 4:2: Values of measured operating parameters (mean ± standard deviation) and *In situ* environmental conditions 65

Table 5:1: Operating conditions per recycled slurry trials 105

Table 5:2: Values of measured operating parameters and *In situ* environmental conditions collated during the RAS feedback trials 108

Table 6:1: Average environmental parameters measured in the inoculated (treatment) and non-inoculated (control) mixed liquor from trial R1 (Chapter 5) incubated under continuous light over a 28 d period to assess biomass production. 153

Table 6:2: Correlation data matrix between all parameters examined within the non-inoculated mixed liquor samples (Control). 162

Table 6:3: Correlation data matrix between all parameters examined within the algae inoculated mixed liquor samples. 163

LIST OF FIGURES

Figure 1.1: Pig numbers in Australia between the periods 1885- 2011. Sourced from: (ABS, 2012), Available in (Buchanan *et al.*, 2013). 3

Figure 1.2: Manure management strategies per housing type. Sourced from (Tucker, 2015) 10

Figure 1.3: Biological processes of anaerobic digestion. Copied from (Girard *et al.*, 2013). Licensed under Creative Commons Attribution 3.0 License (https://creativecommons.org/licenses/by/3.0/). Available from: 12

Figure 1.4: the biological transformation of nitrogen. (1) aerobic oxidation of ammonia to nitrite, (2) aerobic oxidation of nitrite to nitrate, (3) reduction of nitrate to nitrite, (4) reduction of nitrite to nitric acid, (5) reduction of nitric acid to nitrous oxide, (6) reduction of nitrous oxide to di-nitrogen gas (N_2), (7) nitrogen fixation and (8) ANAMMOX the oxidation of ammonia with nitrite to nitrogen gas. Sourced from (Kampschreur *et al.*, 2009) 22

Figure 1.5: The impact of dissolved oxygen level and treatment time at mesophilic temperature on the fate of nitrogen in pig slurry. Sourced from: (Buchanan *et al.*, 2013). 25

Figure 2.1: A schematic diagram of the aeration of anaerobic pig slurry in a modelled aerobic wastewater treatment system. 31

Figure 2.2: A Schematic and pictorial representation of the air diffuser configuration inside the aerobic reactor vessel. 34

Figure 3.1: DO probe location: The DO probe was placed at a depth of either a) 4-6 cm or b) 19- 21 cm below the water's surface. 45

Figure 3.2: Air stone position: Four air stones were attached by silicon tubing to make a ring, with the stones facing, either a) inwards towards the centre of the vessel or b) outwards away from the centre). 45

Figure 3.3: Re-aeration A1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. Data collection occurred every 5 seconds over 0.03h. Position of DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which re-aeration occurred and the linear regression was determined. 49

Figure 3.4: Slope of Re-aeration A1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc/ min at 20.7^{0}C (above). The represented data refers to the linear regression ($p \leq 0.001$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 3.3. 49

Figure 4.1: Average TSS concentrations (g L^{-1}) before (inlet) and after (outlet) aeration in the AWTS under various operating regimes: a) THRT 5 d at 0.5 ± 0.2 mg O_2 L^{-1} (~10% air saturation, 0.8 mg O_2 L^{-1}set point), 20.8 ± 1.1°C (ST1); b) THRT 5 d at 0.7 ± 0.2 mg O_2 L^{-1} (~10% air saturation, 0.8 mg O_2 L^{-1}set point), 20.4 ± 0.7°C (ST2), c) THRT 5 d, 1.3± 0.2 mg O_2 L^{-1} (~20% air saturation, 1.5 mg O_2 L^{-1}set point), 21.4 ± 0.5°C (ST3), d) THRT 5 d, 6.3± 0.8 mg O_2 L^{-1} (~ up to 100% air saturation, uncontrolled 7.5 mg O_2 L^{-1} set point) , 20.0 ± 1.1°C (ST4) and e) THRT 10 d, 5.1± 1.7 mg O_2 L^{-1} (~up to 100% air saturation, uncontrolled 7.5 mg O_2 L^{-1} set point) , 20.9 ± 0.8°C (ST5). 68

Figure 4.2: A comparison of the mean (± SD) ANPS SS concentrations (g L^{-1}) before (inlet) and after (outlet) aeration at a 0.8 mg O_2 L^{-1} (10% saturation) set point and 5 d THRT for trials ST1 and ST2 69

Figure 4.3: Comparison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before (inlet) and after (outlet) aeration with a 5 d THRT and DO set points of 10%, 20% or no control (up to 100% saturation) over a series of three experiments. 70

Figure 4.4: Comparrison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before (inlet) and after (outlet) aeration under continuous aeration with no set DO set point (up to 100% saturation) and either a 5 d or 10 d THRT for trials ST4 and ST5. 72

Figure 4.5: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment at a DO (*in situ*) concentration of 0.5 ± 0.2 mg O_2 L^{-1} at 20.8 ± 1.1°C (approx. 10% saturation) and a 5 d THRT during ST1. A pH of 8.5 ± 0.1 maintained. 75

Figure 4.6: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment at a DO (*in situ*) concentration of 0.7 ± 0.2 mg O_2 L^{-1} at 20.4 ± 0.7°C (approx. 10% saturation) and a 5 d THRT during ST2. A pH of 8.5 ± 0.1 maintained. 75

Figure 4.7: A comparison of TIN and TC levels in both the inlet and mixed liquor outlet analysed during trialsST1 and ST2 when aerated with a 0.8 mg O_2 L^{-1} saturation set point (10%) and 5 d THRT operation configuration. 76

Figure 4.8: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment (outlet) at a DO (*in situ*) concentration of 1.3± 0.2 mg O_2 L^{-1} at 21.4 ± 0.5°C (approx.17-20% saturation) and a 5 d THRT during ST3. A pH of 8.6± 0.0 maintained..... 77

Figure 4.9: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment in an uncontrolled DO environment and THRT of 5 d during ST4. A mean DO (*in situ)* concentration of 6.3 ± 0.8 mg O_2 L^{-1} at 20.0 ± 1.1°C (No DO set point; ~ 100% saturation) and pH 8.4 ± 0.7 achieved over a 46 d treatment period. 79

Figure 4.10: A comparison of outlet N and C levels analysed during trials ST2, ST3 and ST4 operated with a 5 d THRT and DO saturation set points of 10%, 20% or no DO control (100%) set points 79

Figure 4.11: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment in an uncontrolled DO environment and 10 d THRT during ST5. A mean DO (*in situ*) concentration of 5.1 ± 1.7 mg O_2 L^{-1} at 20.9 ± 0.8°C (up to 100% saturation) and pH 8.2± 0.2 achieved over a 41 d treatment period..... 81

Figure 4.12: A comparison of TIN and TC levels in both the inlet and mixed liquor outlet analysed during trialsST4 and ST4 when aerated with an uncontrolled DO set point (up to 100%) and either a 5 or 10 d THRT operation configuration. 82

Figure 4.13: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS within the ANPS before (inlet) and after (outlet) aerobic treatment with a 0.8 mg O_2 L^{-1} (10% saturation) set point and 5 d THRT for trials ST1 and ST2. 85

Figure 4.14: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before (inlet) and after (outlet) aerobic treatment with a 5 d THRT and DO set points of 10%, 20% or no DO control (100% DO) (ST2-4)..... 86

Figure 4.15: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before (inlet) and after (outlet) aerobic treatment at no DO set point (100%) with either a 5 d or 10 d THRT and for trials ST4 and ST5..... 86

Figure 5.1: A schematic diagram of the aeration of ANPS in a modelled AWTS with the inclusion of a returned activated effluent feedback step 104

Figure 5.2: Average TSS concentrations (g L^{-1}) before (inlet) and after (outlet) aeration in the AWTS with 20% RAS feedback under various operating regimes: a) THRT 5 d at 4.1 ± 1.9 mg O_2 L^{-1} (~up to 100% air saturation, uncontrolled), 21.8 ± 2.9°C (R3); b) THRT 7 d, 2.3 ± 2.0 mg O_2 L^{-1} (60-70% air saturation), 22.1 ± 0.8°C (R4), c) THRT 10 d, 1.3 ± 0.5 mg O_2 L^{-1} (20% air saturation), 22.2 ± 0.9°C (R5), d) THRT 10 d, 2.8 ± 1.1 mg O_2 L^{-1} (42-50% air saturation), 20.2 ± 1.4°C (R6), e) THRT 10 d, 3.4 ± 1.5 mg O_2 L^{-1} (66-70%), 20.9 ± 0.8°C (R2) and f) THRT 10 d, 6.4 ± 2.6 mg O_2 L^{-1} (86-100% air saturation) at 21.8 ± 0.6°C (R1)..... 111

Figure 5.3: Comparison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before and after aeration with RAS feedback when operated with a 10 d THRT and the mixed liquor set to maintain DO concentrations of 20%, 50%, 70% or ~100% saturation over a series of four experiments..... 112

Figure 5.4: Comparison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before and after aeration with RAS feedback when operated at a) an uncontrolled DO set point up to 100% saturation with either a 10 d (R1) or 5 d (R3) THRT and b) at 70% saturation with either a 10 d (R2) or 7 d (R4) THRT..... 112

Figure 5.5: A comparison of the mean TSS load when RAS feedback was present to those of the non-recycled trials of Chapter 5 under the same operating parameters; set at 100% DO saturation (uncontrolled) and either a) a 10 d (R1 and ST5) or b) a 5 d (R3 and ST4) THRT. 114

Figure 5.6: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback in an uncontrolled DO environment set to maintain 7.5 mg O_2 L^{-1} and 10 d THRT

during R1. A mean DO (*in situ*) concentration of 6.4 ± 2.6 mg O_2 L^{-1} at 21.8 ± 0.6°C (up to 100% saturation) and pH 7.5 ± 0.5 achieved over a 41 d treatment period.....117

Figure 5.7: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 5.3 mg O_2 L^{-1} (~70% air saturation) and 10 d THRT during R2. A mean DO concentration of 3.4 ± 1.5 mg O_2 L^{-1} at 20.9 ± 0.8°C and pH 7.0 ± 0.7 achieved over a 56 d treatment period. 118

Figure 5.8: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 3.8 mg O_2 L^{-1} (~50% air saturation) and 10 d THRT during R6. A mean DO concentration of 2.8 ± 1.1 mg O_2 L^{-1} at 20.2 ± 1.4°C and pH 7.4 ± 0.8 achieved over a 24 d treatment period. 120

Figure 5.9: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 1.5 mg O_2 L^{-1} (~20% air saturation) and 10 d THRT during R5. A mean DO concentration of 1.3 ± 0.5 mg O_2 L^{-1} at 22.2 ± 0.9°C and pH 8.2 ± 0.4 achieved over a 44 d treatment period. 121

Figure 5.10: A comparison of inorganic-N levels analysed during the aeration of ANPS equipped with 20% RAS feedback and a 10 d THRT during trials R1, R2, R5, and R6, set at 20%, 50%, 70%, and 100% DO saturation set points, respectively.....123

Figure 5.11: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 7.5 mg O_2 L^{-1} (~100% air saturation) and 5 d THRT during R3. A mean DO concentration of 4.1 ± 1.9 mg O_2 L^{-1} at 21.9 ± 2.9°C and pH 8.2 ± 0.4 achieved over a 43 d treatment period. 125

Figure 5.12: A total inorganic-N mass account acquired during the aeration of ANPS equipped with 20% RAS feedback for R4. DO was set to maintain 5.3 mg O_2 L^{-1} (~70% air saturation) and 7 d THRT. A mean DO concentration of 2.3 ± 2.0 mg O_2 L^{-1} and pH 7.6 ± 0.8 at 21.1 ± 0.8°C was observed over a 43 d treatment period. 127

Figure 5.13: A comparison of inorganic-N and carbon concentrations measured in the ANPS before and after aeration with RAS feedback when operated at a) an uncontrolled DO set point up to 100% saturation with either a 10 d (R1) or 5 d (R3) THRT and b) at 70% saturation with either a 10 d (R2) or 7 d (R4) THRT.....128

Figure 5.14; Effect of 20% RAS vs. No-RAS on mixed liquor inorganic N transformation (mean ±SD) before and after aeration at an air saturation level set to 100% and THRT of either a) 10 d (ST5 and R1 or b) 5 d (ST4 and R3). A significant difference in all inorganic-N fractions was detected before and after aeration.....131

Figure 5.15: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before and after aerobic treatment with 20% RAS feedback for each of the four air saturation set points examined (20%, 50%, 70% and 100%) with a 10 d THRT (R1, R2, R5 and R6). 135

Figure 5.16: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before and after aerobic treatment with 20% RAS feedback following a reduction in THRT from 10 d to either 5 d or 7 d at an aeration level set at a) 100% saturation (R1, R3) and b) 70% saturation (R2, R4) DO set points. 135

Figure 5.17: A comparison of the total carbon levels (g L^{-1}) within the ANPS before and after aerobic treatment with an aeration level set to a 100% saturation set point (7.5 mg O_2 L^{-1}) with or without RAS (20%) feedback and a THRT of a) 10 d (R1 and ST5) and b) 5 d (R3 and ST4). 136

Figure 6.1: Chl *a* concentrations measured in the inoculated (treatment) and non-inoculated (control) mixed liquor over a 28 d growth period under continuous light (PAR400-700 nm 26.5 µmol m^{-2} s^{-1}) at 24°C on an orbital shaker (100 rpm). 155

Figure 6.2: Average (mean ± SD) SS (g L^{-1}) and turbidity (NTU) measured in the inoculated (treatment) and non-inoculated (control) mixed liquor over a 28 d growth period under continuous illumination (26.5 µmol m^{-2} s^{-1}) at 20.5 ± 1.1°C and stirred constantly at 100 rpm.....158

Figure 6.3: Average concentrations of inorganic-N fractions measured in the filtered inoculated (treatment) and non-inoculated (control) mixed liquor, stirred constantly at 100 rpm under continuous illumination (26.5 µmol m^{-2} s^{-1}) exposure at 20.5 ± 1.1°C and 100 rpm over a 28 d incubation period. 160

Figure 6.4: Mean carbon concentrations measured in the filtered inoculated and non-inoculated (control) mixed liquor (filtered), stirred constantly at 100 rpm under continuous light exposure (26.5 µmolm^{-2} s^{-1}) at 20.5 ± 1.1°C and 100 rpm over a 28 d incubation period. 160

Figure 8.1: Re-aeration A2 of deoxygenated tap water (3 L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. Data collection occurs every 5 s over 0.03h.Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....174

Figure 8.2: Slope of re-aeration A2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. The represented data refers to the linear regression (p = 6.86e^{-04} ≤0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.1.....174

Figure 8.3: Re-aeration A3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7°C. Data collection occurred every 5 s over 0.03h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....175

Figure 8.4: Slope of re-aeration A3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. The represented data refers to the linear regression (p = 0.001 ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.3.....175

Figure 8.5: Re-aeration B1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. Data collection occurred every 10 s over 0.05h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....176

Figure 8.6: Slope of re-aeration B1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23^{0}C (above). The represented data refers to the linear regression (p = 0.008 ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.5.....176

Figure 8.7: Re-aeration B2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. Data collection occurred every 10 s over 0.05h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....177

Figure 8.8: Slope of re-aeration A3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. The represented data refers to the linear regression (p = 0.01 ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.7.....177

Figure 8.9: Re-aeration B3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. Data collection occurred every 10 s over 0.05h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....178

Figure 8.10: Slope of re-aeration B3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23^{0}C. The represented data refers to the linear regression (p = 0.001 ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.9.....178

Figure 8.11: Re-aeration E1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9°C. Data collection occurred every second over 0.15h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....179

Figure 8.12: Slope of re-aeration E1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9^{0}C (above). The represented data refers to the linear regression (p = 8.10e^{-14} ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.11.....179

Figure 8.13: Re-aeration E2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9°C. Data collection occurred every second over 0.13h. Aeration equipment arranged according to configuration 1;

DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....180

Figure 8.14: Slope of re-aeration E2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9^{0}C (above). The represented data refers to the linear regression ($p = 2.22e^{-14} \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.13.....180

Figure 8.15: Re-aeration D1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 22.0°C. Data collection occurred every 10 s over 2.14 h. Aeration equipment arranged according to configuration 2; DO probe ~4-6 cm below water, with air stones facing outwards (away from centre). The black dots represent the respective data points between which the linear regression was determined.....181

Figure 8.16: Slope of re-aeration D1 of deoxygenated tap water (3L) at a flow rate of 26.31 ccmin^{-1} at 22^{0}C.The represented data refers to the linear regression ($p = 4.44e^{-121} \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.15.....181

Figure 8.17: Re-aeration D2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 22.0°C. Data collection occurred every 10 s over 2.14 h. Aeration equipment arranged according to configuration 2; DO probe ~4-6 cm below water, with air stones facing outwards (away from centre).The black dots represent the respective data points between which the linear regression was determined.....182

Figure 8.18: Slope of re-aeration D2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 22^{0}C. The represented data refers to the linear regression ($p = 4.44e^{-121} \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.17.....182

Figure 8.19: Re-aeration F1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.4°C. Data collection occurred every second over 1.85h. Aeration equipment arranged according to configuration 3; DO probe ~19-22 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....183

Figure 8.20: Slope of re-aeration F1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.5^{0}C. The represented data refers to the linear regression ($p \geq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.19. Position of Do probe ~4-6 cm above the bottom of the vessel, with air stones (bottom) facing inwards (towards the centre).....183

Figure 8.21: Re-aeration G1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.5°C. Data collection occurred every second over 2.6 h. Aeration equipment arranged according to configuration 3; DO probe ~19-22 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.....184

Figure 8.22: Slope of re-aeration G1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.5^{0}C. The represented data refers to the linear regression ($p \geq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the lighter coloured dots shown in Figure 8.21. Position of Position of DO probe ~4-6 cm above the bottom of the vessel, with air stones (bottom) facing inwards (towards the centre).....184

Figure 8.23: Re-aeration C1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.8°C. Data collection occurred every 10 s over 0.63h. Aeration equipment arranged according to configuration 4; DO probe ~19-22 cm below water, with air stones facing outwards (away from centre). The black dots represent the respective data points between which the linear regression was determined.....185

Figure 8.24: Slope of re-aeration C1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.8^{0}C. The represented data refers to the linear regression ($p = 1.09e^{-127} \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.23.....185

Figure 8.25: Re-aeration C2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.8°C. Data collection occurred every 10 s over 0.62 h. Aeration equipment arranged according to configuration 4; DO probe ~19-22 cm below water, with air stones facing outwards (away from centre). The black dots represent the respective data points between which the linear regression was determined.....186

Figure 8.26: Slope of re-aeration C2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at

23.8^{0}C. The represented data refers to the linear regression (p = 7.08e^{-107} ≤0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.25. 186

Figure 8.27: Re-aeration 1A of ANPS (2 L) at a flow rate of 26.31 cc min^{-1} at 20.6°C. Data collection occurred every 5 s over 3.50 h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (away from centre). The black dots represent the respective data points between which the linear regression was determined. 187

Figure 8.28: Slope of re-aeration 1A of ANPS (3L) at a flow rate of 26.31 cc min^{-1} at 20.6^{0}C. The represented data refers to the linear regression (p ≥ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.27 (Dark squares). Position of DO probe ~4-6 cm below slurry surface (BW), with air stones (bottom) facing inwards (towards the centre). 187

LIST OF PLATES

Plate 2.1: Equipment configuration for the aeration of anaerobic pig slurry: a) air flow meter, b) slurry feeder (peristaltic pump), c) solenoid valve, d) filtration flask, e) ANPS inlet pipe, f) air filter, g) aerobic reactor vessel, h) DO probe, i) aerobic pig slurry (APS) outlet pipe, j) pH probe, k) outlet aerobic reservoir, l) Inlet anaerobic reservoir, m) water bath, n) Cu coil, o) air pump, p) recirculating cooler, q) thermostat temperature controller. 32

Plate 2.2: Aerobic reactor vessel..... 33

Plate 2.3: An anaerobic treatment pond at a local South Australian Piggery. Collection sites are shown on the right..... 35

Plate 6.1: Algal growth in mixed liquor samples for the non-inoculated control (left) and inoculated (KOM HRAP water) treatment (Right) samples.....151

SUMMARY

Deriving renewable energy from pig effluent pre-treated in an anaerobic pond and through the production of algal biomass on this waste is a strategy currently being explored by the Australian pork industry. The objective being to lower the environmental impacts and greenhouse gas emissions associated with pork production. Unfortunately, unfavourable concentrations of ammonia (NH_3) and suspended solids (SS) obtain in pig slurry; both can have a toxic and inhibitory effect on pig and algal growth, in high dosages. Additional treatment (post) of the anaerobic pig slurry (ANPS) is advised prior to reuse

Integrated wastewater treatments for combined algal biomass and quality water production are not a new technology; however, there is still a lack of documentation available regarding the aeration of ANPS pertaining to system characterisation and algal production. The development of an enabling technology to facilitate algal growth on ANPS via the oxidation of ammonia to nitrate was examined at a laboratory scale in a low energy system via the incorporation of an aerobic reactor; with system characterisation the main objective.

The laboratory apparatus was characterised over several experiments to establish optimal operating conditions for re-aeration, nitrification, and biomass production. Both equipment configuration (dissolved oxygen (DO) probe and air source (air stone) direction) and aeration parameter combinations (air saturation and retention times (THRT)) were examined either with or without a returned activated sludge (RAS) feedback step. Based on this research aeration was found to be most proficient when air was directed in towards the centre of the vessel with the DO probe placed just below the water's surface (4-6 cm). Once optimally configured, nitrification and removal rates measured 52-79% SS and 20-86% NH_4^+-N respectively. Low nitrification rates were exhibited during the earlier trials at each parameter set. However, the incorporation of a 20% RAS, significantly improved nitrification performances with greater nitrate accumulation detected.

Once suitable conditions were obtained, the algal growth potential was examined in the treated waste signifying a successful growth pattern after 21 days of continuous light exposure. Thus, highlighting the need for an integrated treatment step.

Success of this integrated treatment technology would not only revolutionise the pork industry's outlook on pig effluent as an important potential sustainable source of renewable energy but also enhance profitability with on-site energy, heat, and cleaner wastewater production for subsequent beneficial reuse.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Megan Hawley

4th March 2019

ACKNOWLEDGEMENTS

It is with deepest pride and greatest pleasure that I offer my upmost appreciation to all the people who have helped and encouraged me throughout this thesis, without whom, none of this would have been possible.

Firstly, to my supervisor Professor Howard Fallowfield, for his continued support and guidance over the years. The invaluable knowledge and wisdom he has shared has not only, been of great benefit to this research project, but it has helped to shape the researcher I am today, and for that I am truly grateful. Who knew blowing air through poo could be so much fun.

Secondly, I would like to acknowledge and thank Flinders University, the Australian Government, and the Australian Pork CRC for providing me with a scholarship and top-up scholarship to assist with the funding of the research.

I would like to thank, the technical expertise of these three remarkable men. Raj Indela for his help with the laboratory component of this research and for not complaining (too much) when I filled the lab up with pig slurry (sorry about that Raj). Ivo Svoboda, for his help and guidance during the initial design stage, his knowledge of aeration systems is incredible. Lastly, to Andrew "the Oracle" Hawley, not only for all the electrical and technical advice shared (no matter the hour), but for the construction of the second pump timer also. Thanks dad.

To all the staff and students of the Environmental Health Department at Flinders University, for the many years of friendship, encouragements and for all the weird and wonderful memories we have shared together from our daily lunch time chats about poo to dressing up as zombies and chasing after student as part of a make shift zombie apocalypse. I can certainly say my time with you guys was anything but dull!

A special thanks must go out to all of you wonderful people that came on the exciting venture known as 'slurry collecting' with me. Not the most pleasant of tasks I know, but you all took it your stride, some even came for a return trip, for this, I am truly grateful. To Peter for keeping an eye on things on the rare occasions I could not, 'Arvy' and I thank you.

To Roger Campbell and all the staff and students of the Australian Pork CRC, who warmly welcomed me into their ‘pig family’ with open arms and introduced me to an industry until recently I knew nothing about and for the many great opportunities and experiences being a Pork CRC student entailed. From various training and development courses to conferences, networking events, and farm visits, both my knowledge and professional development skills improved as a result. One of the main things I have learnt throughout this journey is that pig poo is certainly an icebreaker.

A big shout out to all my family and friends, I could not have gotten through this without you guys! Especially, to my mum and dad who have been amazing throughout this whole journey, from their unconditional love and support to always being there through the good, the bad and the downright ugly, whether it was a shoulder to cry on, an ear to listen too or simply a bit of comic relief, I am truly indebted to you both.

Last but in no way least, to my twin sister and best friend Amy (also doing a PhD) who was has been by my side throughout this whole journey making me laugh every step of the way. Thank you for always being an amazing supporter, and for always knowing how to pick me up when I’m down. We did it!

I owe a lot to you all.

Flinders University and the Australian Pork CRC proudly funded this research project.

1. INTRODUCTION

1.1 Introduction

A rise in global population size has evoked a higher demand for fresh produce (Wanapat *et al.*, 2015). This is already evident in Europe, with a 63% increase in animal product consumption over the past 40 years, with Australia following suit (Buchanan *et al.*, 2013, Kleanthous, 2009). Greater production demands have placed an immense strain on natural resources, requiring more than the Earth is able to provide (by approximately 25%) (APL, 2010, Kleanthous, 2009). Alternative water and energy resources have been sought to help elevate this stress, with particular emphasis on the generation of bio-energy. Research identified the reuse of treated animal wastewater to be an ideal candidate (Tucker *et al.*, 2010). Unfortunately, a variety of adverse health risks can arise from the reuse of animal slurries, including water pollution, diseases and greenhouse gas emissions (GHG); a concern for the welfare of both animals and worker (Buchanan *et al.*, 2013, Fallowfield and Garrett, 1985a, Rigolot *et al.*, 2010).

The Australian pork industry, as a large contributor to Australia's livestock production continuously seeks new avenues to enhance pork production with particular emphasis on making the process more environmentally friendly. Reducing feed wastage, environmental impacts, and natural resource demands (i.e. water) are some of the ways currently being explored, with particular emphasis on exploiting pig effluent as a sustainable resource (APL, 2015a, Buchanan *et al.*, 2013, McGlone, 2013, Tucker, 2018). For this to occur it is imperative that effective treatment practices are in place to reduce unwanted health risks, improve effluent quality and maximise exploitation potentials (i.e. heat energy) whilst maintaining a high level of productivity (Buchanan *et al.*, 2013, Calvet *et al.*, 2017).

Research has also been dedicated to look at the use of microalgae grown on treated pig slurry as an alternative feed and biofuel source (Aguirre *et al.*, 2011, Fallowfield, 2013, Fallowfield and Garrett, 1985a, Fallowfield *et al.*, 1999, Strain *et al.*, 1986). Certain challenges, such as elevated ammonia however need to be overcome before this technology can be employed on-farms (Buchanan *et al.*, 2013).

1.2 Australian Pork industry

Farming and agriculture are large driving forces of Australia's economy, with over 131,184 related businesses. Of these, 0.5% (Approx. 687) pertains to the pork industry, with over 1,500 producers (ABS, 2012). The pork industry is an exceedingly intense industry that rapidly adopts and adapts evolving technologies to best maximise production profitability. Globally, the consumption of pork based products is greater than any other meat type at present (McGlone, 2013); consequently, production has intensified to produce 356,000 tonnes of pig meat in 2012-2013 in Australia (ABARES, 2013), with a gross earnings of AU$934 million in 2011-2012 (ABS, 2013a).

Increased consumption and population requirements, has led to an increase in pig numbers across the globe (Chynoweth *et al.*, 1998, FAO, 2013). Global pig numbers for the period of 2001-2011 is outlined in Table 1.1. Australia reflects a similar trend. Population growth has been noted in Australia since 1885 (Figure 1.1), during which numbers averaged around 800,000 pigs. Numbers remained relatively stable, until the onset of World War II, which marked a major milestone in pork production with numbers recorded at 1.8 million. However, after the war, numbers dropped by 55.6% to one million in 1955. It wasn't until the 1960's that populations began to rise at a steady rate to reach a record 3.25 million in 1972, before plummeting to a stable 2.6 million (ABS, 2010, (Buchanan *et al.*, 2013). At present numbers estimate at 2.29 million in 2016 (FAOSTAT, 2017).

Table 1:1: Global populations of pigs (unit: million heads) for the period of 2001-2011. Adapted from (FAO, 2013).

Regions	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Asia	517.7	524.2	526.1	523.4	534.6	545.5	531.9	550.5	575.7	583.1	575.9
Americas	147.3	146.1	146.5	148.7	151.8	154.7	155.5	160.3	158.1	159.6	162.4
Africa	21.7	22.9	22.7	23.8	25.0	25.7	27.0	28.2	29.6	31.0	32.2
Oceania	5.5	5.8	5.6	5.5	5.6	5.6	5.4	5.4	5.3	5.2	5.2
Europe	192.2	194.6	197.8	192.3	190.3	192.9	197.0	190.6	187.3	188.8	187.4
World	884.4	893.6	898.7	893.7	907.3	924.4	916.8	935.0	956.0	967.7	963.1

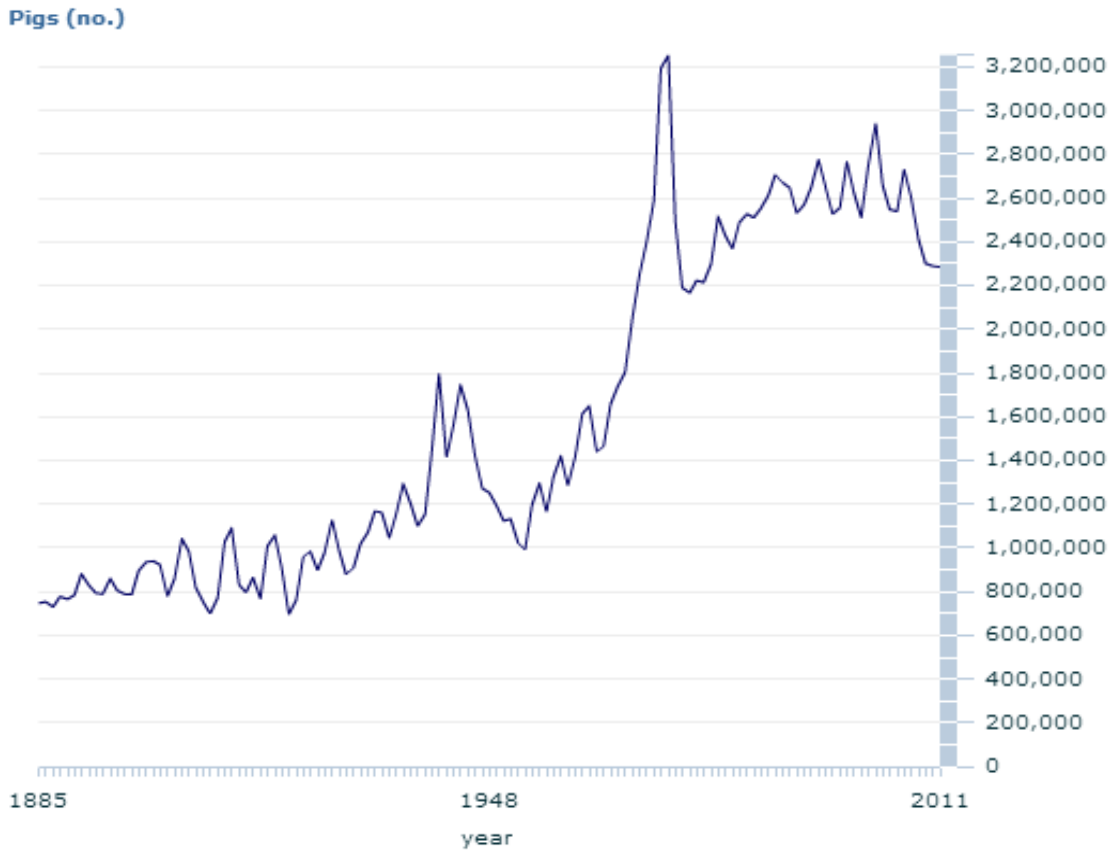

Figure 1.1: Pig numbers in Australia between the periods 1885- 2011. Sourced from: (ABS, 2012), Available in (Buchanan *et al.*, 2013).

Pig production occurs throughout Australia, with primary distribution occurring in grain-growing regions along the Wheat belt of Western Australia and Murray Darling Basin between South East Queensland and the Murray lands of South Australia (Buchanan *et al.*, 2013). Herd numbers per state are outlined in Table 1.2.

Locations of piggeries play a key role in productivity and waste management. Ideal locations for piggeries are determined based on environmental, social, and economical factors. Sites should be positioned within close proximity to abundant potable water resources, power supplies, feed suppliers, markets, and slaughterhouses (Buchanan *et al.*, 2013, Department of Agriculture Fisheries and Forestry, 2012, Tucker, 2018). It is recommended that sites also be positioned within sufficient separating distances from other piggeries and major roads to avoid and prevent disease transmission (Department of Agriculture Fisheries and Forestry, 2012, Tucker, 2018).

Table 1:2: Average herd numbers for June 2009 per state. Adapted from: ABS Agricultural Commodities 2008-09, Available in: (Buchanan *et al.*, 2013)

State	Herd size	Breeding Sows numbers
New South Wales (NSW)	384	62,901
Queensland (QLD)	272	51,412
South Australia (SA)	254	44,029
Victoria (VIC)	281	53,419
Western Australia (WA)	119	28,464
Tasmania (TAS)	42	1,960
Total	1,351	242,185

Due to the intensive nature of the industry, large number of pigs tends to be housed within close proximity to one another. In Australia, there are two types of indoor sheds common to the pork industry. These include; (1) conventional sheds; that collect liquid effluent (faeces, water and urine) into channels underneath slatted flooring (often effluent is flushed out to treatment ponds) and (2) deep litter systems; where the earth's floor is covered by a layer of bedding (e.g. straw) capable of absorbing manure (Buchanan *et al.*, 2013, Murphy, 2011, Murphy *et al.*, 2012, Tucker, 2018, Tucker *et al.*, 2010). Pigs can also be housed outdoors in feedlot and rotational piggeries, where pigs are housed in either pens or paddocks, respectively (Tucker *et al.*, 2010, Tucker and O'Keefe, 2013).

Regardless of accommodation type, it is essential to ensure adequate conditions are maintained to prevent the occurrence of unwanted health risks, such as disease. Therefore, frequent cleaning, effluent disposal, fresh bedding (deep-litter systems) and the removal of waste products (i.e. carcass, feed wastes) is required on a regular basis (Tucker, 2018, Tucker *et al.*, 2010, Tucker and O'Keefe, 2013). This supports the need for efficient wastewater treatment, with wastewater often reused in pig sheds for flushing and wash down water (Buchanan *et al.*, 2013).

1.3 Characteristics of piggery slurry

Piggery slurry; a mixture of faeces, urine, bedding and uneaten food contains high concentrations of organic matter, nutrients (i.e. nitrogen (N), phosphorous (P)), biological oxygen demand (BOD_5), chemical oxygen demand (COD), total solids, and pathogenic and non-pathogenic organisms (Bolton, 2013, Tucker *et al.*, 2010). Table 1.3 illustrates the average effluent (pond) characteristics according to the APL: National Environmental Guidelines for Indoor Piggeries (2018) for conventional piggeries in Australia.

Table 1:3: Pond effluent characteristics from conventional piggeries in Australia, according to the APL: National Environmental Guidelines for Indoor Piggeries (2018). Adapted from: (Tucker, 2018)

Element	Units	Effluent at work[a]	DEEDI data[b]	
			Average	Range
Dry Matter	mg L^{-1}	3623	7900	1100 - 44300
Volatile Solids	mg L^{-1}	1809	1640	480 - 5290
pH		8.0	8.0	7.0 - 8.7
Total Nitrogen or (TKN)	mg L^{-1}	(384)	584	158 - 955
Ammonium Nitrogen	mg L^{-1}	249	144	25 - 243
Total Phosphorus	mg L^{-1}	44	69.7	19.3 - 174.1
Ortho-Phosphorus	mg L^{-1}	28.5	16.3	2.4 – 77.9
Potassium	mg L^{-1}	-	491	128 – 784
Sulphur	mg L^{-1}	22 (9-50)	-	-
Sulphate	mg L^{-1}	26	47.6	13.3 – 87.2
Copper	mg L^{-1}	-	0.09	0.00 – 0.28
Iron	mg L^{-1}	-	0.56	0.09 – 1.61
Manganese	mg L^{-1}	-	0.02	0.00 – 0.05
Zinc	mg L^{-1}	-	0.47	0.16 – 1.27
Calcium	mg L^{-1}	-	20.6	7.3 0– 41.2
Magnesium	mg L^{-1}	-	25.0	6.6 – 72.3
Sodium	mg L^{-1}	603	399	41 – 1132
Chloride	mg L^{-1}	810	19.1	3.6 – 34.4
Conductivity	dS/m	-	6.4	2.5 – 11.7

DEEDI = Department of Employment, Economic Development & Innovation Qld,

TKN = total Kjeldahl nitrogen

[a](Kruger *et al.*, 1995)– samples were collected from piggeries in Queensland, Western Australian and New South Wales

[b]Unpublished data – samples were collected from 10 piggeries in southern Queensland.

1.3.1 Health risks associated with wastewater reuse from piggeries

The composition of slurry, described above can pose a threat to public, animal and environment health if levels are in excess (Mohaibes and Heinonen-Tanski, 2004, Velho *et al.*, 2012).

The presence and degradation of organic matter can lead to nutrient leaching, offensive odours, oxygen depletion in surface waters, pathogenic microorganisms, and GHG emissions such as methane (CH_4), carbon dioxide (CO_2), nitrous oxides (N_2O), hydrogen sulphide (H_2S) and ammonia (NH_3) (Chynoweth *et al.*, 1998, Côté *et al.*, 2006, Fallowfield and Garrett, 1985a, Rigolot *et al.*, 2010). Exposure to these pollutants can have a negative effect on pig and human health. For instance, respiratory diseases, and decreased growth have both been associated to CO_2 (at levels that exceed 1500 ppm) and ammonia exposure. Hydrogen sulphide can lead to difficulty breathing, loss of appetite and a fear of light in pigs (Murphy, 2011).

1.4 Pig manure management

1.4.1 Manure production

Reuse of treated livestock waste can be a beneficial asset to both agriculture and the economy, reducing stress on natural water and fossil fuel derived energy resources (Buchanan *et al.*, 2013, Svoboda and Evans, 1987, Tucker, 2015).

Production boosts has led to an increase in excrement volume, with pigs known to excrete roughly 6% of their weight in manure per day (Imbeah, 1998). The faecal output of pigs varies according to several factors including feed input and piggery system. In Australia, manure production can be predicted via PigBal 4 a nutrient mass balance program designed to predict faecal output of pigs relative to feed inputs capable of calculating the final composition of slurry based on management protocols e.g. wash-water usage and nutrients produced, nitrogen, phosphorous and potassium loads (Buchanan *et al.*, 2013, Casey *et al.*, 1999, McGahan *et al.*, 2010, Tucker, 2018). Validation of this nutrient mass balance approach was performed at a 2500- sow commercial piggery along with another prediction method, Dry Matter Digestibility approximation of manure production (DMDAMP). Table 1.4 depicts the results of a PigBal 4 analysis for a 1000 sow conventional piggery according to the National Environmental Guidelines for Indoor Piggeries (NGEP) (Tucker,

2018).

Table 1:4: Estimated outputs of pig slurry via PigBal 4, for a 1000 sow conventional piggery for each class of pigs according to the National Environmental Guidelines for Indoor Piggeries (NEGIP). Adapted from; (Tucker, 2018)

Pig Class	No. of pigs (SPUs)	Pig weights: in-out (kg)	Total solids (t/yr)	Volatile solids (t/yr)	Nitrogen (t/yr)	Phosphorus (t/yr)	Potassium (t/yr)
Gilts	73 (132)	115-160	17.6	14.3	1.12	0.36	0.31
Boars	54 (86)	115-300	10.0	7.7	0.82	0.25	0.23
Gestating sows	834 (1334)	160-215	154.9	120.5	11.56	3.69	3.42
Lactating sows	165 (411)	215-160	55.4	43.9	4.45	1.12	1.26
Suckers	1,757 (171)	1.4-6.7	41.0	35.2	3.86	0.76	0.60
Weaner pigs	2,776 (1,429)	6.7-30	189.8	163.3	13.89	3.36	2.94
Porkers	1,741 (1,872)	30-55	267.1	225.9	20.60	4.30	5.37
Growers	1,722 (2,544)	55-80	380.0	317.7	28.62	6.96	6.76
Finishers	1,704 (2,915)	80-104	480.6	399.5	31.48	9.03	8.18
Total	10,826 (10,896)	-	1,328	1,328	116.4	29.8	29.1

Notes: Output estimates were calculated for a sorghum-wheat diet using PigBal4 for a conventional piggery with feed wastage values of 20% for suckers, 11% weaners, finishers and porkers, 10% gilts and 5% breeding stock.

Due to increased excrement production and the desire to protect animal and worker welfare, suitable handling practices are essential to reduce any unwanted risks (Tucker, 2015). Such that waste management is vital in the removal of potential disease causing sources, such as the waste itself (Bolton, 2013). Various strategies have been established to (1) degrade organic matter, (2) lower GHG emissions and (3) exploit potential energy benefits (Buchanan *et al.*, 2013). Many comprise of physical, biological and chemical processes (Flotats Ripoll *et al.*, 2012). Figure 1.2 summarises manure and effluent management practices used in Australia (Tucker, 2015).

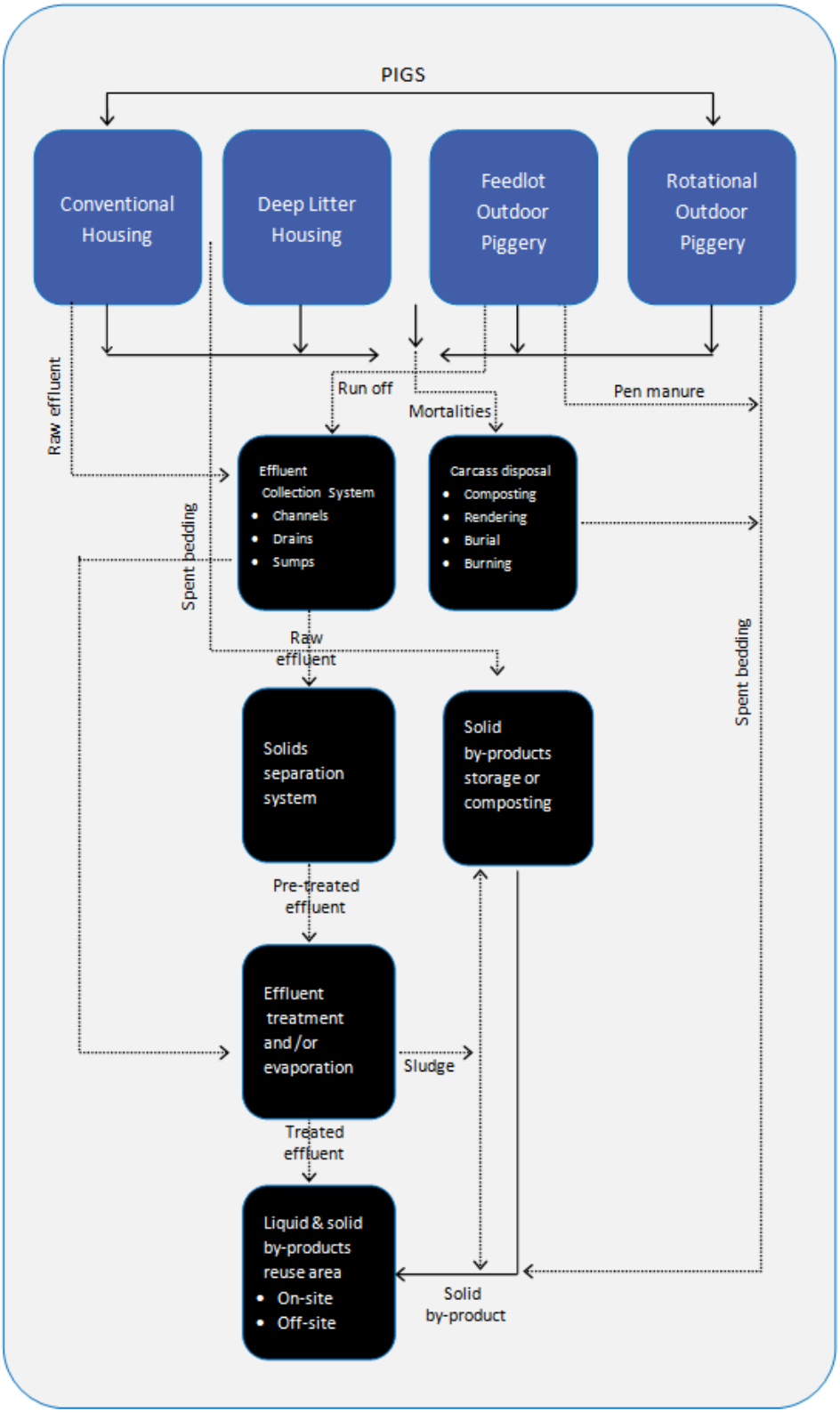

Figure 1.2: Manure management strategies per housing type. Sourced from (Tucker, 2015)

1.4.2 Current treatment strategies

Conventionally, livestock waste is disposed of onto fields or reused within sheds as wash-water (Wadleigh, 1968). In Australia, 78% of piggeries reuse treated effluent within pig sheds, 22% as both wash water and flushing water, whilst 28% account for land application (Tucker *et al.*, 2010). Studies identified certain characteristics, elevated nutrients (nitrogen, phosphorous) and organic load to render it unsuitable for direct discharge into environmental water; as well as inhibitory effects on algal growth and pig health (Buchanan *et al.*, 2013, Tucker *et al.*, 2010). Pre-treatment is therefore an essential requirement prior to discharge and additional treatment in high rate algal ponds (HRAPs) (Buchanan *et al.*, 2013, Fallowfield *et al.*, 1999).

Waste treatment in Australian piggeries as described by Buchanan *et al.* (2013a) can be achieved through a number of options including anaerobic treatment lagoons (59%), direct land use (27%), and various others (6%). A brief overview of reactions and conversions of anaerobic treatment will be discussed in section 1.5.

1.5 Anaerobic digestion of piggery slurry

According to a survey by the Australian Pork Limited (2010), anaerobic ponds are the most common effluent treatment strategy currently available in Australia; used by 83% of producers (APL, 2010, Tucker *et al.*, 2010). Anaerobic digestion (AD); the natural degradation of organic matter within pig slurry is carried out by anaerobic microorganisms in the absence of oxygen, which consumes the converted organic matter to generate energy required for growth and reproduction (Chynoweth *et al.*, 1998, Holm-Nielsen *et al.*, 2009, Lyerly, 2005, Safley Jr and Westerman, 1990). This allows for the formation of CO_2 and CH_4; end products of the conversion (Safley Jr and Westerman, 1990, Sarmiento *et al.*, 2011, van Lier *et al.*, 2008, Zabranska and Pokorna, 2018). Conversions take place via a four-part biochemical process; hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1.3) (Girard *et al.*, 2013).

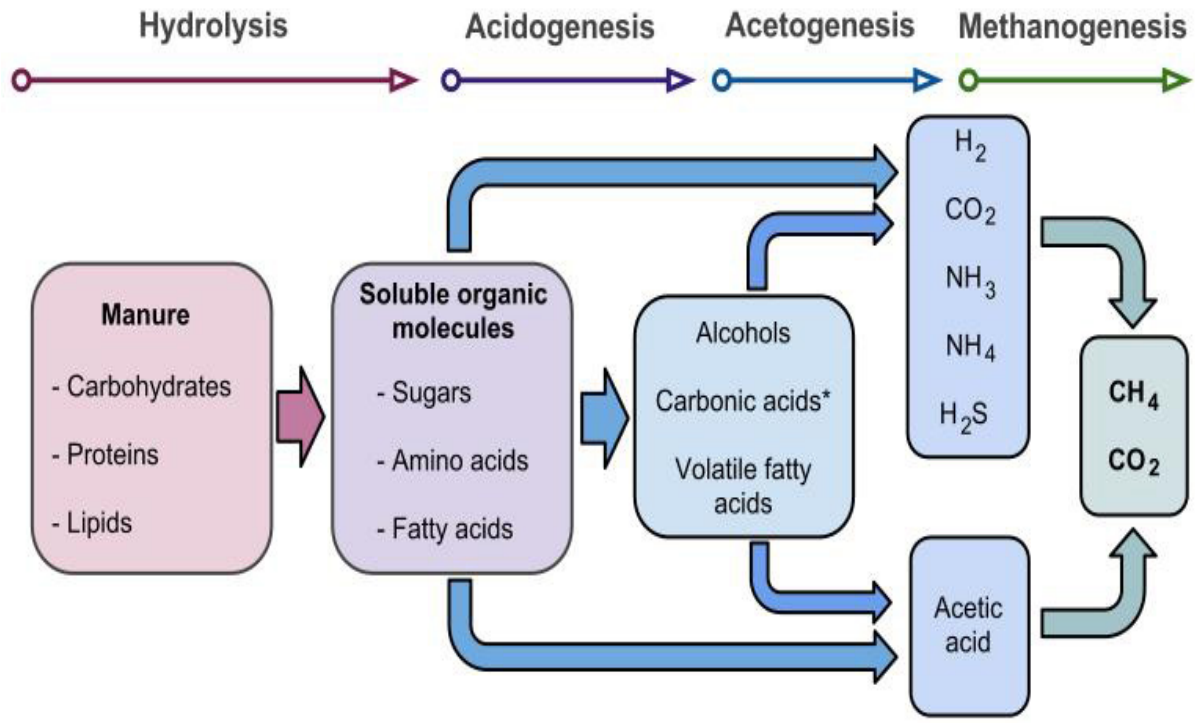

Figure 1.3: Biological processes of anaerobic digestion. Copied from (Girard *et al.*, 2013). Licensed under Creative Commons Attribution 3.0 License (https://creativecommons.org/licenses/by/3.0/). Available from: https://www.intechopen.com/books/biodegradation-engineering-and-technology/biodegradation-in-animal-manure-management

Briefly,

Hydrolysis; the first step in AD converts complex polymers such as proteins, lipids, and carbohydrates into smaller organic molecules (soluble); amino acids, fatty acids and sugars, respectively (Buchanan *et al.*, 2013, Veeken *et al.*, 2000). These conversions are performed via non-methanogenic 'acid forming' anaerobic bacteria.

Acidogenesis / fermentation; Carried out by acidic bacteria, the dissolved organic compounds created through hydrolysis undergo fermentation to form a variety of products; carbon dioxide (CO_2), alcohols (ethanol), hydrogen gas (H_2) and volatile fatty acids (VFA) (i.e. acetate, propionate, succinate and butyrate) (Buchanan *et al.*, 2013).

Acetogenesis; Products formed during fermentation undergo anaerobic oxidation, converting VFA and long chain fatty acids to acetate, hydrogen and CO_2 via acetogenic bacteria (Buchanan *et al.*, 2013).

Methanogenesis; the last stage of AD is the production of methane and CO_2 through the conversion of acetate, hydrogen, CO_2 and other organic matter via a distinct group of anaerobic microorganisms (Archaea); methanogens. Most methanogens are either hydrogen utilizing (25-30%) which reduce CO_2 and H^+ by hydrotropic methanogens or acetate utilizing (70-75%) - the breakdown of acetate into CO_2 and CH_4 (Buchanan *et al.*, 2013, Sarmiento *et al.*, 2011, Zabranska and Pokorna, 2018).

Out of the four stages, methanogenesis is considered to be the most critical to the overall digestive process; due to the biological reactions taking place at a slower pace, and thus making them more prone to environmental changes (Buchanan *et al.*, 2013).

1.5.1 Factors that affect AD

Success of digestion depends on the viability and productivity of different digestive microorganisms involved. It is therefore vital to maintain adequate conditions for the bacteria to thrive (Svoboda, 2003). However, the process of AD can be affected by various factors, that result from operating conditions and waste characteristics (Chen, 1983, van Lier *et al.*, 2008). These include

Temperature;

Each stage of the digestion process can be affected by changes in temperature (Buchanan *et al.*, 2013). Reaction and growth rates of microorganisms during AD are temperature dependent, and accelerate at elevated temperatures (Calvet *et al.*, 2017, Lin *et al.*, 2016, Van Lier *et al.*, 1996). AD processes are known to occur at three temperature ranges, psychrophillic (<10°C), mesophillic (30°C - 40°C; optimal temperature 35°C), and thermophillic (45°C- >65°C; optimal temperature 60°C). For instance, under mesophilic conditions (30-37^{o}C) total solids, chemical oxygen demand (COD), and biological oxygen demand (BOD_5) are reduced by 40%, 50% and 75% during AD to produce a low quality effluent (Buchanan *et al.*, 2013, Svoboda, 2003).

pH:

Studies determined the optimal pH for AD to lie between 6.5-7.5, for methanogenic bacteria, whereas acidogenic and hydrolytic bacteria prefer a pH of 6 (Buchanan *et al.*, 2013).

1.6 Problems and Solutions associated with the reuse of pig slurry

1.6.1 Greenhouse gases

Breakdown of organic matter can lead to GHG emissions, specifically CH_4, CO_2, and hydrogen sulphide (H_2S) from the surface of anaerobic lagoons (Tauseef *et al.*, 2013). Exposure can pose a health risk to animal and worker wellbeing, weakening their immune sensitivity to disease (Buchanan *et al.*, 2013).

Over the years greenhouse gas emissions have been reported to be a significant contributors to environmental pollution and climate change (Kaparaju and Rintala, 2011, Vanotti *et al.*, 2006, Wiedemann *et al.*, 2012). Pork production is responsible for 0.4% of Australia's GHG emissions, 66% the result of uncovered treatment ponds (i.e. anaerobic) (APL, 2004, Tucker *et al.*, 2010). Consequently, the pork industry is vigorously seeking strategies that will reduce emissions from 6-8 kg carbon dioxide equivalents (CO_2e) per kg per hot scored carcass weight ($HSCW^{-1}$) per day (this includes CH_4: a GHG with approximately 23 times more impact than CO_2), to a target of 1 kg CO_2e kg $HSCW^{-1}$ according to a review by Buchanan *et al.* (2013).

Research found that by covering an anaerobic treatment pond, biogas (CH_4; 60-70%, CO_2; 30-40%, H_2; 1-2%, H_2S; 0-0.3%, N; 0-4%) could be harvested for use throughout the industry; effectively reducing atmospheric emissions (Buchanan *et al.*, 2013, Hudson *et al.*, 2007, Lusk and Wiselogel, 1998, Stafford *et al.*, 1980). Interestingly, Wiedemann *et al* (2012) found the incorporation of covered anaerobic ponds (CAP) with combined heat and power (CHP) units could reduce energy requirements and GHG emissions in conventional piggeries by 30% and 55%, to achieve emissions of 2.7 kg CO_2e kg $HSCW^{-1}$ (CAP+CHP). The investigation also identified a potential GHG reduction of 46% could be achieved through the entrapment and flaring of emitted methane as an alternative to fossil fuels (Wiedemann *et al.*, 2012).

Another option currently being investigated by the Australian pork industry is the use of alternative feed sources such as algae (grown on the treated wastewater) that have low GHG emissions (Buchanan *et al.*, 2013, Wiedemann *et al.*, 2012). Successive harvesting of microalgae grown on treated pond water can, not only be used to help further purify water for reuse through solar

disinfection in high rate algal ponds (HRAPs), but can also be used for the production of biofuels as an alternative energy source (Abdel-Raouf *et al.*, 2012, Abou-Shanab *et al.*, 2013, Ayre, 2013, Becker, 1994, Borowitzka and Moheimani, 2013, Gerchman *et al.*, 2017, Gutiérrez *et al.*, 2015). However, several characteristics of pig slurry; total solids (TS) and high ammonia (NH_3) have been shown to have an inhibitory effect on algal growth. It is therefore recommended that a pre-treatment is used prior to additional treatment in HRAPs (Buchanan *et al.*, 2013).

1.6.2 Suspended solids

Treatment in anaerobic lagoons can reduce the solid content of the slurry through sedimentation of settable solids (Chynoweth *et al.*, 1999, Chynoweth *et al.*, 1998, Svoboda, 2003). However, high levels of suspended solids are often retained (Borowitzka and Moheimani, 2013, Buchanan *et al.*, 2013, Fallowfield, 2013). Suspended solids can be any organic or inorganic particles retained within the water that can be trapped via a filter (Spellman, 2013).

Research has shown that the presence of elevated suspended solids within the pond water can have a number of adverse side effects by change the chemical, physical and biological properties of the slurry; a concern for reuse and algal growth (Bilotta and Brazier, 2008, Borowitzka and Moheimani, 2013, Buchanan *et al.*, 2013, Fallowfield, 2013). These include blockages of vital equipment and pipe work, release of contaminants (i.e. heavy metals, pathogens) into the environment and reduced light penetration, depleted DO levels, increased water temperature and strong colouration (Bilotta and Brazier, 2008, Borowitzka and Moheimani, 2013, Buchanan *et al.*, 2013, Fallowfield, 2013). This often makes it expensive and unappealing for reuse, and can inhibit algal growth (Bilotta and Brazier, 2008, Borowitzka and Moheimani, 2013, Buchanan *et al.*, 2013, Fallowfield, 2013).

Strategies to further reduce the suspended solid content from both piggery slurry and other wastewater bodies have been examined throughout the literature (Béline *et al.*, 2008, Bonmatí and Flotats, 2003, Buchanan *et al.*, 2013, Ginnivan, 1983, Zhu *et al.*, 2004). Strategies include aeration, centrifugation, filtration and clarification, sedimentation, activated sludge processes and the addition of coagulants (i.e. alum), (Burton, 1992, Sneath *et al.*, 1988, Zhu *et al.*, 2004).

1.6.3 Ammonia

Contrary to methane emissions, which can be converted for a multiple of uses, elevated nitrogen (N) and phosphorous (P) concentrations are often stored within the digestate of anaerobically treated pig slurry (ANPS) (Portejoie *et al.*, 2003, van de Graaf *et al.*, 1995, Vanotti *et al.*, 2006, Zhang *et al.*, 2011). During anaerobic digestion, protein degradation converts organic-N to ammonia, increasing the concentration of ammonia (Ek *et al.*, 2011, Sialve *et al.*, 2009). Free ammonia concentrations exist in equilibrium with ammonium ions (NH_4^+-N), which is triggered by a rise in pH (Sialve *et al.*, 2009). Research shows high ammonia concentrations (Table 1.8) to be toxic to algal biomass, pig and worker health; a growth inhibitor (Ayre, 2013, Bernet *et al.*, 2000, Buchanan *et al.*, 2013, Chynoweth *et al.*, 1998, Craggs *et al.*, 2014, Fallowfield and Garrett, 1985a, Moreaux *et al.*, 2000, van de Graaf *et al.*, 1995)

Table 1:5: The toxicity effect of NH_3-N on anaerobic treatment. Source: (McCarty, 1964)

Ammonia concentration (mg NH_3-N /L)	Effect on anaerobic treatment
50-200	Favourable
200-1000	No adverse effects
1500- 3000	Inhibitory at high pH
>3000	Toxic

Presence of ammonia in piggeries can arise from numerous sources, the main from pig waste products, urea, and faeces. In Australian piggeries, average ammonia levels within pig sheds vary from three to 20 ppm according to the research carried out by Banhazi and Cargrill (1996) and Murphy (2011). Pig sheds often adopt slatted floors to capture excreted waste products in channels below the floor, and flushed out to large treatment ponds. Studies found ammonia emissions to rise from these slatted floors, with exposure levels greatest at pig height (15 – 20 ppm) (Banhazi and Cargill, 1996, Cargill and Skirrow, 1997, Murphy, 2011, Murphy *et al.*, 2012).

High levels of ammonia exposure can lead to the prevalence of clinical and sub-clinical diseases. Ammonia reacts with mucosal surfaces of the respiratory tract and eye, reducing disease immunity (Brockmeier *et al.*, 2002, Chynoweth *et al.*, 1998, Colina *et al.*, 2000, Murphy, 2011). Clinical signs of ammonia exposure include; salivation, sneezing, coughing and appetite loss (Moreaux *et al.*, 2000, Murphy *et al.*, 2012). Murphy (2011) identified a link between ammonia exposure and an increase in bacteria populations of the lungs (pigs).

Disease prevalence from ammonia exposure can ultimately influence pig production and activity, increasing overall cost of production and feed; the result of delayed weight attainment e.g. growth and slaughter (FAO, 2009). A review by Buchanan *et al.* (2013) indentified an annual AU$600K loss because of inadequate wastewater treatment.

This introductory analysis signifies the dire need for efficient effluent treatment strategies, designed to reduce unwanted health and economic risks (Moreaux *et al.*, 2000, van de Graaf *et al.*, 1995).

1.6.3.1 Solutions

Various reduction strategies have been examined in the past, dedicated to the reduction of unwanted slurry characteristics and pathogen eradication. Included in these were shed ventilation improvements, dietary modifications, and effluent treatment (Murphy, 2011).

Increased ventilation (airflow) within these sheds was found effective at decreasing airborne ammonia (Kim *et al.*, 2007, Murphy *et al.*, 2012). Best results were obtained when ventilation rates were increased to 60% (Kim *et al.*, 2007, Murphy, 2011). However, results were unsuccessful at pathogen eradication (Murphy, 2011). Diet has a large influence on slurry characteristics, such that

it is valuable to modify feed components. Reducing the amount of protein consumed could lower the concentration of nitrogen excreted (Buchanan *et al.*, 2013, Murphy *et al.*, 2012).

Integration of treatment processes

Several effluent treatment strategies have been explored to minimise potential risks associated with the reuse of treated piggery slurry. However the ability to convert nitrogen into its various forms would be of value and an advantage of aerobic treatment (AT) (Zhang *et al.*, 2011). A brief overview of AT is discussed in section 1.7.

For a number of Australian producers, AD is a key process in the treatment of pig slurry. Buchanan *et al.* (2013) proposed an integration of AD processes with aerobic treatment (AT) and high rate algal ponds (HRAPs), as a means of not only managing the high ammonia and solid content of ANPS but would also improve the quality of water for reuse and microalgae biomass production. Of which the latter could be harvested and processed as an alternative fertiliser and fuel source.

This concept was supported by research conducted in Italy by Bartone (2009). Bartone (2009) looked at the integration of biological treatments, AD and AT to treat raw pig manure through denitrification. It was found that an S. Anna sequential batch reactor (SBR) plant involving anoxic-anaerobic phases showed good COD (99%), P (96%) and N (98%) removal potential (Bortone, 2009). Also concluded from the investigation is the use of sequential biological treatment process, which could reduce energy production costs by ~60%, a benefit to the industry.

Pourcher *et al.* (2007) also assessed a similar treatment approach, looking at pathogen eradication, through an integrated aerobic treatment and anaerobic storage system in France. Results identified a greater reduction rate of 3.1 $\log_{10}$ *Escherichia coli* and 1.4 $\log_{10}$ *Enterococci* was achieved through the combined treatment train, than by anaerobic storage alone; no detectable reduction. Despite a treatment being the reverse than proposed in this investigation (AD followed by AT), results provide evidence to suggest effective pathogen eradication can be achieved through N removing techniques (Pourcher *et al.*, 2007).

Like most research, both Poucher *et al.* (2007) and Bortone (2005) conducted these investigations using raw pig slurry. However, since a lot of the water used on farm is sourced from the anaerobic

treatment ponds questions have arisen regarding the impact of using anaerobically pre-treated pig slurry (ANPS) in an integrated aerobic treatment train for algal growth, with very little work performed in this area. This will be the focus of current investigation, with focus on managing the high ammonia and solid content.

1.7 Aerobic treatment

Aerobic treatment (AT) an effective biological process for the disinfection of wastewater is considered a viable option for the integrated treatment of piggery slurry (Buchanan *et al.*, 2013, Evans *et al.*, 1979, Evans *et al.*, 1980, Pourcher *et al.*, 2007). Despite greater running costs compared to AD, organic matter degradation occurs more rapidly reducing the size of aerated systems required. Other benefits include the removal of BOD, pathogen elimination, enhanced odour control, and solid separation; these assist in metabolic heat recovery and the prevention of ammonia inhibition (Buchanan *et al.*, 2013, Burton and Sneath, 1995, Svoboda and Evans, 1987, Svoboda and Fallowfield, 1989).

The ability to convert nitrogen into its various forms is an area of great interest to the pork industry and an advantage of aerobic treatment. As mentioned 78% of Australian pork producers reuse treated effluent within pig houses, thus the ability to reduce ammonia to a non-toxic form; nitrate (NO_3^-) consequently removing the phytotoxic effects of ammonia at high pH is imperative (Buchanan *et al.*, 2013, Tucker *et al.*, 2010). Without adequate treatment, excess NH_3-N levels can give rise to a number of concerns when reintroduced back into the environment (section 1.5.2). AT attempts to target these problems by manipulating nitrogenous compounds, pathogen eradication and lowering BOD, COD and offensive odour levels, to enhance the quality of effluent produced (Bernet and Béline, 2009, Buchanan *et al.*, 2013, Svoboda and Evans, 1987).

Throughout aerobic treatment two main substrates, carbon and nitrogen are oxidised; metabolic heat is released during this process (Svoboda *et al.*, 2013, Svoboda and Evans, 1987, Svoboda and Fallowfield, 1989). Heat recovered, can be used for a number of hot water purposes in and around the farm, including space heating, and the replacement of electric heat pads in weaner houses (Buchanan *et al.*, 2013, Svoboda and Fallowfield, 1989).

The biological process of AT can be broken down into a three-stage process according to Svoboda (2003) and Buchanan *et al.* (2013). The three-stages are: (1) metabolism of dissolved components (i.e. organic acids, sulphur molecules, low molecular weight proteins, and idoles) by aerobic microbial populations, (2) hydrolysis of suspended matter by bacterial enzymes, and (3) decomposition of nitrogenous compounds (i.e. proteins, urea) into CO_2 and NH_3^-.Products of nitrogen decomposition are further oxidised to NO_2^- and NO_3^- and reduced to nitrogen gas. The final product results in a slurry low in BOD, solids, pathogens (eradicated), but high in readily available plant nutrients.

1.7.1 Carbon oxidation

During aerobic treatment, heterotrophic bacteria degrade carbonaceous compounds within the slurry, for biomass biosynthesis and energy production (with an associated formation of carbon dioxide and water); thereupon-releasing energy, 40% of which is lost as heat (Svoboda *et al.*, 2013). Unlike anaerobic digestion, which requires heat to preserve the temperature of slurry, aerobic treatment produces a large quantity of heat, an advantage in reducing treatment time and potential treatment cost offset by reusing the generated heat for on-farm purposes (i.e. heating in weaner sheds, hot water, etc.) (Svoboda, 2003).

1.7.2 Ammonification

Ammonification is the conversion of organic nitrogen (RNH_2) to ammonia (NH_3) during the decomposition of faecal matter via a process known as hydrolysis. Heterotrophic microorganisms stimulate hydrolysis (Equation 1.1).

$$RNH_2 \leftrightarrow NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad \textbf{Equation 1.1}$$

Several factors are known to affect the rate of Ammonification, these include; (1) pH, (2) temperature, (3) dissolved oxygen (DO) concentration, (4) moisture content, and (5) microorganism population (Burton and Turner, 2003).

1.7.3 Ammonia oxidation

Research has been conducted to look at both the effectiveness of AT on animal waste and the factors which affect the process (Buchanan *et al.*, 2013, Evans *et al.*, 1983, Evans *et al.*, 1986, Svoboda *et al.*, 2013, Svoboda, 1995, Svoboda and Evans, 1987, Svoboda and Fallowfield, 1989). Due to elevated ammonia concentrations observed in piggery waste, the transition of nitrogenous components is of great significance with focus directed towards the conversion of ammonia to nitrate (non-toxic) (Buchanan *et al.*, 2013). Reduction of ammonia can be achieved via a multi-step process, nitrification (Barati Roshvanlo *et al.*, 2014, van de Graaf *et al.*, 1995, Zoppas *et al.*, 2017).

Nitrogen removal; nitrification

Nitrogen in piggery slurry comes in many forms, ammonia in both gaseous (NH_3) and ionic form (NH_4); nitrite (NO_2); nitrate (NO_3) and organic nitrogen as urea, and amino acids and compounds (Svoboda, 2003).

Elevated levels of the various nitrogen forms can have a potentially adverse effect on both environment and animal health. It is therefore, valuable to recognise the various transformations of nitrogen (N) during the nitrogen cycle (Figure 1.4). Removal of nitrogen occurs via a multistep process induced by different bacterial groups (Amoo and Babalola, 2017).

Figure has been ***removed due to copyright restrictions.***

It can be found in KAMPSCHREUR, M. J., TEMMINK, H., KLEEREBEZEM, R., JETTEN, M. S. M. & VAN LOOSDRECHT, M. C. M. 2009. Nitrous oxide emission during wastewater treatment. Water Research, 43, 4093-4103. On page 4097. https://doi.org/10.1016/j.watres.2009.03.001

Figure 1.4: the biological transformation of nitrogen. (1) aerobic oxidation of ammonia to nitrite, (2) aerobic oxidation of nitrite to nitrate, (3) reduction of nitrate to nitrite, (4) reduction of nitrite to nitric acid, (5) reduction of nitric acid to nitrous oxide, (6) reduction of nitrous oxide to di-nitrogen gas (N_2), (7) nitrogen fixation and (8) ANAMMOX the oxidation of ammonia with nitrite to nitrogen gas. Sourced from (Kampschreur *et al.*, 2009).

Nitrification

Nitrification is an important part of the nitrogen cycle, and is responsible for the conversion of ammonia to nitrate via two autotrophic bacteria groups – ammonia oxidising (*Nitrosomonas spp.)* and nitrite oxidising bacteria (*Nitrobacter spp*.) (Svoboda, 2003). This conversion takes place in two phases (1) nitritation and (2) nitration as represented by Equations 1.2 and 1.3 (Amoo and Babalola, 2017, Buchanan *et al.*, 2013, Svoboda, 2003, Vanotti and Hunt, 2000).

Nitritation:

$NH_4^+ + 1.5\ O_2 \rightarrow$ *Nitrosomonas spp.* $\rightarrow NO_2^- + H_2O + 2H^+ \ \Delta H = -300$ KJ/ mole.....**Equation 1.2**

Nitration:

$NO_2^- + 0.5\ O_2 \rightarrow$ *Nitrobacter spp.* $\rightarrow NO_3^- \ \Delta H = -75$ KJ/ mole .....**Equation 1.3**

Factors that affect the oxidation of ammonia

As the microbial oxidation process is dependent on the metabolic activity of the organisms, the processes of nitrification are susceptible to changes in various environmental conditions; pH, temperature of treatment, treatment time, and dissolved oxygen (DO) (Svoboda *et al.*, 2013).

pH

The rate of nitrification activity can be affected by pH levels. For instance as pH decreases the rate of nitrification decreases; as nitrous acid produced during the reaction acts as an inhibitor to both nitrifying bacterial groups (*Nitrosomonas* and *Nitrobacter*). In prior research, it was found that when pH is less than 5.5 with a DO level of greater than 15% saturation, nitrification stops. The optimum pH required for nitrification lie between 7.2 and 8.2, 6.3 and 9.4 for *Nitrobacter spp.* and between 6 and 9 for *Nitrosomonas spp.* Caution should be taken when pH exceeds 9.0 due to the formation of free ammonia, an inhibitor of nitrite (Svoboda *et al.*, 2013).

Temperature

Temperature is a crucial factor in nitrification, with activity by nitrifying bacteria optimal at temperatures in the mesophilic range between 30-35°C (Willers *et al.*, 1998). When temperatures are below 5°C activity is minimal and >45°C activity is stopped (Neufeld *et al.*, 1986, Svoboda *et*

al., 2013).

Sludge retention time

The sludge retention time required for nitrification must be long enough to accommodate for the long generation time of nitrifying bacteria and prevent process termination caused by the organisms to be washed out. This is more apparent for shorter retention times. 2.5-3 days were identified as the minimal generation time for these organisms when temperature is between 15-40°C (Svoboda, 2003).

Dissolved oxygen

During aerobic treatment, the speciation of inorganic-N in the mixed liquor is influenced by the amount of DO present (Buchanan *et al.*, 2013, Svoboda, 2003). The ability to manipulate the speciation of nitrogen could be a potential benefit during the growth of microalgae in treated piggery slurry according to Buchanan *et al.* (2013). At DO levels of 10% saturation and greater, NH_3^--N oxidation occurs via nitrifying bacteria, The development of said nitrifying bacteria (essential to the process) are found to grow at an aeration rate of 1% saturation after 3 days (Svoboda *et al.*, 2013). A loss of nitrogen to the atmosphere as nitrogen gas is found to occur between saturation levels 0.1-10%. This accounts for approximately 10% of nitrogen produced. However, as DO saturation levels rise above 10% NO_2^--N, and NO_3^--N oxidation occurs, with only organic nitrogen and NH_3^--N present at low DO (<0.1%) (Buchanan *et al.*, 2013, Svoboda, 2003, Svoboda *et al.*, 2013).

A correlation has been found between the amount of oxygen required and the time needed to treat the slurry (days); the effects of which are illustrated in Figure 1.5.

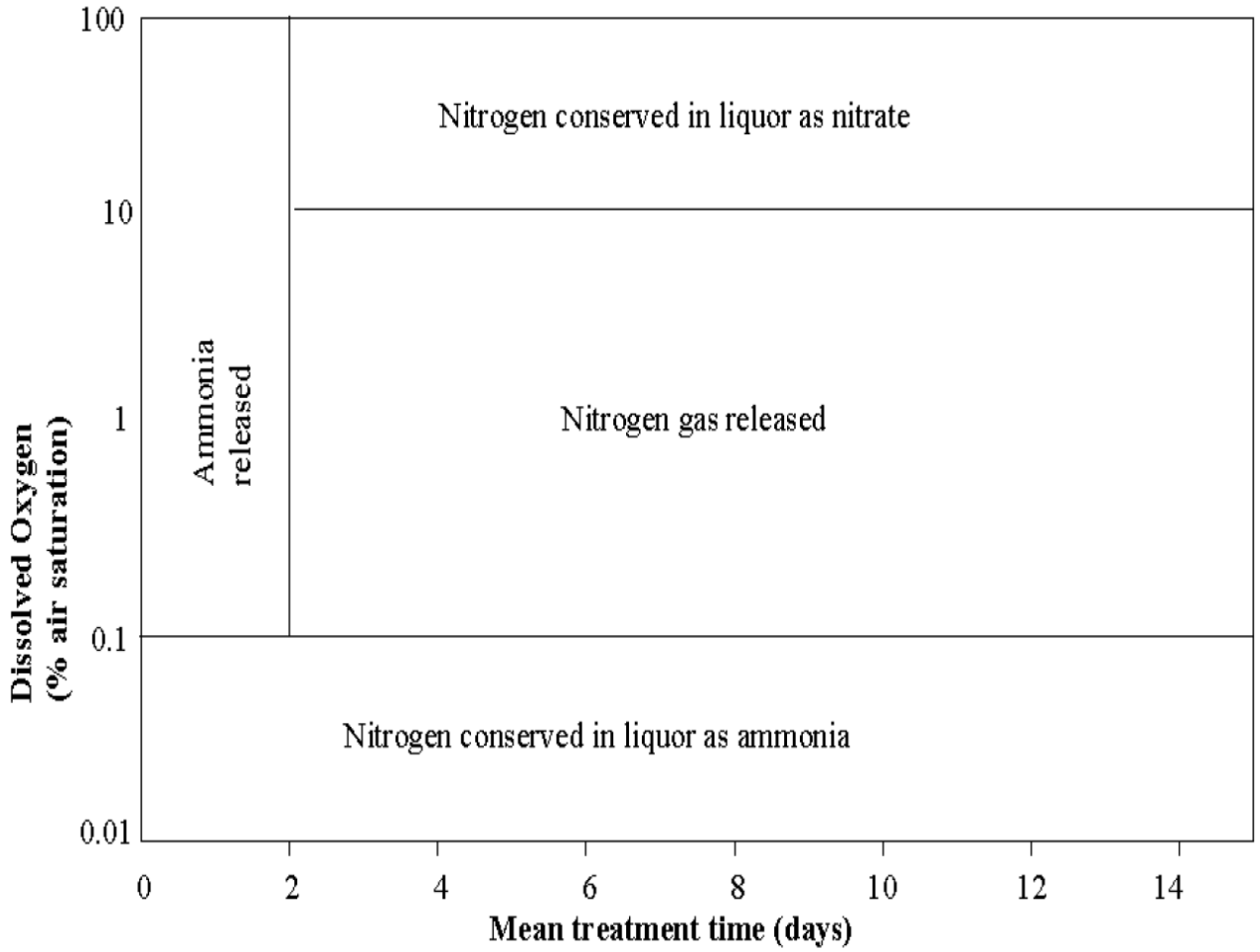

Figure 1.5: The impact of dissolved oxygen level and treatment time at mesophilic temperature on the fate of nitrogen in pig slurry. Sourced from: (Buchanan *et al.*, 2013).

Research found that to be able to treat raw pig slurry to an optimal quality, a residence time of 20 days at mesophilic temperatures would be required if treated through anaerobic digestion. However, for the same temperature range and a DO level greater than 0.1% or 10% saturation, aerobic treatment can achieve a residence time of 5 days (Svoboda *et al.*, 2013). Svoboda *et al.* (2013) identified that to enable further treatment by algal culture and the prevention of nitrogen loss, nitrogen should be available in a non-toxic oxidised form, such as nitrate. It was therefore suggested, that a continuous aeration treatment is run over 5 days at DO saturation levels greater than 20% and a temperature of 35^{o}C to best decrease this loss (Svoboda, 2003).

Unlike past research, which focussed heavily on the treatment of raw pig slurry, little is known about the implications of using anaerobically pre-treated pig slurry in a nitrifying aerobic reactor in

Australia (Bortone, 2009, Buchanan *et al.*, 2013, Pourcher *et al.*, 2007). It is suggested that by using anaerobically pre-treated slurry in a nitrifying aerobic reactor, energy and oxygen requirements needed to drive nitrification will be lowered, as AD pre-treatment would have oxidised carbon components beforehand. Shorter retention times may also result if a portion of the active bacteria released in the treated effluent is recirculated through the aerobic reactor (Buchanan *et al.*, 2013).

As such the incorporation of aerobic treatment into an integrated treatment train designed for the eradication and oxidation of pathogenic microorganisms and ammonia in pig slurry, could add some value to the reduction of environmental impacts generated through pig production, as well as to enable algal growth on piggery waste.

1.8 Project objectives and scope

1.8.1 General objectives

The research to be conducted through this investigation will look to verify the efficiency of integrated aerobic treatment technologies of pig slurry from an anaerobic lagoon, to enable subsequent algal growth on treated effluent through the oxidation of ammonia to nitrate. More specifically, the project aims to:

- Establish a suitable equipment set up for the aeration of ANPS in a small scale AWTS by evaluating the influence of equipment configuration on the re-aeration of tap water that will assist in characterising the system
- Identify optimal aeration regimes to operate an integrated AWTS by assessing the influence of aeration conditions (i.e. DO saturation and THRT) on nitrification and SS removal in ANPS, with or without the inclusion of a RAS feedback step.
- Evaluation of the microalgae growth potential of aerated ANPS prior to application in a HRAP

1.8.2 Research Questions

- What is the influence of aerating anaerobic pig slurry on the oxidation of ammonia?
- What are the optimal operating conditions for sustainable nitrification of anaerobically pre-treated effluent?

2. GENERAL MATERIALS AND METHODOLOGY

This chapter outlines general methods and specialised equipment used throughout this study. Methods relating to specific research aims are provided in respective chapters.

2.1 Aerobic wastewater treatment system

A laboratory scaled aerobic wastewater treatment system (AWTS) was built at the Environmental Health Laboratories, Flinders University, South Australia (SA) to aerobically modify anaerobically pre-treated pig slurry (ANPS) to improve reuse water quality and enable the growth of microalgae (Figure 2.1 and Plate 2.1). For this, the equipment had to be able to perform and handle adequate aeration of ANPS and water circulation to 1) ensure solids remained in re-suspension and 2) perform essential biological processes for nitrification.

2.1.1 System for the aerobic treatment of anaerobically pre-treated piggery slurry

A 3 L cylindrical aerobic reactor was constructed from a piece of polyvinyl chloride (PVC) pipe (25 cm H x 16 cm diameter; internal) sealed at one end (Plate 2.2 and Figure 2.2). The cylinder had a wet surface area: volume ratio of 0.32. Inlet and outlet feed pipes (3 mm silicon tubes, later swapped to 6 mm tubes) were positioned 25 cm and 15 cm respectively from the bottom of the vessel, maintaining a working volume of 3 L throughout the entire investigation. Bubble diffusers (4 x air stones) were placed inside the reactor 2 mm from the bottom (Figure 2.2). ANPS was stored, in a 3 L glass conical flask placed in a refrigerated recirculating water bath (Model RC2, Ratek, Pty Ltd) maintained at 4-7^{o}C using an RS EDT 1411 Thermostat controller. The surface of the water bath was insulated with bubble-wrap. The ANPS was delivered every 4 h (03:30h, 07:30 h, 11:30h. 15:30h, 19:30h, 23:30h) by a calibrated, variable speed, peristaltic pump (Watson Marlow, model 503s R/L) controlled by digital timer (Arlec Compact Digital Time Switch, PC697); the pump run time was determined by the required hydraulic retention time.

The aeration of the ANPS was via a subsurface diffusion aerator. A Welch Thomas vacuum piston, dry air pump (model 2522C-02) was used to blow air, continuously or intermittently, through cylindrical (¾ inch) aquarium air stones attached by 4 mm plastic “T” pieces to silicon tubing (8mm diameter) in a ring formation (13 cm diameter; Figure 2.2) inside the reactor. The air stones were

positioned to face inwards towards the centre of the vessel (Chapter 3 Figure 3.2). The dissolved oxygen saturation within the mixed liquor in the reactor was controlled using an ABB DO transmitter controller (model 4640/500) connected to a DO probe. The DO saturation was controlled in various experiments at 10%, 20%, 50%, 70%, or 100% saturation set points at room temperature (19-26°C). When, the DO in the mixed liquor exceeded these set values the air supply to the vessel was bypassed via a solenoid valve (Model 35A–AAA–DDBA-1BA, MAC Valves, New Zealand) activated by the DO transmitter controller. The reactor remained bypassed until DO concentrations dropped below the set value, when the air supply was resumed. Airflow rate was adjusted using a 150 mm variable area air flow meter (Model F150 Porter, USA) and air pressure valve. An air gap, created using a flask, was incorporated to prevent potential slurry back syphoning into the aeration pump during inactive periods. Aerobically treated effluent was gravity discharged from the outlet into a glass 3 L, conical flask stored in the refrigerated water bath (above).

2.2 Collection of anaerobically pre-treated slurry

Stock ANPS was collected several times during the investigating period from an on-site anaerobic lagoon at a local SA piggery (Plate 2.3). The lagoon operates on a fill and draw system receiving effluent flushed from the nearby sheds (slated flooring), over an 11-12 month period between February to mid-January the following year, where the pond is emptied and contents land spread. Collection took place from one of two sites at the pond; near the inlet pipe or from the opposite end of the pond depending on accessibility (Plate 2.3). DO pH and temperature (^{0}C) was measured on site using portable probes (YSI model 55 Dissolved oxygen and Temperature meter, Xylem and Jenway 350 pH meter).

To collect samples a 1 L plastic bottle attached to an extendable sampling rod “mighty gripper” was dipped (inverted) into the pond approximately 0.1 m below the pond’s surface following the 2007 Environmental Protection Authority (EPA) guidelines (Duncan *et al.*, 2007). Care, was taken to not gather any crust or debris residing on the pond’s surface. Acquired liquor (20L) was transported to the ENVH Laboratories. Additional 3 L was collected into 1 L plastic bottles. Slurry was stored in a cold room at ~2-4^{o}C until required.

Water quality analyses were carried out for DO, pH, total suspended solids (TSS), ammonia-nitrogen (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N) using standard wastewater analysis methods described by American Public Health Association (APHA, 1992).

2.3 Maintenance of a nitrifying activated sludge inoculums for the aerobic reactor

A nitrifying bacterial population was cultivated and maintained in a laboratory fume hood to provide a source of nitrifying bacteria should the aerobic reactor mixed liquor require seeding. Activated sludge (AS) was collected from Bolivar wastewater treatment plant Adelaide, SA by SA Water staff. In an attempt to acclimatise the nitrifiers to the high levels of NH_4^+-N found in the ANPS, 200mL of ANPS was added to 1.8 L activated sludge (AS), which was then aerated continuously through an air stone ring attached to a small aquarium air pump to establish an initial nitrifying bacterial population. An ammonium enriched feed source was added every 3-4 days. At the start of each aerobic reactor experiment the mixed liquor comprised 10% (v/v) of the nitrifying inocula and 90% ANPS

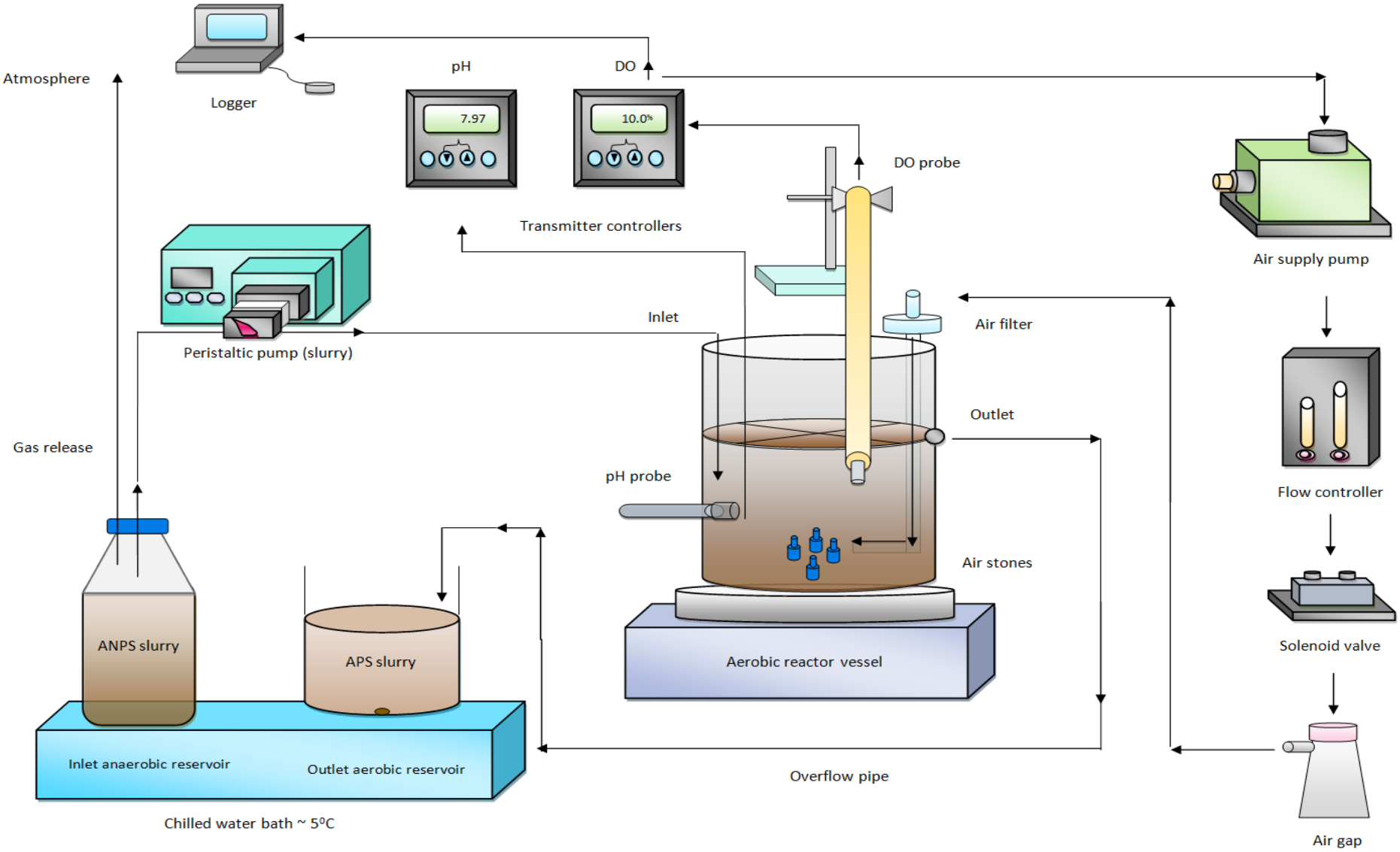

Figure 2.1: A schematic diagram of the aeration of anaerobic pig slurry in a modelled aerobic wastewater treatment system.

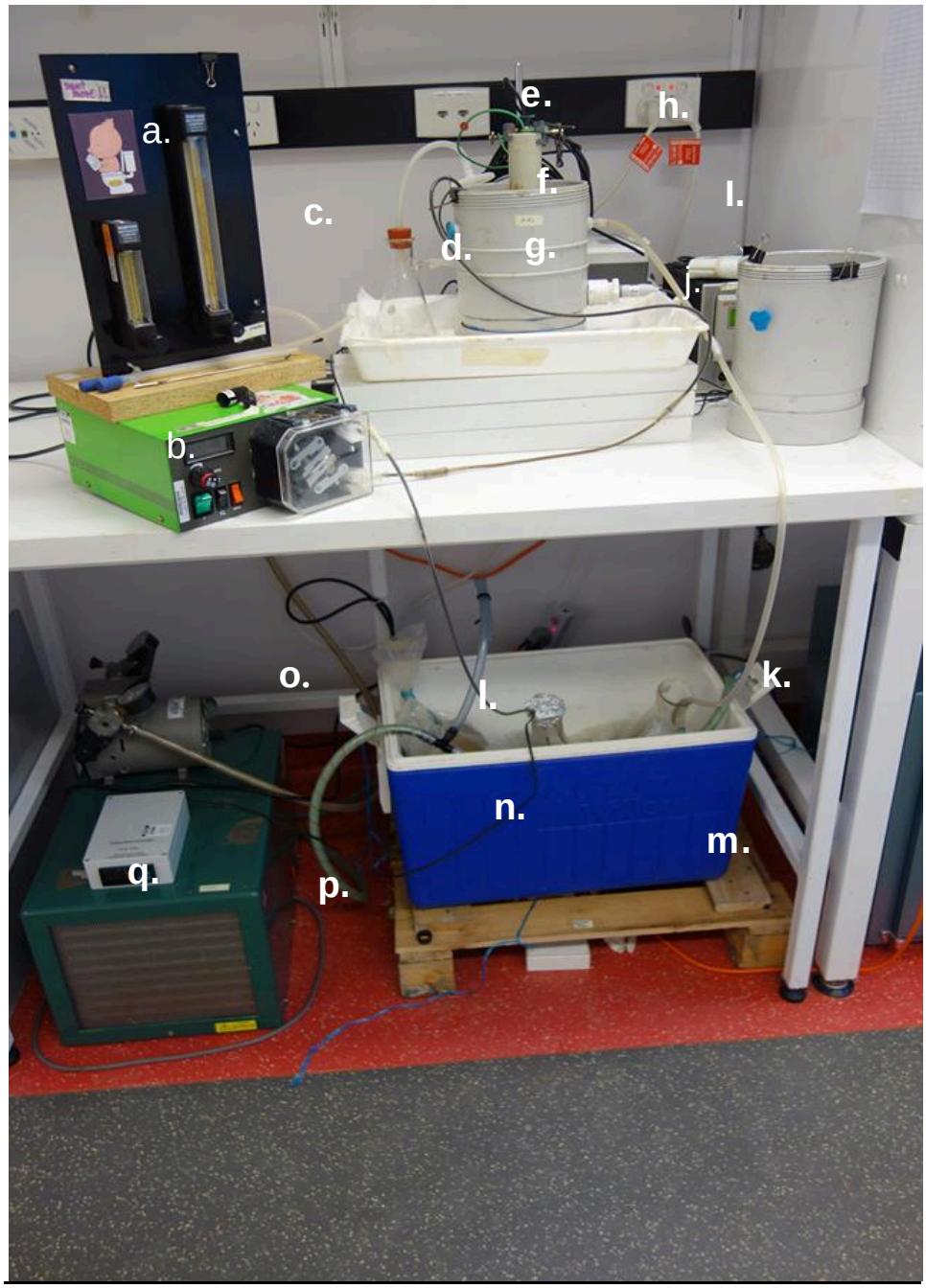

Plate 2.1: Equipment configuration for the aeration of anaerobic pig slurry: a) air flow meter, b) slurry feeder (peristaltic pump), c) solenoid valve, d) filtration flask, e) ANPS inlet pipe, f) air filter, g) aerobic reactor vessel, h) DO probe, i) aerobic pig slurry (APS) outlet pipe, j) pH probe, k) outlet aerobic reservoir, l) Inlet anaerobic reservoir, m) water bath, n) Cu coil, o) air pump, p) recirculating cooler, q) thermostat temperature controller.

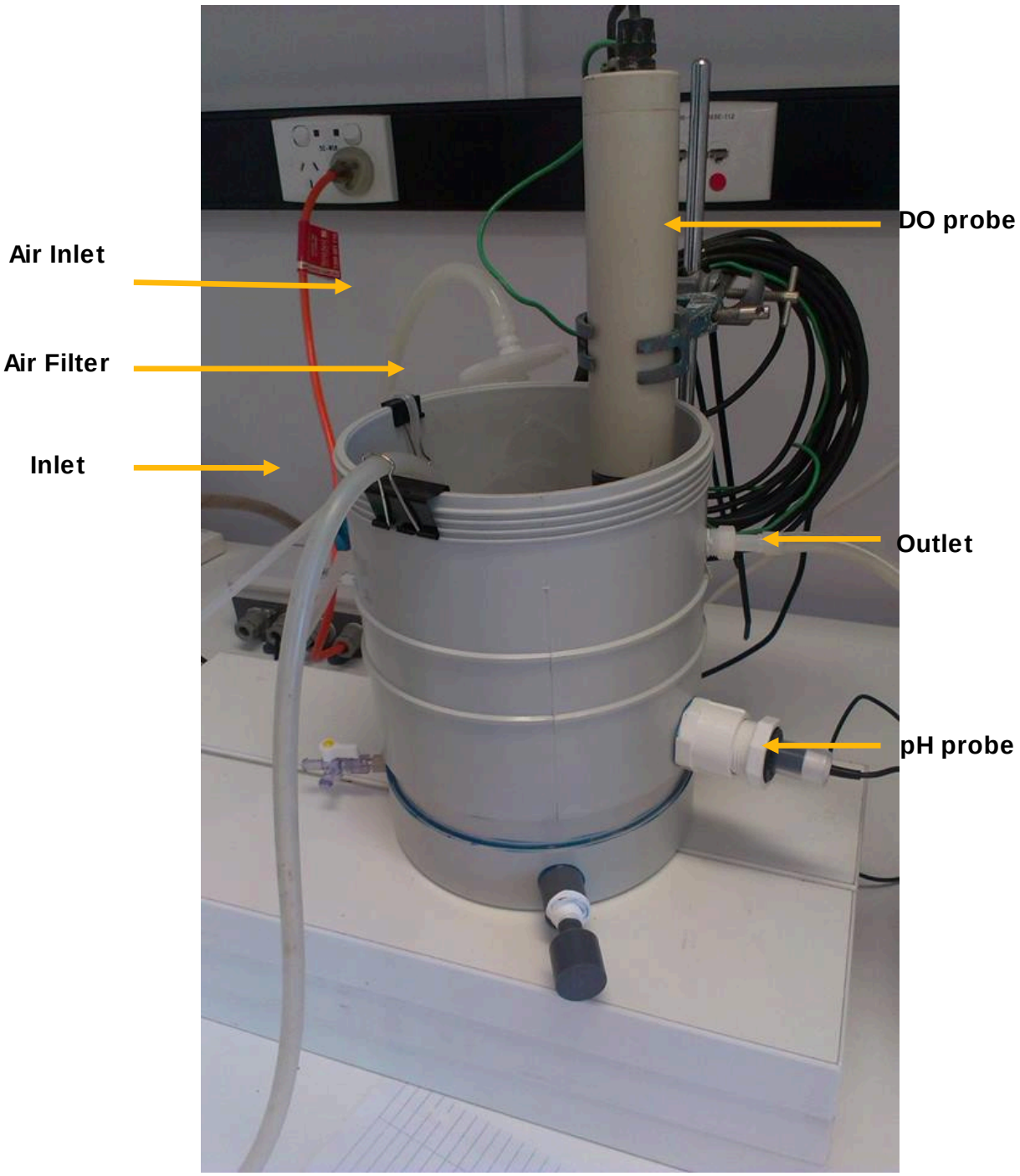

Plate 2.2: Aerobic reactor vessel

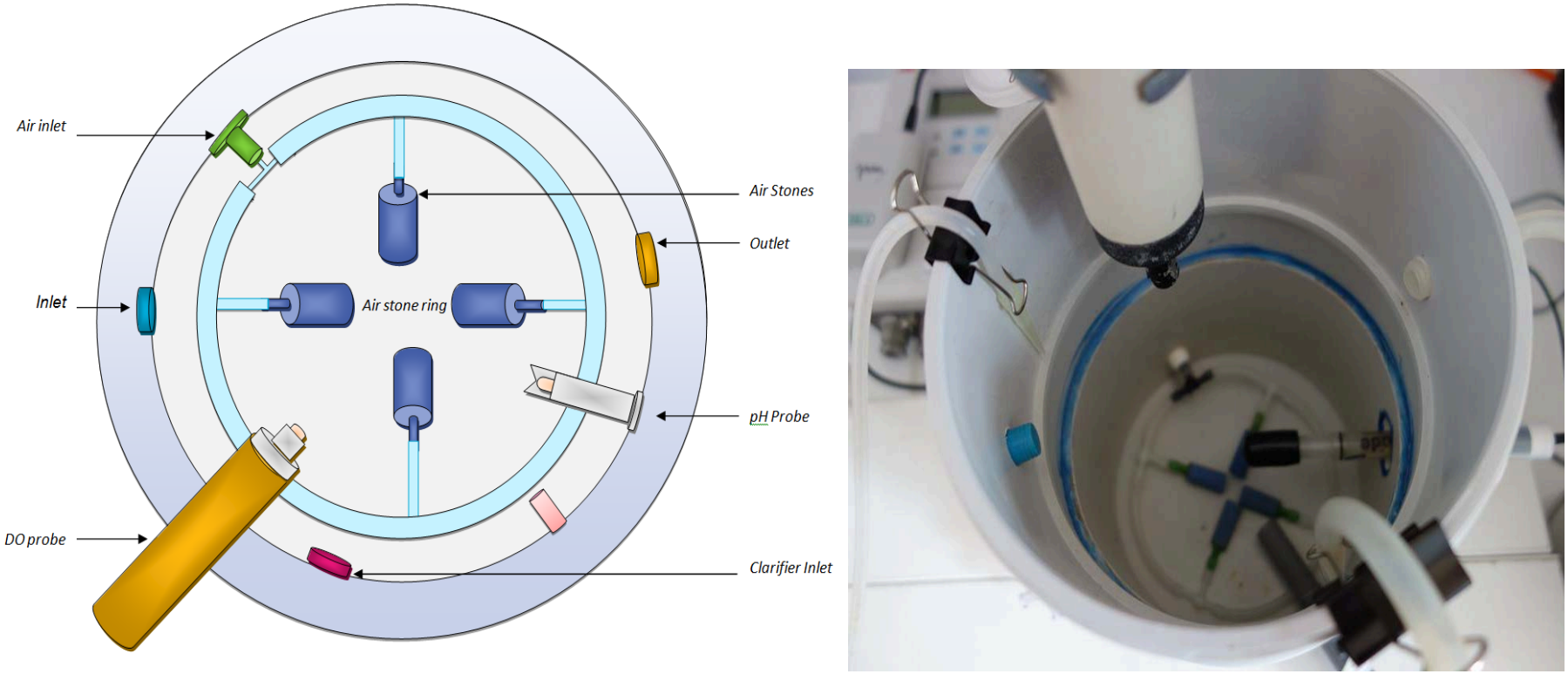

Figure 2.2: A Schematic and pictorial representation of the air diffuser configuration inside the aerobic reactor vessel.

Plate 2.3: An anaerobic treatment pond at a local South Australian Piggery. Collection sites are shown on the right.

2.4 Aerobic wastewater treatment system – sampling and slurry analysis

2.4.1 Inlet ANPS

200 mL ANPS was collected every 5, 7 or 10 days (depending on the operational THRT) and stored, frozen (-20°C) in 40 mL aliquots until required for analysis.

2.4.2 Aerobically treated pig slurry

200 mL aerobically treated slurry was collected and stored, frozen (-20°C) in 40 mL aliquots until required for analysis. Outlet sampling took place every 2-3 days unless otherwise stated.

2.5 Water quality analysis

2.5.1 Physical

2.5.1.1 pH

The aerobic reactor mixed liquor pH was measured using a pH probe (Inode pH electrode IJ44C, Australia) attached to an ABB Kent-Taylor 4600 pH meter.

Portable handheld pH probes (Jenway 350 pH meter and HANNA Instruments HI 9025 microcomputer waterproof pH meter) were also used on occasion to measure pH.

2.5.1.2 Dissolved Oxygen

Dissolved oxygen was measured (mg L^{-1}) using a calibrated ABB DO probe with an ABB oxygen capsule (8012170) sensor attached to an ABB Dissolved oxygen transmitter 4600 controller. DO concentrations were logged electronically every 5 minutes using a T-TEC A™ data logger. Logged data was downloaded and graphed using the T-TEC A™ and Microsoft Excel (2007) software.

Occasionally DO was determined using portable handheld DO meters (Jenway 970 Dissolved Oxygen meter; YSI model 55 Dissolved oxygen and Temperature meter, Xylem and a HANNA Instruments HI 9143 microprocessor auto calibration DO meter).

2.5.1.3 Temperature

Temperature (°C) was measured several times a day in both the ARV and WB from the screen of the DO transmitter controller, portable DO, or pH probes or from a manual handheld thermometer.

2.5.2 Chemical analysis

Nutrient concentrations were determined using both manual and automated methods, with modifications (APHA, 1992). Analytical testing was carried out in duplicate and triplicate. Mean ± standard deviation (SD) values recorded.

2.5.2.1 Total Suspended solids (TSS)

Triplicate, 40 mL aliquots of the respective wastewater samples were filtered through 90 mm diameter, dried (105°C; 18-24 h), glass microfiber filters (GFC; LabServ, LBSOGF 090, Australia). The filters were dried overnight (105°C; 18-24h) and reweighed. The mean TSS (g L^{-1}) was determined from triplicate determinations according to Equation 1 (APHA, 1992).

$$\frac{(W_{final}) - (W_{inital})}{V_{sample}\,(L)} = SSgL^{-1} \quad \text{.....Equation 2.1}$$

Where,

W_{final} = final weight (g) of filter paper and slurry residue

$W_{initial}$ = Initial weight (g) of filter paper

V_{sample} = Volume of filtered samples (L)

2.5.2.2 Determination of inorganic and total nitrogen

2.5.2.2.1 Ammonium-N (NH_4^+-N)

Ammonium nitrogen (g NH_4^+-NL^{-1}) was measured at 630 nm using a FiaStar™ 5000 automated analyser (FOSS, Sweden) according to the APHA standard methods(1992). Pre- and post-aeration samples were diluted 500 and 250 times, respectively using Milli-Q® water.

2.5.2.2.2 Nitrite –N (NO_2^--N)

NO_2^--N was measured using the NO_2/NO_3 cassette from a FiaStar™ 5000 automated analyser (FOSS, Sweden) according to the APHA standard methods (1992). Pre- and post-aeration Samples were diluted 10 and 250 times, respectively using Milli-Q® water. Duplicate 2 mL injections were extracted per sample and analysed for NO_2^--N at 543 nm. The mean and standard

deviation concentration was recorded, tabulated and graphs produced in Excel (2007). Results are given in g L^{-1}.

Occasionally, manual NO_2^--N concentrations were analysed by adding 400 µl colour reagent to duplicate 5 mL samples, vortex mixed and left for 20 mins to develop colour. Manual NO_2 colour reagent was prepared prior to analysis by dissolving into 80 mL Milli-Q® water, phosphoric acid (20mL), sulphanilamide (1.0 g) and n-1-naphthelene diaminedihydochloride (0.08 g); an additional 28.75 mL distilled water was added. Once colour developed (20 min) samples were transferred into a glass micro-cuvette and absorbance read at 543 nm via a Shimadzu UV probe spectrophotometer (UV-1800, USA).

2.5.2.2.3 Nitrate (NO_3^-- N)

NO_3^--N was measured using the NO_2/NO_3 cassette from a FiaStar™ 5000 automated analyser (FOSS, Sweden) according to the APHA standard methods (1992). Pre- and post-aeration samples were diluted 10 and 250 times, respectively using Milli-Q® water. To determine NO_3^--N, the total oxidised nitrogen (TON) or sum of NO_3 - NO_2 was first determined. A cadmium reductor was used to reduce NO_3^- in the sample to NO_2^-. A diazo compound was formed from the reaction of the NO_2^- when a sulphanilamide reagent was added (APHA standard methods(1992) chemistry adapted for machine use (FOSS)). The absorption was then measured at 543 nm in duplicate 2 mL injections per sample. NO_3^--N concentrations were determined by subtracting the NO_2^--N concentration (mg L^{-1}) derived in Section 2.5.2.2.4 from the TON value using Equation 2.

$$\mathbf{C_{NO_3}} = (\boldsymbol{C_{TON}}) - \mathbf{C_{NO_2}} \quad \textbf{Equation 2.2}$$

Where

C_{NO3}= Concentration (g L^{-1}) of NO_3-N in sample

C_{TON}= Concentration (g L^{-1}) of TON in sample

C_{NO2} = Concentration (g L^{-1}) of initial NO_2-N in sample

2.5.2.2.4 Total Nitrogen (TN)

TN is representative of the sum of NH_4^+-N, NO^-_2-N, NO_3^--N and organic- N in the sample. Wastewater samples were diluted 100 times with Milli-Q® water. Total Nitrogen (g TN L^{-1}) was measured using a Shimadzu TOC-$L_{CSH/CSN}$ 500 analyser (Shimadzu Corporation, Japan) according to the manufacturer's instructions.

A TN mass balance was performed to track the input, transformation, and accumulation of nitrogen during nitrification using Equation 2.3. Organic-N was determined via an indirect method by subtracting the NH_4^+-N, NO_2^--N, and NO_3^--N concentrations from the TN values obtained.

$$\mathbf{TN} = \boldsymbol{C_{NH_4-N}} - \boldsymbol{C_{NO_2-N}} - \boldsymbol{C_{NO_3-N}} - \mathbf{C_{RNH_2-N}} \quad \textbf{Equation 2.3}$$

Where,

TN= Concentration (g L^{-1}) of TN in sample

C_{NH4-N} = Concentration (g L^{-1}) of NH_4-N in sample

C_{NO2-N} = Concentration (g L^{-1}) of NO_2-N in sample

C_{NO3-N} = Concentration (g L^{-1}) of NO_3-N in sample

C_{RNH2-N} = Concentration (g L^{-1}) of organic-N in sample

2.5.2.3 Total Carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) analysis

Wastewater samples were diluted 100 times with Milli-Q® water.TC, TOC and IC was measured (g C L^{-1}) using a Shimadzu TOC-$L_{CSH/CSN}$ 500 analyser (Shimadzu Corporation, Japan).

A carbon mass balance was performed to track the input, conversion, and accumulation of carbon during nitrification using Equation 4.

$$\boldsymbol{TC} = \boldsymbol{C_{TC}} - \boldsymbol{C_{TOC}} - \boldsymbol{C_{IC}} \quad \textbf{Equation 2.4}$$

Where,

C_{TC} = Concentration (g L^{-1}) of TC in sample

C_{TOC} = Concentration (g L^{-1}) of TOC-C in sample

C_{IC} = Concentration (g L^{-1}) of IC-C in sample

2.6 Statistical analysis

Data was analysed using Microsoft Excel (2010) (Microsoft Corporation, USA) and R i386 3.3.1 (R Development Core Team, 2016). The mean ± standard deviation (SD) was reported and used for all data. Prior to the application of parametric and non-parametric analyses data was assessed for normality. Normality test used included a Shaprio- Wilk test of normality, histograms and Quantile-Quantile plots (Q-Q plots). Data that satisfied normality were assessed using parametric tests; independent t-tests (test), paired t-tests, and an analysis of variance; one-way ANOVA. Data that satisfied normality were assessed using parametric tests; independent t-tests (two-sample t-test or Welch two-sample t-test), paired t-tests and an analysis of variance; two-way ANOVA. Data not considered normal were assessed using non-parametric (Wilcoxon rank sum test, Kruskal Wallis rank test). Due to the data variability, the level of significance was accepted at 95% and above, where p-values ≤ 0.05, unless otherwise stated. When, applicable, p-values were reported as the "actual test" p-value except for values 0.001 (reported as ≤ 0.001).

Specific hypothesis to be tested include

1. Null hypothesis
    a. No significant difference in either SS or ammonia levels before or after integrated treatment

2. Alternative hypothesis
    a. Integrated aerobic system will reduce SS and ammonia levels in anaerobically pre-treated pig slurry

3. COMMISSIONING THE LABORATORY SCALED AEROBIC REACTOR VESSEL.

3.1 Introduction

The central purpose of incorporating an aeration step to a wastewater treatment regime is to supply the oxygen required for essential; microorganism growth, nitrogen management, heat recovery, odour and pathogen control, as well as to provide sufficient circulation within the treatment system(Buchanan *et al.*, 2013, Burton, 1992, Evans *et al.*, 1982, Roman and Mureşan, 2014, Svoboda and Fallowfield, 1989). A poor aeration regime can lead to insufficient mixing, excessive foam production, an uneven treatment and high power and capital costs (Burton, 1992, Burton and Farrent, 1998, Burton and Sneath, 1995, Cumby, 1987a, b, c, Sneath *et al.*, 1990). Management of these factors is considered fundamental for an optimal performance. Thereby, establishing suitable operating conditions and apparatus configuration is key (Burton, 1992, Cumby, 1987a, b, c, Svoboda *et al.*, 2013).

According to a review by Burton (1992) the first step in attaining an adequate aeration processes is to outline the desired treatment objectives, benefits and constraints of the system taking into consideration the concerns and requirements of the farmer, codes of practice and the various legislations associated with the treatment and reuse of piggery slurries. For the purpose of this investigation the system needed to therefore be able to 1) perform and handle adequate aeration of piggery slurry, 2) be able to provide sufficient mixing and airflow to keep solids in re-suspension and 3) perform essential biological processes for nitrification.

There are various aerator types on the market for the on-farm treatment of agricultural wastes, and include: compressed air, pumped liquid, mechanical surface or subsurface, and combined mechanical / compressed air aerators (Cumby, 1987c). Factors such as cost, practicality, accessibility, and performance potentials also have to be considered. Svoboda *et al.* (2013) recommended venturi jet aerator with either an external pump site through which air is blown into the liquor through a hole in the tank wall or via an internal submersible pump to for the treatment of ANPS; the first option preferable.

To ascertain favourable conditions for microbial growth in aerobic reactors, there must be an adequate oxygen supply within the mixed liquor (Tribe *et al.*, 1995). Determination of the volumetric mass transfer coefficient (k_La) described as the effectiveness in which oxygen is supplied to a body of water within an aeration vessel through mixing or sparging for a known set of operating conditions, provides important information about a reactors performance (Burton, 1992, Moutafchieva *et al.*, 2013, Tribe *et al.*, 1995). This can be measured either by physical or chemical techniques. The collected information can then be used to optimise control parameters (Tribe *et al.*, 1995). Factors such as tank geometry, equipment configuration, impeller or sparger type, air flow rates, temperature and agitation are known to affect k_La (Özbek and Gayik, 2001).

Therefore to treat ANPS and reduce the subsequent risk of NH_3 exposure; a modelled AWTS as proposed by Buchanan *et al.* (2013) was used to simulate the nitrification processes on a laboratory-scale. For efficient aeration, characterising the system for an ideal equipment configuration and air saturation (DO) levels was required.

This chapter presents the findings from a series of re-aeration experiments performed in the laboratory scaled AWTS described in Chapter 2. The purpose of this research was to examine the effect of equipment configuration on aeration performance at a laboratory scale to assist in the characterisation of an integrated AWTS for optimal treatment. The specific aims of these experiments were to:

- Determine the influence of equipment placement (DO probe height and direction of air stones) on the re-aeration (k_La values) of tap water in the AWTS
- Establish an ideal equipment placement for optimal aeration
- Once configured, determine at what DO concentration (mg O_2 L^{-1}) ANPS reaches complete air saturation at, to assist in the characterisation of an integrated AWTS at a laboratory scale.

3.2 Methods and materials

Configuration of the AWTS was based on the need for regular aeration and combined addition – extraction of ANPS from the reactor.

Prior to conducting the research on the aeration of ANPS, the design and equipment configuration of the system was evaluated; for probe position height (DO probe) and airflow direction (air stones), that best suited the aeration of ANPS. The four configurations for which the re-aeration rate was determined (k_La) are presented in Table 3.1.

Table 3:1: The different equipment configurations assessed

Configuration	DO depth	Air stone orientation to vessel centre
1	Top (4-6 cm below surface)	In
2	Top (4-6 cm below surface)	Out
3	Bottom (19-22 cm below surface)	In
4	Bottom (19-22 cm below surface)	Out

3.2.1 Experimental set up and operational configuration

The laboratory-scaled AWTS described in Chapter 2.1.1 was used to characterise the aeration of ANPS on a laboratory-scale (Figure 2.1). Aeration protocols were, carried out as described in Chapter 2.1.1 with modifications.

3.2.1.1 DO probe position

Aeration was controlled by the amount of DO in the mixed liquor at any given time. Air supplied to the reactor activated, or deactivated, accordingly (Chapter 2.1.1). DO levels were monitored frequently via an ABB DO probe (equipped with an ABB oxygen sensor capsule) suspended in the mixed liquor via a retort stand and clamp. This allowed adjustment to probe height and maintenance to be carried out with ease.

The DO probe was positioned to the side of the reactor (in an area of relatively low bubble production) at a height of either 1) 4-6 cm or 2) 19-21 cm from the bottom of the vessel (Figure 3.1). This was to prevent bubbles from striking the sensor membrane and giving false or inaccurate

readings. Due to the size of the reactor, a DO probe cap guard was not used. However, one could be applied to a larger system if required.

DO concentrations were electronically logged (mA) in 1, 5 or 10 second intervals using a T-TEC A™ data logger (4-20 mA range) (refer to Chapter 2.5.1.2). Equation 3.1 was used to convert values to mg DO L^{-1}.

$$\boldsymbol{DO_{mgL^{-1}} = (DO_{mA} - 4) \times 1.25} \quad \text{.....Equation 3.1:}$$

Where,

$DO_{mg\ L^{-1}}$ = concentration of DO in mg L^{-1}

DO_{mA} = concentration of DO from data logger in mA

1.25 = slope

Slope = DO probe range mg L^{-1} (0-20) divided by DO logger range mA (20-4)

3.2.1.2 Air stone position

Air stones were configured at the bottom of the vessel to face either; towards (in) or away (out) from the vessel's centre (Figure 3.2) in order to establish which orientation maximum bubble displacement, mixing and re-aeration rates could be best achieved.

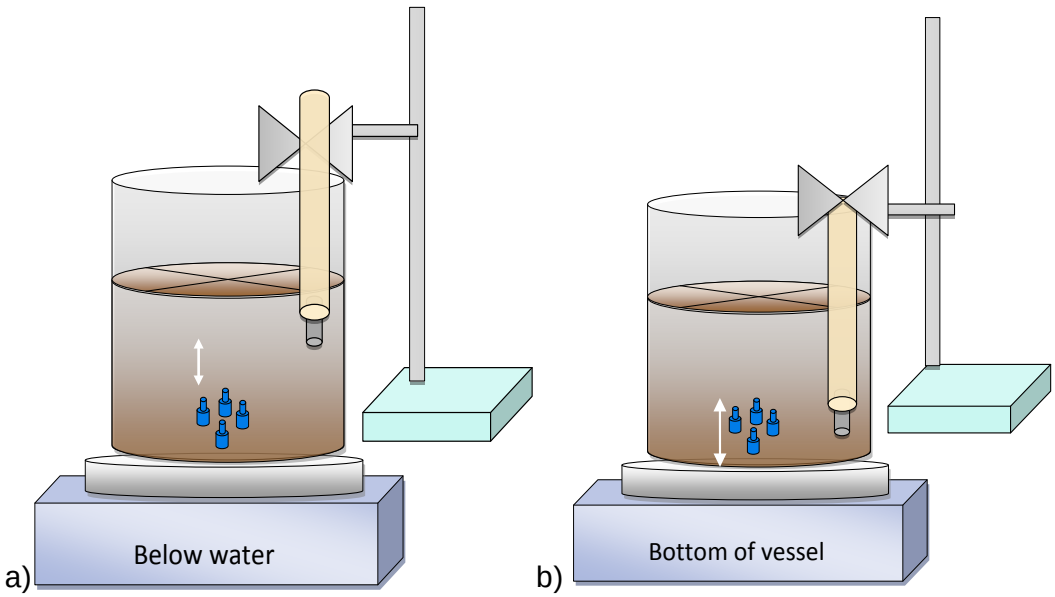

Figure 3.1: DO probe location: The DO probe was placed at a depth of either a) 4-6 cm or b) 19- 21 cm below the water's surface.

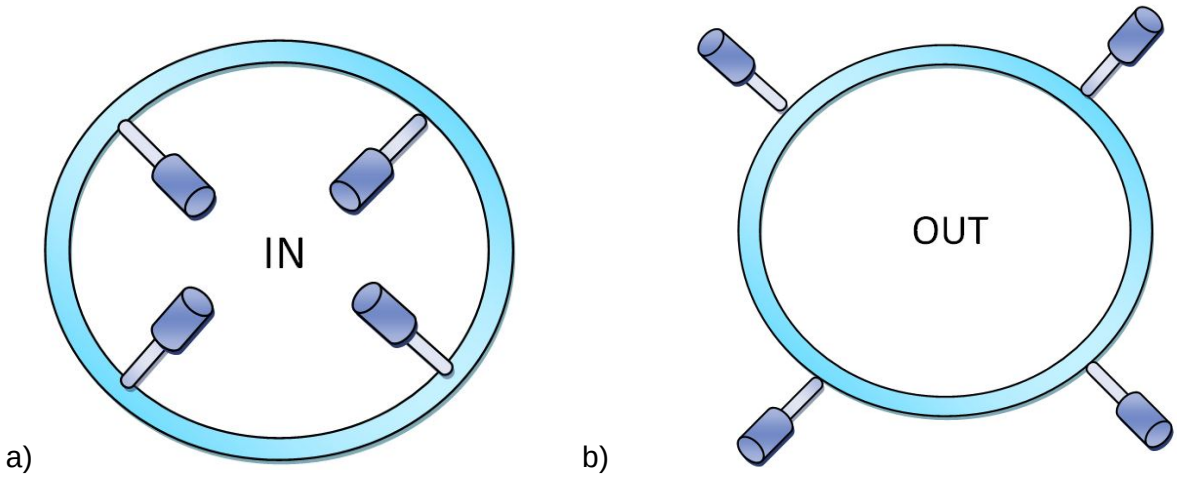

Figure 3.2: Air stone position: Four air stones were attached by silicon tubing to make a ring, with the stones facing, either a) inwards towards the centre of the vessel or b) outwards away from the centre)

3.2.2 Air saturation and re-aeration rates

Oxygen saturation values oxygen transfer rates (k_La) were determined to establish ideal operating parameters (aeration level and THRT) of the AWTS.

Re-aeration rates and the subsequent oxygen saturation values were established by measuring the time it took deoxygenated water to reach complete air saturation (100%). This was carried out per equipment configuration mentioned in 3.1.1 (Table 3.2). Two water sources were examined; deoxygenated tap water and ANPS.

3.2.2.1 Tap water re-aeration

Re-aeration was analysed first in 3 L deoxygenated tap water (0.0 mg DO L^{-1}) sourced directly from the cold-water tap in the laboratory and deoxygenated via the addition of sodium sulphite (Na_2SO_3) (0.217 g 3 L^{-1}). Once deoxygenated, an air supply was activated and aeration occurred as described in Chapter 2.1.1.

Air was blown continuously through the diffusers at a rate of 26.31 cc min^{-1} until complete saturation was reached. Saturation time was monitored via a stopwatch. The re-aeration process was performed in duplicate per equipment configuration unless stated otherwise (Table 3.2).

Temperature and DO concentrations were measured as outlined in Chapter 2, with concentrations logged via a T-TEC A™ data logger every 1, 5 or 10 seconds) accordingly. Manual DO levels were taken directly from the DO transmitter controller and used to visually observe when saturation was reached. Aeration ceased accordingly. Logged data was downloaded, tabulated and graphs produced via the T-TEC A™ software and Microsoft Excel (2007). Results were converted to mg L^{-1} using Equation 3.1.

Oxygen transfer rates (k_La; mg O_2 L^{-1} h^{-1}) were established from the slope of the aeration curves produced in 3.2.2. Aeration curves were generated by plotting the concentration of DO (mg O_2 L^{-1}) against time (h). Additional data beyond the point of saturation was discarded. The estimated slope was taken from the approximate first point of incline to the point of saturation i.e. the infliction at the point of saturation (as indicated via the different coloured dots on the corresponding graphs of Figure 3.3 and Appendix A). A linear line of best fit was plotted using Microsoft Excel 2007 and the

equation of the line determined.

Table 3.2: Equipment configuration for the re-aeration of deoxygenated water per test in the model AWTS. DO probe was placed either 4-6 cm or 19-21 cm below waters (BW) surface and airflow direction either in towards or away from (out) the vessels centre.

Test no.	Flow rate (cc/min)	Duration of run (hr)	Temperature (°C)	Position	
				DO probe depth (BW)	Air stones
A[1]	26.31	0.03	20.7	4-6 cm	IN
B[1]	26.31	0.05	23.0	4-6 cm	IN
C[4]	26.31	0.63	23.8	19-21 cm	OUT
D[2]	26.31	2.41	22.0	4-6 cm	OUT
E[1]	26.31	0.11	21.9	4-6 cm	IN
F[3]	26.31	1.85	20.4	19-21 cm	IN
G[3]	26.31	2.6	20.5	19-21 cm	IN

Note: [1,2,3 or 4] refers to the configuration type described in Table 3.1

3.2.2.2 Anaerobic pig Slurry re-aeration

Once an optimum probe and stone position was identified, in this case configuration 1; 4-6 cm below the water surface (19-22 cm from the bottom of the vessel) and inward facing respectively (Table 3.1), the re-aeration protocol described in 3.2.2.1 was repeated using ANPS. Addition of Na_2SO_3 was not required due to the anaerobic nature of the slurry.

3.2.3 Data analysis

Data were analysed for statistical significance as described in Chapter 2.6 using R (2.15.1) (R Development Core Team, 2011) and Excel 2007 (Microsoft Corporation, USA).

3.3 Results of re-aeration tests

3.3.1 Tap water re-aeration

The ability to aerate a body of deoxygenated tap water varied greatly depending on the configuration of aeration apparatus (Tables 3.2 and 3.3). To determine optimal performance, four configuration sets were examined as described in Table 3.1.

Aeration plots of DO (mg O_2 L^{-1}) against time (h) produced per run (i.e. 1, 2 or 3 runs per test) are presented in Figure 3.3 and Appendix A and demonstrated the influence of apparatus configuration on aeration performance with particular emphasis on DO probe depth and air stone ring placement (configuration; stone + probe). An example of this is presented in Figure 3.3, which presents the re-aeration capability of the system during run 1 of Test A. Equipment was arranged according to configuration 1 (see Table 3.1). DO levels was shown to have reached approximate saturation at 6.9 mg O_2 L^{-1} after 0.03 h of continuous aeration at 20.7°C during run 1 of test A.

In most cases, a definitive lag phase was observed at the start of each run and took between 0.008–0.06 h (0.48–3.6 min) before a measurable increase in DO was recorded. This was due in part to the addition of Na_2SO_3 in excess at the start of each run, to ensure all oxygen was removed as per standard practice. Consequently, time was required to match the oxidation requirements of Na_2SO_3 before saturation could be achieved. In tap water, saturation was reached after 0.02–1.64 h of continuous aeration at 21-24^{o}C across the seven experiments (Figure 3.3 and Appendix A).

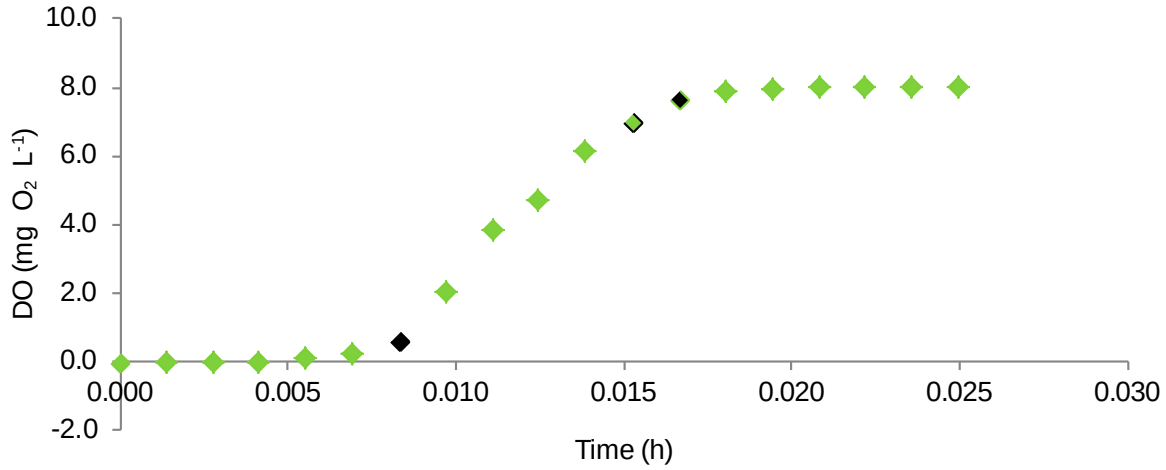

Figure 3.3: Re-aeration A1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. Data collection occurred every 5 seconds over 0.03h. Position of DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which re-aeration occurred and the linear regression was determined.

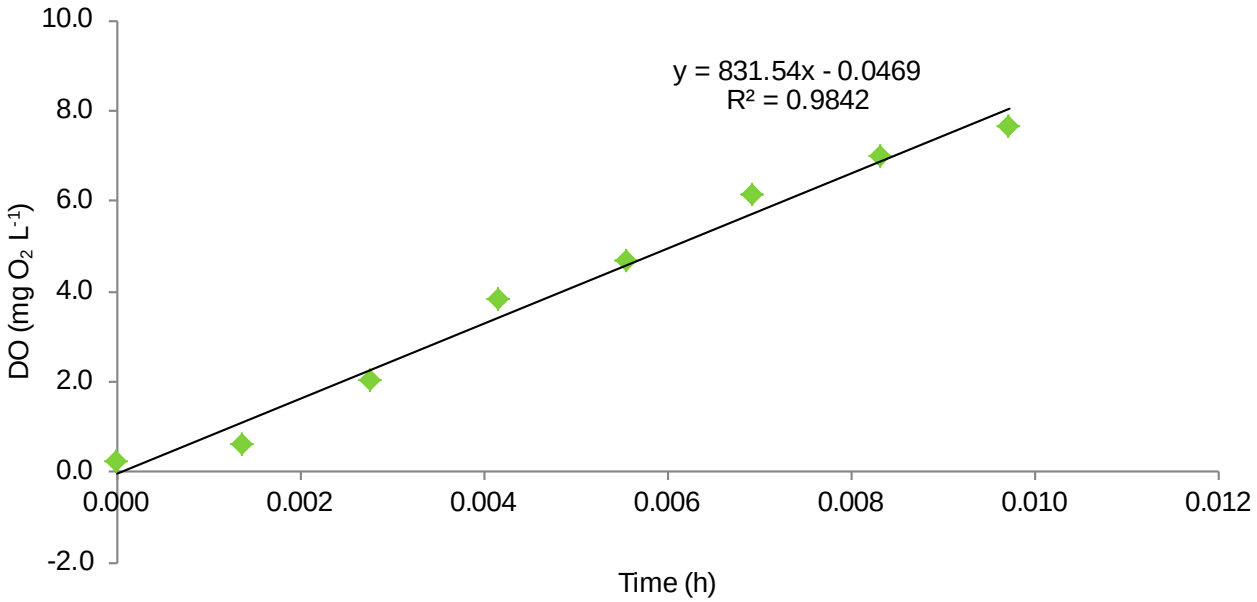

Figure 3.4: Slope of Re-aeration A1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc/ min at 20.7^{0}C (above). The represented data refers to the linear regression ($p \leq 0.001$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 3.3.

Tables 3.2 and 3.3 summarises the oxygen transfer rates (k_La) per configuration type (stone direction + probe depth). For each configuration, oxygen transfer rates (K_La; mg O_2 L^{-1} h^{-1}) were calculated from the slope of each individual aeration curve (mg O_2 L^{-1}) against time (h) described above, sub-plotted from the initial point of incline to the point of inflection, as demonstrated in Figure 3.4 and Appendix A.

Re-aeration was observed at a much faster rate, when the air stones faced 'in' towards the centre of the vessel with the resultant oxygen transfer rates 61.5 times larger on average than those observed when the stones pointed 'out' from the centre, the difference between stone direction statistically significant ($p \leq 0.05$) (Table 3.2). This was more noticeable, in experiments A, B and E, when the DO probe was placed towards the top of the vessel (configuration 1) than when placed at the towards the bottom (experiments C, F and G) (Table 3.3). Mean k_La values were noted to be significantly higher for configuration 1 than those at configurations 2 (out + top), 3 (in + bottom) and 4 (out + bottom) ($p \leq 0.05$). However, no statistically significant differences in k_La values were observed between configurations 2 and 3, 2 and 4 or 3 and 4 ($p \leq 0.05$).

Based on these results the rate of re-aeration and DO measurements appeared to be influenced by apparatus configuration within the system. Re-aeration was detected highest for configuration 1 when the air stones faced "in" to the centre and DO measurements recorded at the highest aeration point. This configuration used throughout the remaining experiments to determine under what operating conditions optimal aeration is achieved.

Table 3.3: Oxygen transfer rates (k_La) obtained at different DO probe depths and air stone ring directions in 0% saturated water (tap) in order to characterise the modelled AWTS for optimal performance.

Test	No.	Air flow(cc min^{-1})	Logger interval (Sec)	Aeration Duration (h)	Saturation time(h)	Equipment position: Air Stone	Equipment position: DO probe (BW)	Mean Slope (KL_a) (mg $O_2 L^{-1} hr^{-1}$) per DO probe position: Equation of line	R^2	Slope	Mean	Per stone direction	per stone + DO position
E[1]	1	26.31	1	2.19	0.11	In	Top	y = 1038x - 0.09	0.98	1038	1095.5 ± 81.32	779.12 ± 423.28	992.06 ± 184.35
	2	26.31			0.08			y = 1153 x - 0.10	0.98	1153			
A[1]	1	26.31	5	0.025	0.04	In	Top	y = 831.5 - 0.05	0.98	831.5	953.10 ± 194.98		
	2	26.31			0.01			y = 1178x + 0.36	0.97	1178			
	3	26.31			0.01			y = 849.8x + 1.11	0.9	849.8			
B[1]	1	26.31	10	0.047	0.02	In	Top	y = 932.8x - 0.47	0.95	932.8	906.13 ± 74.76		
	2	26.31			0.02			y = 963.9x + 0.05	0.96	963.9			
	3	26.31			0.02			y = 821.7x - 0.08	1	821.7			
F[3]	1	26.31	1	1.85	0.50	In	Bottom	y = 13.18x + 0.51	0.98	13.2	13.20 ± 0.00		11.27 ± 2.74
G[3]	1	26.31	1	2.6	0.47	In	Bottom	y = 9.33x + 0.17	1	9.33	9.33 ± 0.00		
C[4]	1	26.31	10	0.63	0.70	Out	Bottom	y = 14.98x - 0.10	1	14.98	15.11 ± 0.18	12.67 ± 3.29	15.11 ± 0.18
	2	26.31			0.60			y = 15.24x + 0.17	0.99	15.24			
D[2]	1	26.31	10	2.41	2.03	Out	Top	y = 8.15x + 0.64	0.98	8.15	10.23 ± 2.94		10.23 ± 2.94
	2	26.31			1.24			y = 12.31x +0.40	0.99	12.31			

Note: * Stone position refers to whether stones are facing towards the vessel's centre (In) or away from (out) and DO probe refers to whether the probe is ~4-6 cm (top) or 19-22 cm (bottom) below water surface (Below water= BW):* [1,2,3 or 4] refers to the configuration type described in Table 3.1

3.3.2 Anaerobic pig slurry re-aeration

Using configuration 1 identified in section 3.4.1(stones directed towards the centre and DO probe 4-6 cm below water), re-aeration rates of ANPS was then examined. Estimated K_La values in ANPS are presented in Table 3.2. Air saturation in ANPS was reached after 1.42 h of continuous aeration at 20.6°C in the modelled system as shown in Appendix B; an air saturation constant of ~ 7.5 mg O_2 L^{-1} identified. This value was then used as a control parameter to manage the quantity of DO available in the vessel at any time to assist in the characterisation of the system for optimal performance.

Table 3.4:Oxygen transfer rate (K_La) of ANPS in the modelled AWTS obtained when using the proposed "best case" configuration; air stones pointed "in" towards the centre of the vessel* and a DO probe depth 4-6 cm below the water surface (configuration 1).

	Air flow(cc min^{-1})	Logger interval (Sec)	Aeration duration(h)	Saturation time (h)	Saturation value (mg O_2 L^{-1})	Slope (KL_a) (mg O_2 L^{-1} hr^{-1}) per DO probe position		
						Equation of line	R^2	Slope
S1	26.31	5	3.50	1.42	7.5	y = 9.67x + 0.20	0.99	9.67
Note: *air stone ring was located 2cm above the bottom of the reactor								

3.4 Discussion

The aim of this research project was to characterise an integrated AWTS at a laboratory scale for the treatment of ANPS. System design plays an important role in achieving an efficient aeration regime for optimal performance in the system (Özbek and Gayik, 2001, Svoboda *et al.*, 2013, Thakre *et al.*, 2008). Research has reported that performance of an aerator is affected by several factors (Cumby, 1987a, b, c, 1990, Özbek and Gayik, 2001). These include, apparatus configurations within the tank, DO concentrations, airflow (i.e. direction), bubble displacement, energy efficiency and the type of aerator used (Cumby, 1987a, b, c).

In order to achieve a high degree of nitrification and SS removal in the system while keeping costs to a minimum, optimal operating conditions such as THRT and air saturation levels are of great importance. The system was characterised for optimum output based on an ideal equipment configuration, achievable aeration, and saturation rates of the system (Bicudo and Svoboda, 1995, Droste, 1997, Svoboda *et al.*, 2013). Equipment configuration was based on the need to identify a suitable probe location for recording DO measurements (i.e. towards the surface) that would be both economical and practical (i.e. longer cable length, easier access). Whilst, at the same time establishing a preferential airflow pattern (direction of air stones at the bottom of the vessel) that will enable competent aeration and recirculation of the aerated water to occur.

Four apparatus configurations were examined for DO probe depths; either 4-6 cm or 19-22 cm below the water surface and air stones orientation; either towards (in) or away (out) from the centre of the air stone ring (Figures 3.1 and 3.2).

During this investigation, data on DO saturation and oxygen transfer rates (k_La) were collected in the mixed liquor of the AWTS for two water types. First in clean water (tap) to optimize equipment configuration (Table 3.1) as per standard protocol (Özbek and Gayik, 2001, Pittoors *et al.*, 2014, Zhen *et al.*, 2003, Zhou *et al.*, 2013), then in ANPS to determine the saturation value to be used as a DO set point under the ‘best case scenario’ in the proceeding chapters.

3.4.1 Tap water re-aeration

This information obtained at a laboratory scale determined the oxygen transfer rates achievable at the four configuration positions examined within the reactor (Table 3.1). A large variation in tap water k_La values were observed throughout the investigation with results shown to fall into two distinct categories; high (821.7 – 1178 mg O_2 L^{-1} h^{-1}) and low K_La (8.2 –15.2mg O_2 L^{-1} h^{-1}) (Table 3.3). Factors such as geometric influence, bubble displacement, density, and location are a likely cause (Ashley *et al.*, 1992, Chen *et al.*, 1992, Fändriks, 2011).

Average aeration rates in the reactor ranged between 8.2 – 1153.0 mg O_2 L^{-1} h^{-1} (Tables 3.3) and were generally noted higher at a shallower DO depth (4-6 cm below the water surface). Particularly when paired with an airflow direction towards the centre of the vessel (tests A, B, and E). This is due in part to an efficient airlift and recirculation pattern occurring when the air stone faced inwards (Fujie *et al.*, 1992). In that as air rises through the tank to the surface, it is dispersed outwards and recirculated back through the reactor as it comes out of the liquid phase. Resulting in a considerable amount of aeration towards the surface of the water (Chen *et al.*, 1992). This is reflected in the DO measurements observed closer to the surface.

Conversely, re-aeration was detected 1.3 times slower and 60.4 times lower on average at the deeper probe depths (19-22 cm below the water surface) than those closer to the surface (Table 3.3). This was observed for both air stone directions. One possible explanation could be that at the deeper depths the water was not being aerated as well as it was at the surface. In that, the probe may not have necessarily measured the entire DO through the water column at the bottom of the vessel as rapidly as it had towards the surface where greater air dispersion took place. This could have been attributed to the geometry of aeration in the system, relating to the positioning of the probe within the tank (Cumby, 1987a, b, c). It is possible then that a slight dead zone had occurred. This could explain the prolonged aeration observed for configurations 3 (in + bottom) and 4 (out + bottom) (Table 3.3). The aeration data also suggest that an insufficient circulation had occurred in the reactor when the air stones were directed towards the sides of the vessel irrespective of DO depths. In fact, re-aeration rates were observed 62.8 times lower and took 4.9 – 82.0 times longer to reach saturation during experiments C and D when air stones pointed

outwards (Figure 3.2b) than those of A, B, E, F and G where the stones faced inwards (Figure 3.2a) (Table 3.3). Air directed outwards would have struck the walls of the vessel as it rose before dropping back down again; creating an uneven dispersal of the aerated water. Longer re-aeration rates resulted. Higher treatment costs may incur, correspondingly. This highlights the importance of airflow direction and the location of measurements (DO probe height).

Aeration is considered one of the main cost factors associated with the treatment of wastewater and can account for up to 50-90% of the processes energy requirements (Thakre *et al.*, 2008, Wesner *et al.*, 1978). Whilst cost and energy efficiency of the system was of little significance on this small scale and thus lied outside the scope of research for this work. As a result, identification of an ideal configuration for this study was made more on the assumption of practicality than efficiency. However, these factors would certainly come in to effect at a larger scale and is something to consider in the future when up scaling (Fändriks, 2011, Svoboda *et al.*, 2013).

Based on these findings, it is evident from the results that the location of essential aeration apparatus has a significant influence over the rate of re-aeration in the system, with aeration most proficient when air stones pointed towards the centre and DO measured 4-6 cm below the water's surface (19-22 cm from the bottom). Whilst, either probe locations could justifiably be used, it was decided upon deliberation, to go with the 4-6 cm depth paired with the inward orientated stones of configuration 1 and consequently used for all experimentation in the system that followed

3.4.2 ANPS re-aeration

Once a suitable configuration was established, aeration rates were assessed in the ANPS. ANPS was shown to yield an aeration rate of up to 9.4 ± 0.5 mg O_2 L^{-1} h^{-1} at an airflow rate of 26.31 cc min^{-1} (Table 3.4) when aerated in accordance to the proposed "best case" scenario of configuration 1 (stone; in and 4-6 cm DO probe depth) (Table 3.1). These were slightly lower (with the exception of the 4.4 mg O_2 L^{-1} h^{-1} at 0 cc^{-1} min^{-1}) than those detected in the diluted raw slurry of Ginnivan (1983) and could be attributed to the variations in design, mode of aeration, measurement location (unspecified in Ginnivan (1983)) and material used (i.e. diluted raw slurry vs. non diluted ANPS). In that Ginnivan (1983) identified that aeration rates of 4.4, 15.7, 23.6, and 118.4 mg O_2 L^{-1} h^{-1} could

be achieved in raw diluted pig slurry at an airflow rate of 0, 1.26, 1.78, and 8.33 vv^{-1} min^{-1} through hollow tube aeration at 20°C. Whilst both aeration protocol and slurry type differed in the work by Ginnivan (1983) to those in the current study, the oxygen transfer data provided a rough estimate of the type of ranges to expect in pig slurry.

It was also interesting, that although configuration was the same as those used for experiments A, B and E in tap water (Tables 3.3), oxygen transfer rates were noted significantly lower (106.1 times lower) in ANPS (Table 3.4). Again, this is most likely due to the nature and potentially higher oxygen demands of the slurry compared to tap water. The information obtained provides an insight on the how the system behaves when the intended pig slurry was used.

The second part of the characterisation process was to identify the air saturation coefficient of ANPS. On average ANPS was observed to have reached saturation at around 7.5 mg, O_2 L^{-1} at 20.6°C in the laboratory scaled ARV after 1.42 h of continuous aeration (Appendix B). This saturation value formed the basis of the DO set point values used in the proceeding chapters (Chapters 4 and 5). Saturation was noted slightly lower than that observed in the works by Ginnivan (1983) in a 1:1 diluted raw slurry; 8.7 mg O_2 L^{-1} at 20.6^{o}C, this was to be expected due to variations in slurry type, composition and handling practices slight variations are to be expected.

3.4.3 Future research

As a fitting aeration set up has now been identified, the next phase of the investigation is to characterise the system for optimal operation and nitrification performance to treat ANPS; improved water quality for reuse and algal growth the end goal. One approach would be to run the system at different variants of the air saturation level identified in Section 3.3.2 (7.5 mg O_2 L^{-1}) and THRTs. Saturation levels and THRTs to be used should be based on the requirements of nitrification as reported in the literature (Gerardi, 2002, Svoboda *et al.*, 2013, Svoboda, 1995, Svoboda and Evans, 1987). The purpose of which would be to establish under which conditions a high degree of nitrification can be achieved for the lowest aeration and treatment time practical whilst still remaining economically viable. This will be examined in more detail in Chapters 4 and 5.

3.5 Conclusion

A good system design is fundamental to achieving the desired outcomes of a competent integrated AWTS. The findings from this chapter support this notion through the assessment of physical location placement and apparatus arrangement at a laboratory scale for the treatment of ANPS. It was evident from the results obtained from a series of re-aeration experiments in both tap water and ANPS that the physical location of both DO probe and air stones within the air stone ring influenced aeration performance.

In particular, the study was able to identify that saturation and aeration rates could be achieved at a much faster when DO measurements were taken towards the surface of the water (depth; 4-6 cm) and an airflow directed towards the centre (at the bottom) of the aerating vessel that at any of the other combinations.

This study was unique in that it was able to demonstrate the successful re-aeration of ANPS for this system with saturation reached at a DO concentration of 7.5 mg O_2 L^{-1} after 1.42 h of continuous aeration. This value can now be used to assist in the characterisation of the modelled integrated AWTS. Development of an optimised operation regime for the treatment of ANPS for reuse and algal growth is the next phase to be examined for the treatment of ANPS. This will be discussed in further details in the succeeding chapters (Chapters 4 and 5).

Information derived during this investigation can also assist in the design and operation of a larger on-farm pilot system.

4. THE EFFECT CHANGING AERATION LEVELS ON SUSPENDED SOLIDS REMOVAL AND AMMONIA OXIDATION IN ANPS WITH A 5 AND 10-DAY RESIDENCE TIMES.

In this Chapter, some of the work and results presented have been published in a peer review journal.

Citation: Hawley M. C., Svoboda I., Fallowfield H. J. (2015) Aeration of anaerobic pig slurry for ammonia oxidation. *Animal Production Science* **55**, 1452-1452. https://doi.org/10.1071/ANv55n12Ab061

4.1 Introduction

The production of algal biomass on treated pig waste, offers a number of advantages in the enhancement of pig slurry as a sustainable resource (Aguirre *et al.*, 2011, Buchanan *et al.*, 2013, Fallowfield, 2013, Fallowfield *et al.*, 1999, Jiménez-Pérez *et al.*, 2004, Strain *et al.*, 1986). Included, is the improvement of water quality, energy production, nutrient removal and a potential feed alternative (Barlow *et al.*, 1975, Borowitzka and Moheimani, 2013, Buchanan *et al.*, 2013, Craggs *et al.*, 2011, Fallowfield, 2013, Mohedano *et al.*, 2012).

Development of an algal culture on animal wastewater is reliant upon a suitable nutrient load (nitrogen and phosphorous), light availability, temperature and oxygen levels (Becker, 1994). Unfortunately, pig slurry possesses a number of characteristics such as suspended solids (SS), ammonia (NH_3), and a dark colouration, which can have inhibitory effects on both algae and pig production (Borowitzka and Moheimani, 2013, Mobin and Alam, 2014). This presents a major challenge for reuse.

Effluent with a rich colouration, and a high SS and ammonium (NH_4^+-N) load is typical of anaerobic lagoons; the predominant treatment method for pig slurry in use in Australian piggeries currently (Buchanan *et al.*, 2013, Environment Protection Agency *et al.*, 2000). SS and colour restricts the

amount of light made available to microalgae for photosynthesis, along with the potential to cause blockages in vital equipment (Boersma *et al.*, 1975, Craggs, 2005, Fallowfield, 2013, Groeneweg *et al.*, 1980).

Despite, NH_4^+-N being the preferred nitrogen source for algal growth, at high pH elevated levels of NH_3-N can be toxic to most algal species, pigs, and adversely affect worker health (Crofts, 1966, Yuan *et al.*, 2011). A study by Murphy (2011) identified the reuse of anaerobic lagoon wastewater in pig sheds to increase the concentrations of airborne bacteria and NH_3-N; a subsequent decrease in pig and worker health resulted. Pre-treatment is therefore required.

Aerobic treatment is fundamentally an efficient treatment process in the management of domestic, industrial, and animal wastewaters (Evans *et al.*, 1980, Pourcher *et al.*, 2007). Inclusion of aerobic treatment, offers the potential for nitrogen manipulation, solids removal, metabolic heat recovery, and odour control (Buchanan *et al.*, 2013, Burton and Sneath, 1995, Svoboda and Evans, 1987, Svoboda and Fallowfield, 1989). If paired with the implemented anaerobic digestion and high rate algal ponds (HRAPs), inclusion of aerobic treatment could provide an added advantage in the enhancement of pig waste as a sustainable resource (APL, 2015b, Buchanan *et al.*, 2013, McGlone, 2013). This makes it an ideal secondary treatment, preceding algal production in HRAPs.

For efficient biological wastewater treatment, knowing how the treatment system responds under different operating conditions is vital in the implementation of new technologies. Literature pertaining to this area is limited for nutrient removal in aerated ANPS, with the focus directed primarily to the reuse and dilution of raw slurry (Barlow *et al.*, 1975, Béline *et al.*, 2008, Bonmatí and Flotats, 2002, Bortone, 2009, Buchanan *et al.*, 2013). The current study focussed on establishing ideal operating parameters to run an integrated anaerobic-aerobic treatment system (AWTS) at laboratory scale for the treatment of ANPS. DO saturation and THRT the focus.

This chapter presents the findings from a laboratory scaled AWTS, operated under different operating combinations based on the growth requirements of nitrifying bacteria (Gerardi, 2002, Svoboda *et al.*, 2013) and the DO saturation value obtained in Chapter 3 (7.5 mg O_2 L^{-1}). The

purpose of which, to determine the influence of DO saturation and exposure time on SS removal, NH_3 oxidation, and the subsequent quality of ANPS for reuse: optimisation the goal. Outcomes from this research will help to develop guidelines for the implementation of this system in the field.

The particular aims of the current study were:

- To identify optimal aeration conditions for SS removal and nitrification in the AWTS
- To assess the influence of aeration conditions (i.e. DO saturation (%) levels and THRT) on SS removal in aerated ANPS
- To assess the influence of aeration conditions (i.e. DO saturation (%) levels and THRT) on nitrification conversions in aerated ANPS

It was, hypothesised that there would be no difference in the level of SS removal and ammonia oxidation irrespective to any alteration in aeration conditions (i.e. DO saturation levels and slurry retention times).

4.2 Methods

4.2.1 Experimental set up and operational configuration

To establish ideal operating parameters, slurry based trials were conducted in the modelled AWTS; the impact of air saturation and theoretical hydraulic retention time (THRT) on SS removal and nitrification the focus. Construction and operation of the system occurred as outlined in Chapters 2 and 3. To allow for maximum air input, the AWTS remained uncovered throughout the duration of research unless stated otherwise, the inlet reservoir, an exception (Plate 2.1).

Prior to trial commencements, vital equipment (DO and pH probes, water bath (WB) thermostat, air flow meter, and peristaltic pump) was, calibrated and set accordingly.

For the purpose of this chapter two experimental conditions were assessed; aeration level (DO saturation) and slurry retention time (THRT) over a set of five trials. The slurry trials were, referred to as ST with a corresponding trial number (i.e. ST1). Operational parameters for each individual trial are outlined in Table 4.1.

Table 4:1: Operating conditions for each slurry trials

Trial number	1	2	3	4	5
THRT (days)	5	5	5	5	10
Controlled Aeration level (% saturation)	10%	10%	20%	100%	100%
Controlled Aeration set-point (DO mgL^{-1})	0.8	0.8	1.5	>7.5	>7.5
Running time (days)	25	39	36	46	41

4.2.1.1 Aeration regime

Aeration level is an important factor to consider when designing the treatment process as it provides the oxygen input needed for the oxidation of NH_4^+-N to NO_3^--N. For an efficient AWTS design, it was imperative to identify at which level nitrification was achieved best in the system. These levels needed to be both economical and suitable for nitrification while optimising the DO requirement. Thus, aeration was provided via an air stone ring, connected to a Welch Thomas vacuum piston dry air pump and an ABB DO transmitter controller (4640/5000) as outlined in Chapter 2.1.1, at an air flow rate of 26.31 cc min^{-1}. Aeration was DO dependent and controlled according to set concentrations at 10% (0.8 mg O_2 L^{-1}), 20% (1.5 mg O_2 L^{-1}), and 100% (>7.5 mg O_2 L^{-1}) of the air saturation value derived in Chapter 3: ~7.5 mg O_2 L^{-1}. Set point concentrations were selected based on the air saturation value of ANPS identified in Chapter 3 (7.5 mg O_2 L^{-1}) and from the recommended DO saturation range (10-100%) required to oxidise NH_4^+-N to NO_3^--N in pig slurry according to Evans *et al.* (1986) and Buchanan *et al.* (2013). Saturation levels per trial are outlined in Table 4.1. As concentrations exceeded the set value, a solenoid valve switched the incoming air into bypass until the DO concentration subsided; aeration was then resumed.

4.2.1.2 Residence time

NH_4^+ oxidising bacteria are slow growing organisms, that require a treatment time of 2.5-14 days (d) as recommended by (Evans *et al.*, 1979). Outside of this range would not be considered economically viable (Evans *et al.*, 1979, Evans *et al.*, 1986). To encourage nitrification and avoid nitrifier washout, a THRT of 5 d was selected, later extended to 10 d at room temperature (19-23^oC). A mean slurry flow rate of 100 mL and 50 mL every 4 h (2.5 h for ST1 and 2 only) was used to achieve a 5 d and 10 d THRT, respectively whilst maintaining a 3 L working volume (Table 4.2). Liquid in excess of this working volume was gravity dispensed from the outlet into the outlet aerobic reservoir (Plate 2.1) for collection.

4.2.1.3 Feedstock and inocula- Seeding the reactor

The reactor was seeded with 100 mL in 11 minutes (50 mL in 3 minutes for ST5, only) of stock ANPS stored in the “inlet” (topped up per THRT) every 4 h (2.5 h for trials 1 and 2 only) via a calibrated variable speed peristaltic pump (Watson Marlow, model 503s R/L) as outlined in Chapter

2.1.1. Pump run time was controlled via an Arlec Compact Digital Time Switch, PC697 set according to the THRT requirements.

Prior to the commencement of the trials, the reactor was initially inoculated with a slurry mix of 2.7 L ANPS and 300 mL ASAPS (Chapter 2.3). ASAPS was added to help kick-start the nitrification process. Additional inoculations were, supplemented when required.

4.2.2 Sampling

240mL of APS and ANPS was collected every 2-3 d and 5-10 d from the outlet and ANPS stock supply, respectively (refer to Chapter2.4). Samples were stored frozen (-20^{o}C) until required for analysis.

4.2.3 Water Quality analysis

4.2.3.1 Environmental conditions

In situ measurements were, recorded several times a week (multiple times per day) for DO, pH, and temperature of the mixed liquor as described in Chapter 2.5. Average values (± SD) were, recorded per parameter per day. Electronic measurements for DO and temperature were also recorded via two T-tech data loggers in 5-minute intervals (Chapter 2.5.1.2 and 2.5.1.3). Due to a fault with the DO transmitter, logged data was not available during trials 5 and 6.

4.2.3.2 Water quality parameters

In order to investigate the effect of aeration operating conditions on SS removal and the inter-conversions within the inorganic-N pool, water quality parameters (TSS, NH_4^+-N, NO_2^--N, NO_3^--N, TN, TC, TOC and IC) were analysed as described in Chapter 2.5.

4.2.4 Statistical Analysis

All data were analysed for statistical significance as described in Chapter 2.6.

4.3 Results

4.3.1 Trial conditions

Values of both controlled operating parameters and measured environmental conditions are given in Table 4.3. Environmental conditions pertained to those of the mixed liquor inside the AWTS. Both *in situ* and electronic measurements recorded. A fault with the DO sensor capsule prevented electronic data collection and aeration control to occur during trials 5-6. Consequently, trials 4-5 were operated under continuous aeration and were therefore considered to have an uncontrolled air saturation level of up to 100% saturation (~7.5 mg O_2 L^{-1}).

Experimentally the measured mean DO concentrations in the mixed liquor were found to be remarkably similar to the required set point values used (0.8 and 1.5 mg O_2 L^{-1}) during trials ST1, 2 and 3 as shown in Table 4.3. An indication that successful (fairly) DO control occurred within the mixed liquor. Under continuous aeration (no set DO set point) DO concentrations averaged 6.32 ± 0.81 (~86%; ST4) and 5.11 ± 1.66 mg O_2 L^{-1} (~68%; ST5), respectively (Table 4.3).

Over the period of experimentation, the mean pH in the mixed liquor ranged from pH 8.2 to 8.6 at 20.0 ± 1.1 to 21.4 ± 0.5°C (Table 4.3). This was consistent with the pH range expected for nitrification; 7.2-9.0 (Alleman, 1985, Gerardi, 2002, Kutty *et al.*, 2011a, Prakasam and Loehr, 1972, Shammas, 1986, Svoboda *et al.*, 2013) and fluctuated with the degree of nitrification.

Table 4:2: Values of measured operating parameters (mean ± standard deviation) and *In situ* environmental conditions

Trial no.	THRT (d)	Experiment Duration (d)	DO (mg O_2 L^{-1})			Temperature (^oC)		pH	
			Set point	Mean	n	Mean	n	Mean	n
1	5	25	0.75	0.5 ± 0.2	28	20.8 ± 1.1	18	8.5± 0.1	18
2	5	39	0.75	0.7 ± 0.2	30	20.4 ± 0.7	23	8.5 ± 0.1	23
3	5	36	1.5	1.3 ± 0.2	32	21.4 ± 0.5	26	8.6 ± 0.0	26
4	5	46	7.5	6.3 ± 0.8	25	20.0 ± 1.1	25	8.4 ± 0.7	25
5	10	33	7.5	5.1 ± 1.7	21	20.9 ± 0.8	22	8.2 ± 0.2	22

4.3.2 Suspended solid removal

Results of TSS removal over time were obtained for each operational configuration analysed, (Figure 4.1). Inlet TSS concentrations (mean ± standard deviation) varied between 0.79 ± 0.13 and 1.15 ± 0.73 g L^{-1} in the ANPS pre-treatment. The highest concentrations reported were detected during ST4 (1.15 ± 0.73 g L^{-1}).

Figure 4.1 showed that under aerobic conditions over 50% of TSS were removed post treatment, (Two-sample t-test, $p \leq 0.05$; Wilcoxon rank sum test, $p \leq 0.05$), irrespective of operational conditions (DO saturation or THRT). More specifically:

The effect of aeration level on SS removal

Under aerobic conditions, up to 52% of TSS were removed when DO maintained ~ 10% saturation (0.5 ± 0.2 mg O_2 L^{-1} and 0.7 ± 0.2 mg O_2 L^{-1}) during ST1 and ST2, respectively when operated with a THRT of 5 d (Figure 4.1a and b). The difference between inlet and outlet TSS concentrations was found to be statistically significant for both trials under this operational configuration. Outlet

TSS reported significantly lower post treatment, (ST1; 0.39 ± 0.07 g L^{-1} and ST2; 0.50 ± 0.04 g L^{-1}) than those reported in the ANPS pre-treatment (0.81 ± 0.07 g L^{-1} and 1.04 ± 0.21 g L^{-1}; t (12) = 11.10, $p \leq 0.001$; W= 94, $p \leq 0.001$, respectively). An indication that aerobic treatment of ANPS resulted in a significant decrease in SS.

Since ST1 and ST2 were operated using the same set aeration parameters (10% DO set point and a 5 d THRT) independent t-tests were performed to compare the differences in inlet and outlet TSS concentrations between the two trials (Figure 4.2). SS concentrations measured 1.3 times higher in ST2 (1.04 ± 0.21 g L^{-1} and 0.39 ± 0.07 g L^{-1}) than ST1 (0.81 ± 0.07 g L^{-1} and 0.39 ± 0.07 g L^{-1}) on average for both inlet and outlet concentrations, respectively. Unfortunately, a statistical significance was detected between the two inlets (t (10) = -2.35, p = 0.04 ≤ 0.05), but not the outlets (W = 48, p = 0.34 ≥ 0.05), an indication that possible confounding due to inlet variation occurred ($p \leq 0.001$). The variation in inlets however, was not reflected in the amount of SS removed on average, both trials removed >52% post treatment.

As this was an adaptive design study, the first experimental run (ST1) was regarded as an exploratory experiment and all comparisons made for the experimental condition (10% DO saturation and 5 d THRT) performed using ST2 only.

An increase in DO to 1.3 ± 0.2 mg O_2 L^{-1} at 21.4 ± 0.5^{o}C (set at 1.5 mg O_2 L^{-}`; ~20% saturation) yielded a slight improvement in TSS removal, with 57% removed over a 32d period in ST3 (Figure 4.1c). This was 6% higher than that of ST2 at 0.74 ± 0.21 mg O_2 L^{-1} (Figure 4.3). 0.43 ± 0.03 g L^{-1} of suspended solids was detected at the end of the aeration period, with an average of 0.35 ± 0.04 g L^{-1} measured in the treated outlet during the trial (Figure 4.1c). Like those of ST1 and ST2; outlet TSS concentrations (0.34 ± 0.06 g L^{-1}), were reported significantly lower post aeration than pre-aeration (0.79 ± 0.13 g L^{-1}) by nearly 0.45 g SS L^{-1} (2.32 times) (t (20) = 10.87, $p \leq .001$) in ST3.

A similar reduction trend to those at low DO was also evident when DO was maintained at 6.3 ± 1.1 mg O_2 L^{-1} in an uncontrolled DO environment set to 100% saturation and a 5 d THRT (Figure 4.1d). Over 74% of TSS were removed at 20.0 ± 1.1^{o}C during ST4, with outlet TSS significantly

lower post aeration than in the inlet by up to 3.9 times (1.44 ± 1.61 g L^{-1} to 0.37 ± 0.05 g L^{-1}; W = 144, $p \leq 0.05$) (Figure 4.1e). An increase in DO up to 6.3 ± 1.1 mg O_2 L^{-1} yielded a removal rate of 0.23–0.34 g SS L^{-1} and 0.34 g SS L^{-1} greater than that at 10% and 20% saturation, respectively (Figure 4.3).

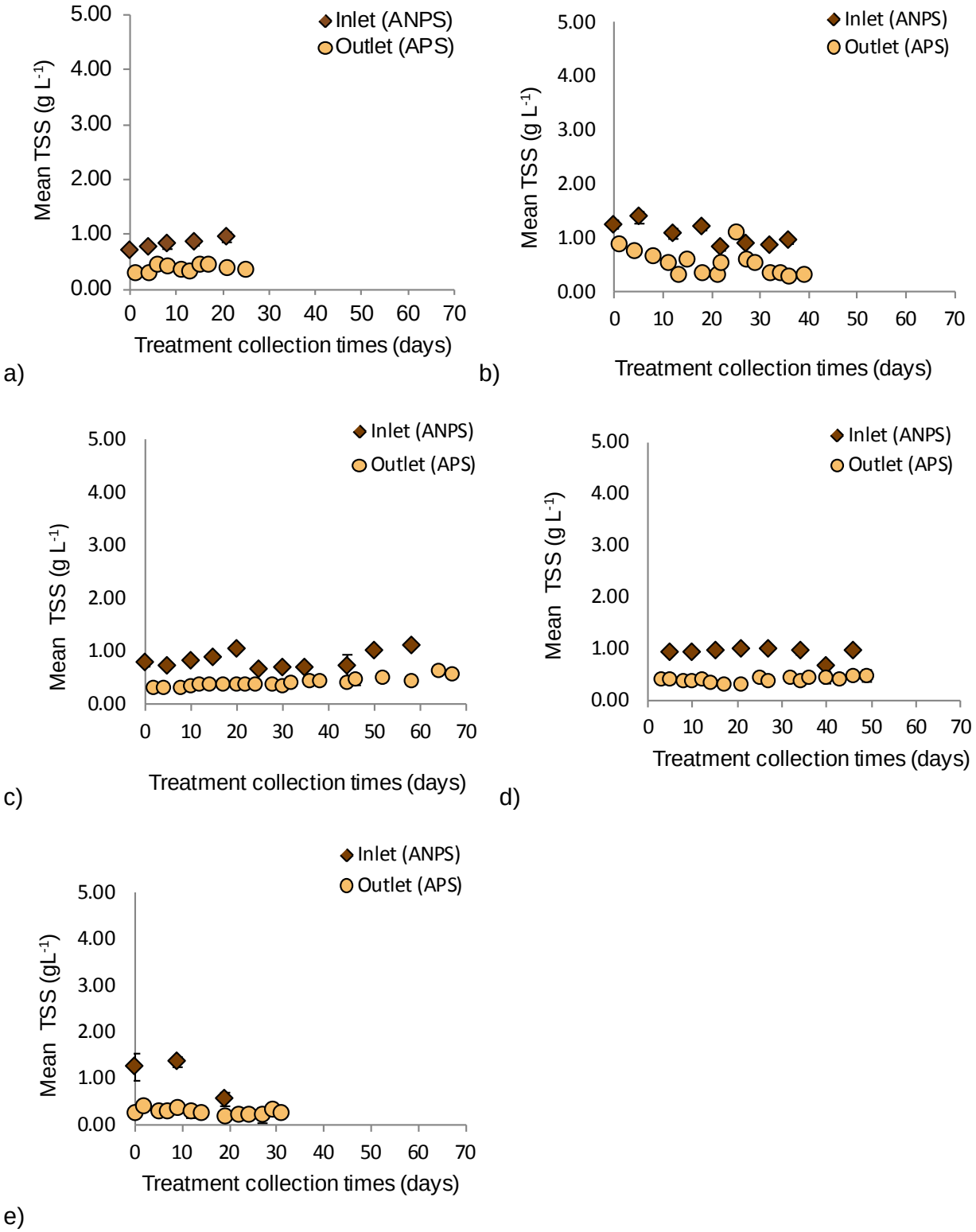

Figure 4.1: Average TSS concentrations (g L^{-1}) before (inlet) and after (outlet) aeration in the AWTS under various operating regimes: a) THRT 5 d at 0.5 ± 0.2 mg O_2 L^{-1} (~10% air saturation, 0.8 mg O_2 L^{-1} set point), 20.8 ± 1.1°C (ST1); b) THRT 5 d at 0.7 ± 0.2 mg O_2 L^{-1} (~10% air saturation, 0.8 mg O_2 L^{-1} set point), 20.4 ± 0.7°C (ST2), c) THRT 5 d, 1.3± 0.2 mg O_2 L^{-1} (~20% air saturation, 1.5 mg O_2 L^{-1} set point), 21.4 ± 0.5°C (ST3), d) THRT 5 d, 6.3± 0.8 mg O_2 L^{-1} (~ up to 100% air saturation, uncontrolled 7.5 mg O_2 L^{-1} set point) , 20.0 ± 1.1°C (ST4) and e) THRT 10 d, 5.1± 1.7 mg O_2 L^{-1} (~up to 100% air saturation, uncontrolled 7.5 mg O_2 L^{-1} set point) , 20.9 ± 0.8°C (ST5).

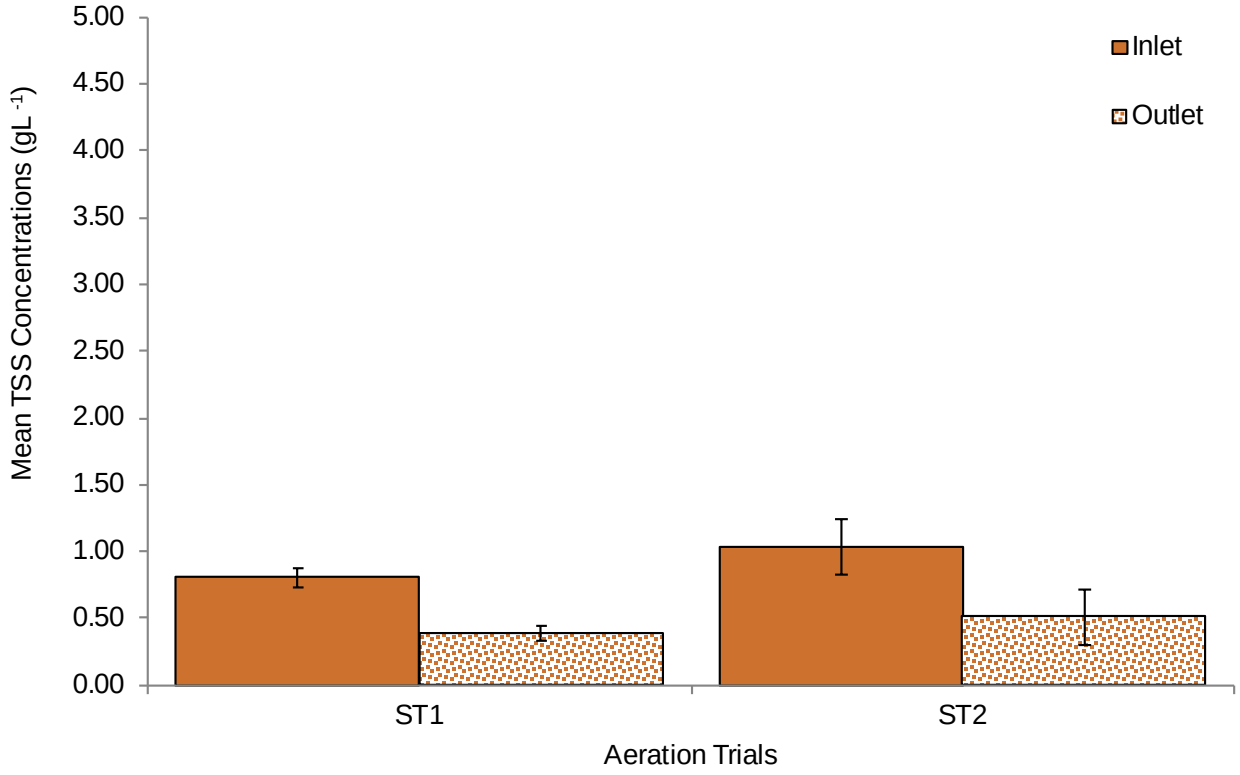

Figure 4.2: A comparison of the mean (± SD) ANPS SS concentrations (g L^{-1}) before (inlet) and after (outlet) aeration at a 0.8 mg O_2 L^{-1} (10% saturation) set point and 5 d THRT for trials ST1 and ST2 .

Figure 4.3 presents a comparison between mean inlet and outlet TSS concentrations across the three experimental conditions operated with a 5 d THRT. Despite a slight improvement in TSS removal, results showed the effect of increasing aeration set points from 0.8 (10%) to 1.5 (20% saturation) then no control (up to 100%) mg O_2 L^{-1} to have had no statistical significance on the mean TSS load post treatment (Kruskall-Wallis Chi-square (2) = 2.90, $p \geq 0.05$) when a 5 d THRT was maintained (Figure 4.3). Each displayed a clear reduction in SS of greater than 50%. However, it should be noted that a slight variation in inlet TSS concentrations was observed across the four trials. Concentrations measured 1.0-1.3 times lower in ST3 (0.79 ± 0.12 g L^{-1}) and 1.1–1.3 times higher in ST2 (1.04 ± 0.21 g L^{-1}). The difference in the overall behaviour exhibited was minimal based on these aeration rates. No significant difference was exhibited in inlet SS concentrations between each of the trials run with a 5 d THRT (ST2, ST3, and ST4) (Figure 4.3). Consequently, the null hypothesis that a change in aeration levels would have no significant impact on the removal of TSS was therefore, accepted.

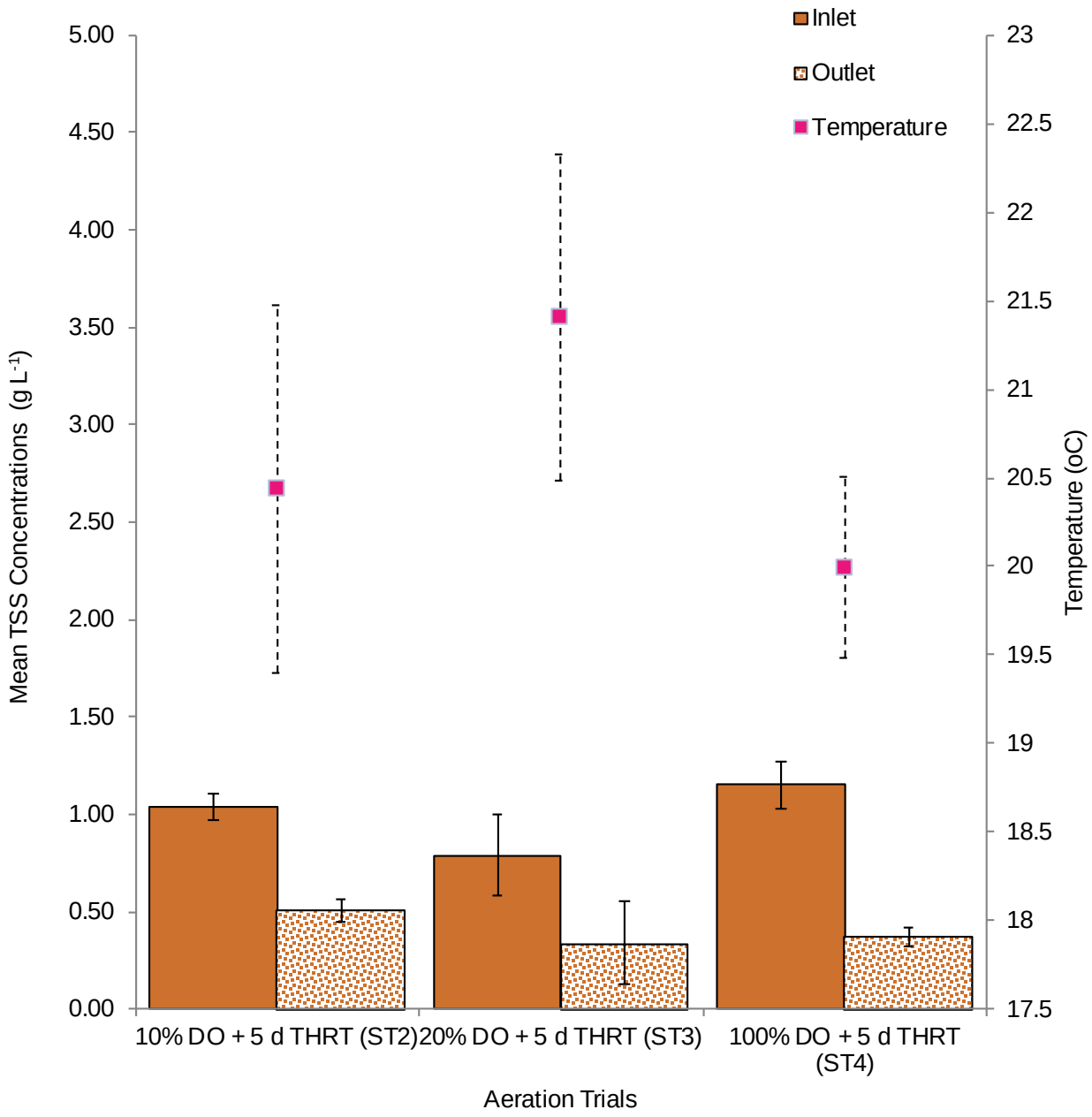

Figure 4.3: Comparison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before (inlet) and after (outlet) aeration with a 5 d THRT and DO set points of 10%, 20% or no control (up to 100% saturation) over a series of three experiments.

The effect of THRT on SS removal

THRT was increased from 5 d to 10 d during ST5 to assess SS removal performance at a longer THRT (Figure 4.1e). During the trial period (41 e), the average SS concentration decreased from 0.96 ± 0.40 g L^{-1} to 0.28 ± 0.06 g L^{-1} at 5.1 ± 1.7 mg O_2 L^{-1} (uncontrolled DO set point of 7.5 mg O_2 L_{-}^{-1} ~100% saturation at 20.9 ± 0.8 °C) (W = 52, $p \leq 0.001$) (Figure 4.1e). This equates to a removal of 71%, the highest removal achieved across the four operation parameter sets assessed in ST2-ST5.

A comparison of mean TSS concentrations before and after aeration with an uncontrolled DO set point of 7.5 mg O_2 L^{-1}, 100% air saturation) during ST4 and ST5 is presented in Figure 4.4. An increase in THRT from 5 d to 10 d resulted in mean outlet TSS levels significantly lower (1.32 times) in ST5 (10 d THRT; 0.28 ± 0.06 g L^{-1}) than ST4 (5 d; 0.37 ± 0.05 g L^{-1}), when aerated at the higher saturation range (62-100% DO) (t (27) = 4.68, $p < 0.001$). A 3% increase in SS removal resulted.

Slight variations in mean inlet SS concentrations were also detected across the two THRT, but were identified as not statistically significant, despite SS noted 1.20 times higher in ST4 (1.15 ± 0.73 g L^{-1}) than ST5 (0.96 ± 0.40 g L^{-1}; W = 0.94, p = 0.88 ≥ .05).

Thus, the null hypothesis that a change in THRT would have no significant effect on TSS removal was rejected.

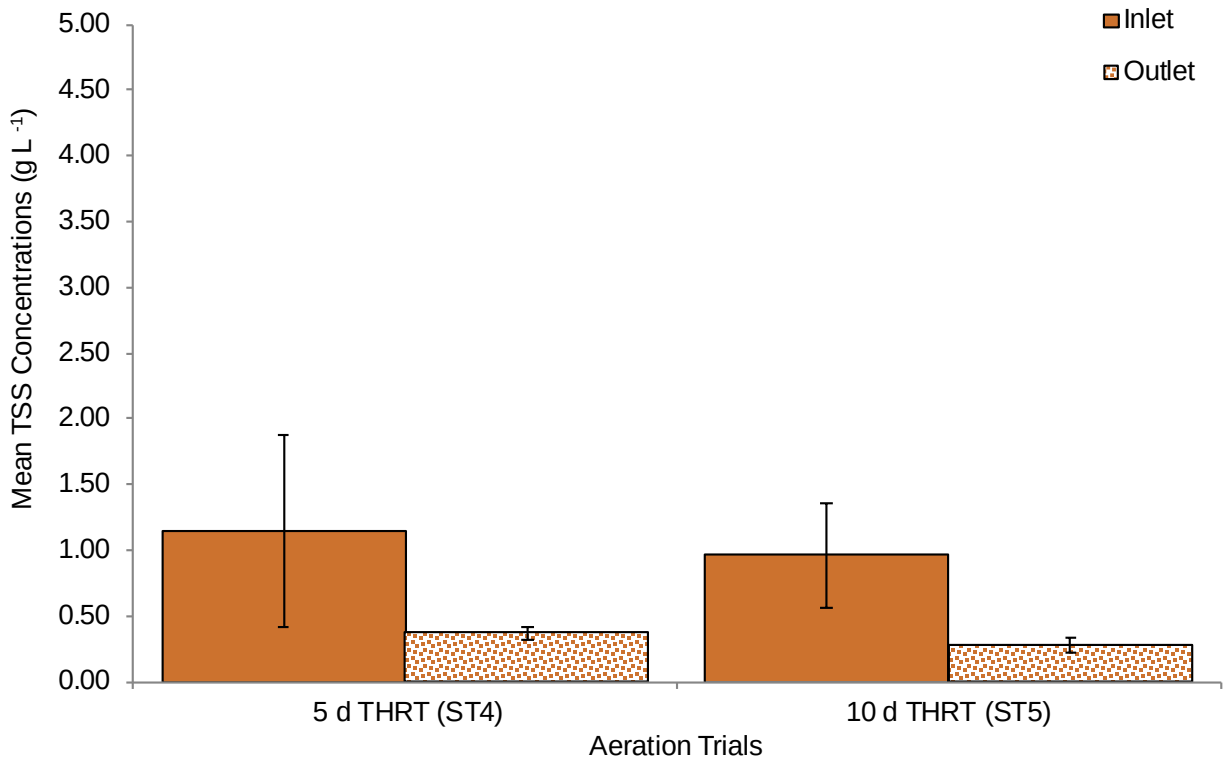

Figure 4.4: Comparrison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before (inlet) and after (outlet) aeration under continuous aeration with no set DO set point (up to 100% saturation) and either a 5 d or 10 d THRT for trials ST4 and ST5.

4.3.3 Effect of DO level and THRT on ammonium oxidation and nitrification

Summarised in Figures 4.5–4.8 are the results of a TIN mass balance for each of the operating configurations highlighted in Table 4.1, assessed over a series of five experiments. TIN accounted for 67-100% of the TN content measured.TN concentrations ranged from 0.87-2.19 g L^{-1} in the inlet and 0.61-1.34 g L^{-1} in the treated mixed liquor across the five trials. A TIN mass balance was generated by comparing the quantifiable N (NH_4^+-N, NO_2^--N, and NO_3^--N) content of both the inlet and treated outlet in order to evaluate the inter-conversion of inorganic-N fractions following subsequent treatment in the modelled AWTS of Chapter 2.

The TIN content, was observed to be on average 1.1–1.7 times higher in the inlet (0.96-1.77 g L^{-1}) than those observed in the outlet (0.87–1.33 g L^{-1}). NH_4^+-N the predominate form, accounting for up to 98-100% of the inlet TIN content. Outlet TIN comprised of 32-100% NH_4^+-N, <BLOD (below the level of detection)–45% NO_2^--N and <BLOD–23% NO_3^--N (Figure 4.5–4.9). The higher TIN content in the inlet was found to be statistically significant to those of the outlet for trials; ST3 (*t* (20)

= 3.81; $p < 0.001$), ST4 (t (23) = 2.26; p = 0.03 ≤ 0.05) and ST5 (t (15) = 3.99; $p < 0.001$).

During aeration up to 24–76% of the inlet NH_4^+-N content was removed on average, with between <BLOD–33% of the inlet NH_4^+-N oxidised to NO_2^--N (3–33%) and NO_3^--N (<BLOD-17%) in the outlet post treatment, 28-96% conserved as NH_4^+-N (Figure 4.5–4.9). The remaining 9-36% unaccounted for, most likely lost through volatilisation. The effect of change to the aeration operating concentrations (DO and THRT) on nitrogen transformation was assessed per configuration parameters.

Operation was first assessed at a controlled DO set point of 0.8 mg O_2 L^{-1} (~10% saturation, 0.5 ± 0.2 mg O_2 L^{-1} maintained) at 20.8 ± 1.1°C, pH 8.5 ± 0.1 and a 5 d THRT (ST1). Aeration yielded a reduction in NH_4^+-N of up to 0.42 g L^{-1} (29%) post treatment, as demonstrated in the inorganic-N transformation plot presented in Figure 4.5 (inlet TIN; 1.46 ± 0.77 g L^{-1} and outlet TIN; 1.33 ± 0.52 g L^{-1}). Less than 0.53 g NH_4^+-N $L^{-1}$was detected at the end of the trial period (25 d). The difference between inlet (1.46 ± 0.77 g L^{-1}) and outlet (1.04 ± 0.51 g L^{-1}) NH_4^+-N concentrations was found to be not statistically significant at the 95% confidence interval, even though outlet NH_4^+-N measured on average 1.6 times lower post treatment (t (12) = 1.24 p = 0.24 ≥.05). Of the reduced NH_4^+-N, 57% was converted to NO_2^--N (0.24 g L^{-1}) and 12% to NO_3^--N (0.05 g L^{-1}) (Figure 4.5). A slight increase in NO_2^--N and NO_3^--N concentrations was detected over time, correspondingly, reaching a maximum of 0.47 g NO_2^--N L^{-1} (d 25) and 0.11 g NO_3^--N L^{-1} (d 8).

In ST2, little to no nitrification was detected when operated under the same operating parameters as ST1, with 2.9% of NH_4^+-N oxidised to NO_2^--N (0.04 ± 0.01 g L^{-1}); NO_3^--N <BLOD) (Figure 4.6). A mean DO concentration of 0.7 ± 0.2 mg O_2 L^{-1} at 20.4 ± 0.7°C was maintained (Table 4.3). Figure 4.6 presents the inorganic-N transformation plot for ST2. The mean TN and TIN content for ST2 averaged 0.98 ± 0.18 g L^{-1} and 0.98 ± 0.67 g L^{-1} g L^{-1} in the inlet and 0.70 ± 0.16 and 0.99 ± 0.37 g L^{-1} in the outlet respectively, majority of which was in the form of NH_4^+-N. These values were significantly lower (1.3-2.2 times) than those of ST1 (inlet; 2.19 ± 0.16, 1.46 ± 0.77 and 1.46 ± 0.77 g L^{-1} and outlet; 1.34 ± 0.27, 1.33 ± 0.52 and 1.6 ± 0.77 g L^{-1}, respectively) (Figures 4.5 and 4.7) when operated under similar aeration conditions. The differences between the two trials was

statistically significant for TN and NO_2^--N ($p \leq 0.05$), but not for TIN, NH_4^+-N, or NO_3^--N ($p \geq 0.05$) (Figure 4.7). This was true for both inlet and outlet samples. The higher TN but similar NH_4^+-N content in ST1 suggests that there may have been some organic-N in the inlet, which might not have been present in ST2, and could explain why nitrification was lower in ST2. Nevertheless, up to 20% of NH_4^+-N was removed post treatment; the lower NH_4^+-N concentrations in the outlet (1.01 ± 0.27 g NH_4^+-N L^{-1}) differed significantly to those in the inlet (1.26 ± 0.21 g NH_4^+-N L^{-1}) by up to 1.3 times (t (19) = 2.09; p = 0.051 ≥ *0.05*).

Evaluation of the dataset for both ST1 and ST2 shows a loss in TN (39% and 29%), TIN (9% and 18%), and NH_4^+-N (29% and 20%), post treatment, respectively. These losses identified to be the likely result of organic-N or nitrogen loss through NH_4^+-N volatilisation (Figures 4.5 - 4.7). Removal was observed 10% higher in ST1 than ST2 for TN and NH_4^+-N, but 9% lower for TIN.

Figure 4.7 presents the inorganic-N transformation data collated for the 10% aeration set point during ST1 and ST2. Greater NH_4^+-N and NO_2^--N oxidation was exhibited during ST1 than ST2 (Figure 4.7). Denoted by the significantly higher NO_2^--N (6.0 times; W = 117, $p \leq 0.001$) and NO_3^--N (>12.5 times; W = 120, $p \leq 0.001$) content of ST1 (0.24 ± 0.13 g NO_2 L^{-1} and 0.05 ± 0.04 g NO_3^--N L^{-1}) post-aeration compared to ST2 (0.04 ± 0.01 g NO_2 L^{-1} and <BLOD g NO_3^--N L^{-1}), respectively. As seen with the SS content, a variation in inlet N concentrations was observed between the two trials, attributed to the variable nature of the ANPS used. TN, TIN, and NH_4^+-N concentrations were reported 2.23, 1.13 and 1.16 times higher in ST1 than ST2, respectively. A statistical significance was detected between the two trials for TN, organic-N and NO_2^--N ($p \leq 0.05$), but not for TIN, NH_4^+-N, and NO_3^--N ($p \geq 0.05$).

Regardless, aeration at 10% DO successfully removed up to 29% of NH_4^+-N. Unfortunately, incomplete nitrification resulted with only <BLOD - 0.05 g NH_4^+-N L^{-1} oxidised through to NO_3^--N. An indication of an insufficient nitrifying population, nutrient levels (DO, N and C), or an additional oxygen demand within the system occurred, which could have confounded nitrification within the system. One solution was to increase the amount of DO maintained in the system. DO was subsequently increased from 10% saturation to 20% saturation (see below).

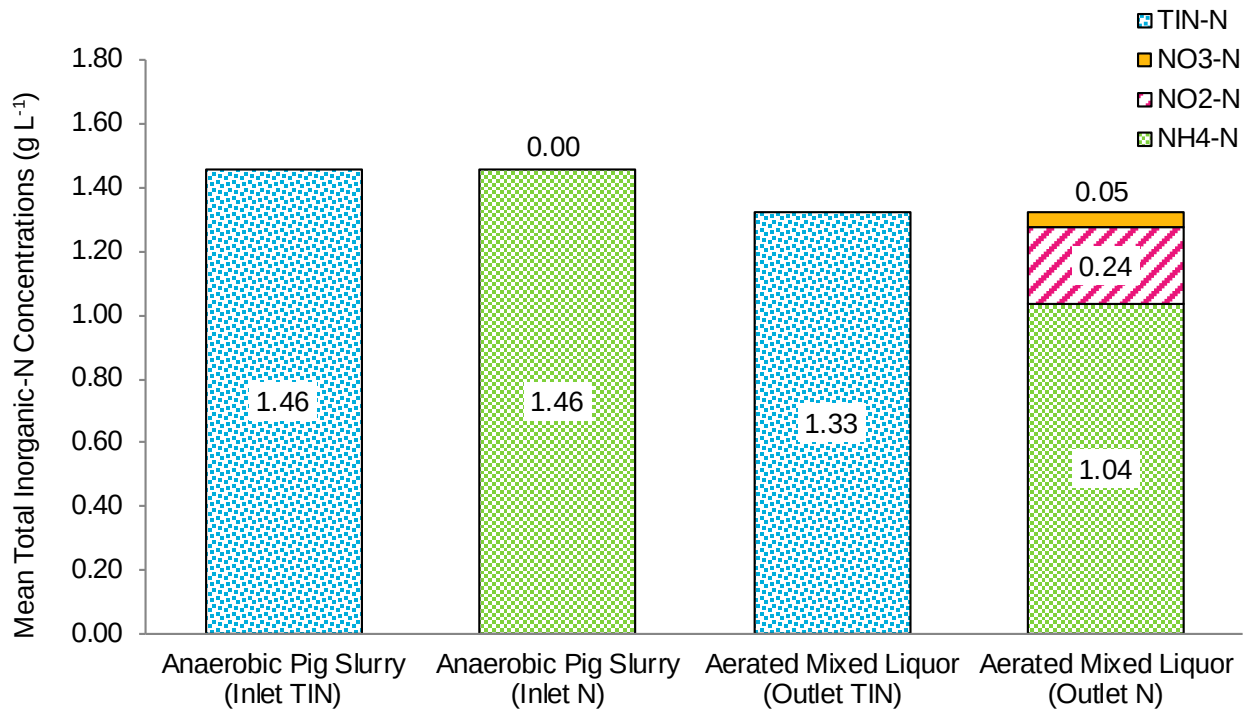

Figure 4.5: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment at a DO (*in situ*) concentration of 0.5 ± 0.2 mg O_2 L^{-1} at 20.8 ± 1.1°C (approx. 10% saturation) and a 5 d THRT during ST1. A pH of 8.5 ± 0.1 maintained.

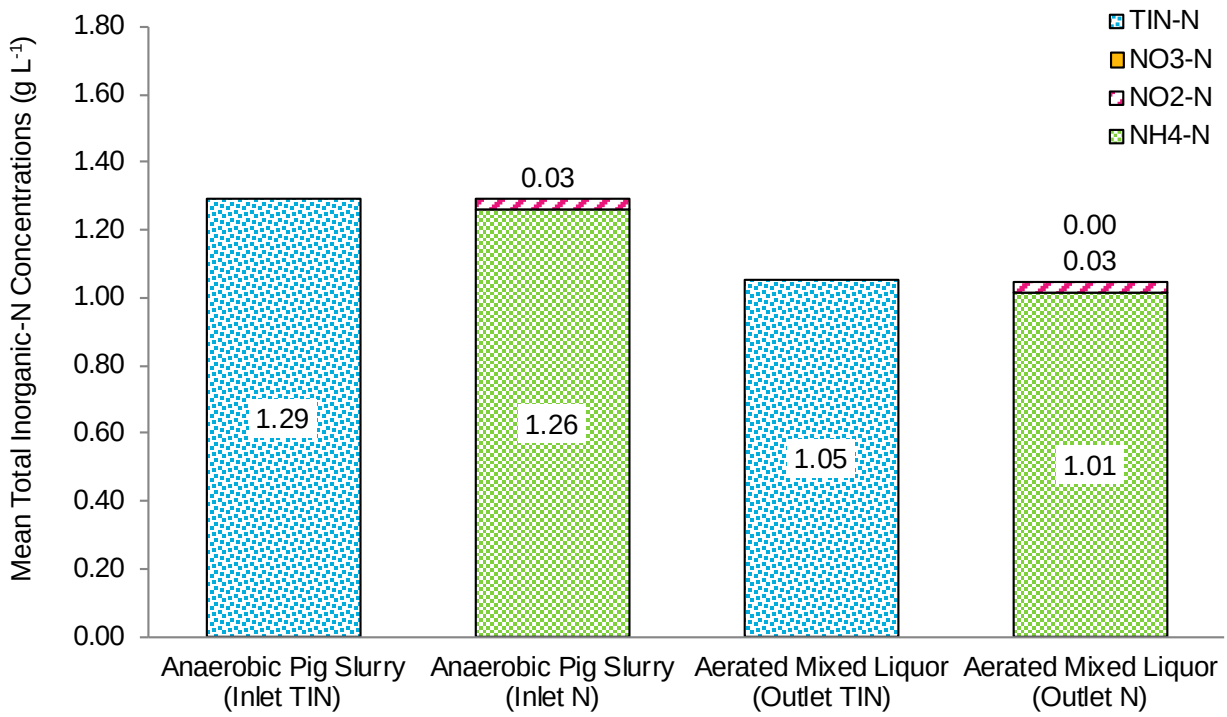

Figure 4.6: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment at a DO (*in situ*) concentration of 0.7 ± 0.2 mg O_2 L^{-1} at 20.4 ± 0.7°C (approx. 10% saturation) and a 5 d THRT during ST2. A pH of 8.5 ± 0.1 maintained.

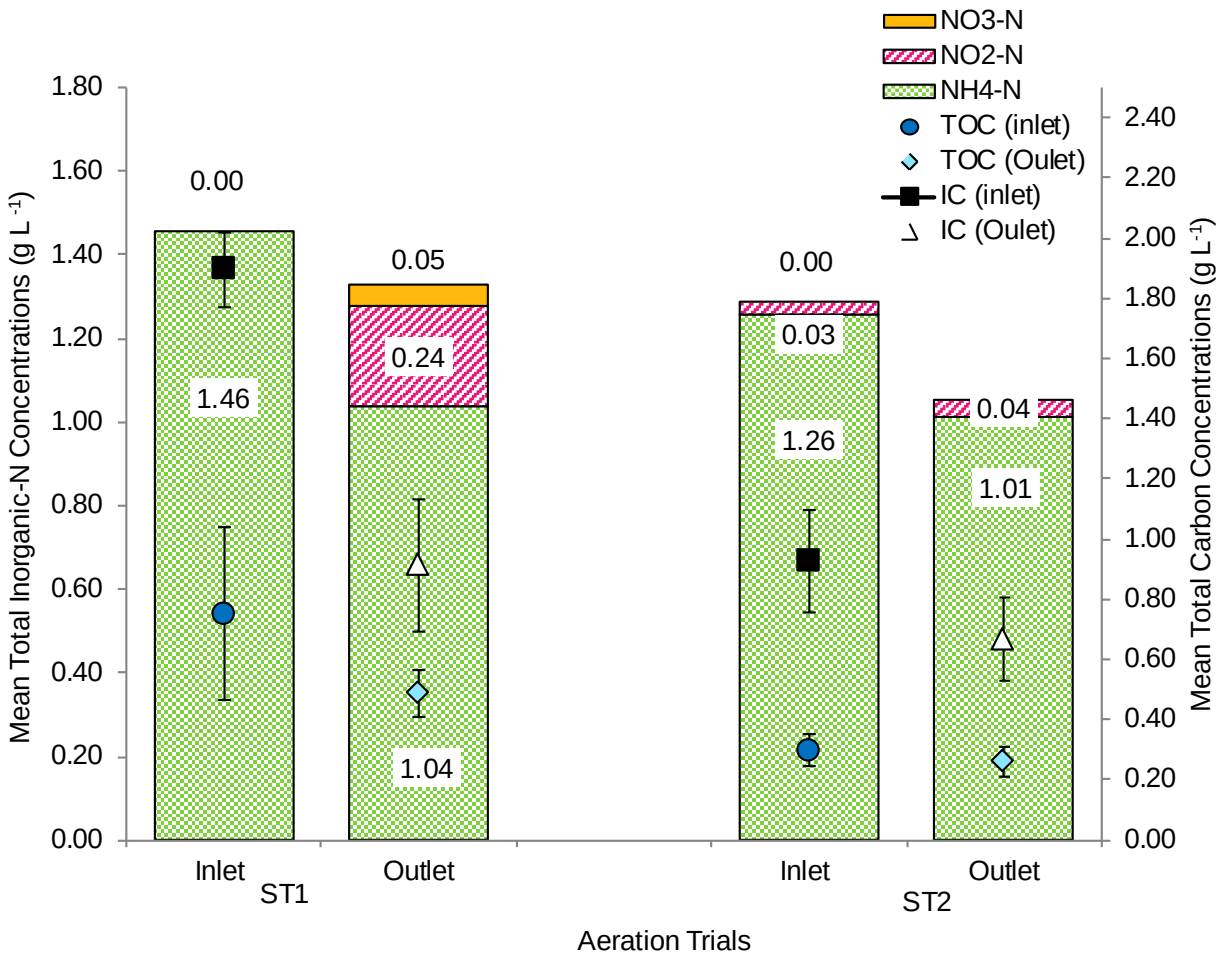

Figure 4.7: A comparison of TIN and TC levels in both the inlet and mixed liquor outlet analysed during trialsST1 and ST2 when aerated with a 0.8 mg O_2 L^{-1} saturation set point (10%) and 5 d THRT operation configuration.

Figure 4.8 presents the results of an inorganic-N transformation following an increase in DO concentrations to a set point of 1.5 mg O_2 L^{-1} (~20% air saturation) during ST3, 1.3 ± 0.2 mg O_2 L^{-1} at 21.4 ± 0.5°C (*In situ*) maintained. THRT remained at 5 d. Over the 36 d experimentation period, 55% of NH_4^+-N was removed in ST3, oxidised to NO_2^--N (21%,) and NO_3^--N (0.04%). This oxidation trend was not dissimilar to that at 0.7 ± 0.2 mg O_2 L^{-1}. In fact, an increase in DO up to 1.3 ± 0.2 mg O_2 L^{-1}demonstrated a slight improvement in NH_4^+-N removal of up to 30% compared to the 20% removal achieved at the 0.8 mg O_2 L^{-1} set point (~10% DO; ST2) (Figures 4.6, 4.8 and 4.10). Mean NH_4^+-N concentrations measured on average 1.3 times higher in the inlet and 1.3 times lower in the outlet samples of ST3 than those of ST2 but were not statistically significant (t (7) = 2.24, p = 0.06 ≥ 0.05; inlet and t (22) = 1.59, p = 0.13 ≥ 0.05; outlet) (Figures 4.6, 4.8 and

4.10). Thus, it appears that Inlet variation between the two trials N content was unlikely to have had a statistical influence on nitrification.

Unfortunately, no real improvement in NO_3^- accumulation was observed (Figure 4.8). The results presented in Figure 4.8 indicate that nitrification had stopped at NO_2^--N, with low NO_3^--N levels (<BLOD–0.02 g NO_3^--N L^{-1}) conserved. The high pH 8.6 ± 0.0 detected, suggests NH_4^+ volatilisation had occurred. This coincided with the results depicted in ST2. The findings suggest that nitrite oxidisers were not present during aeration at the minimum end of the aeration spectrum.

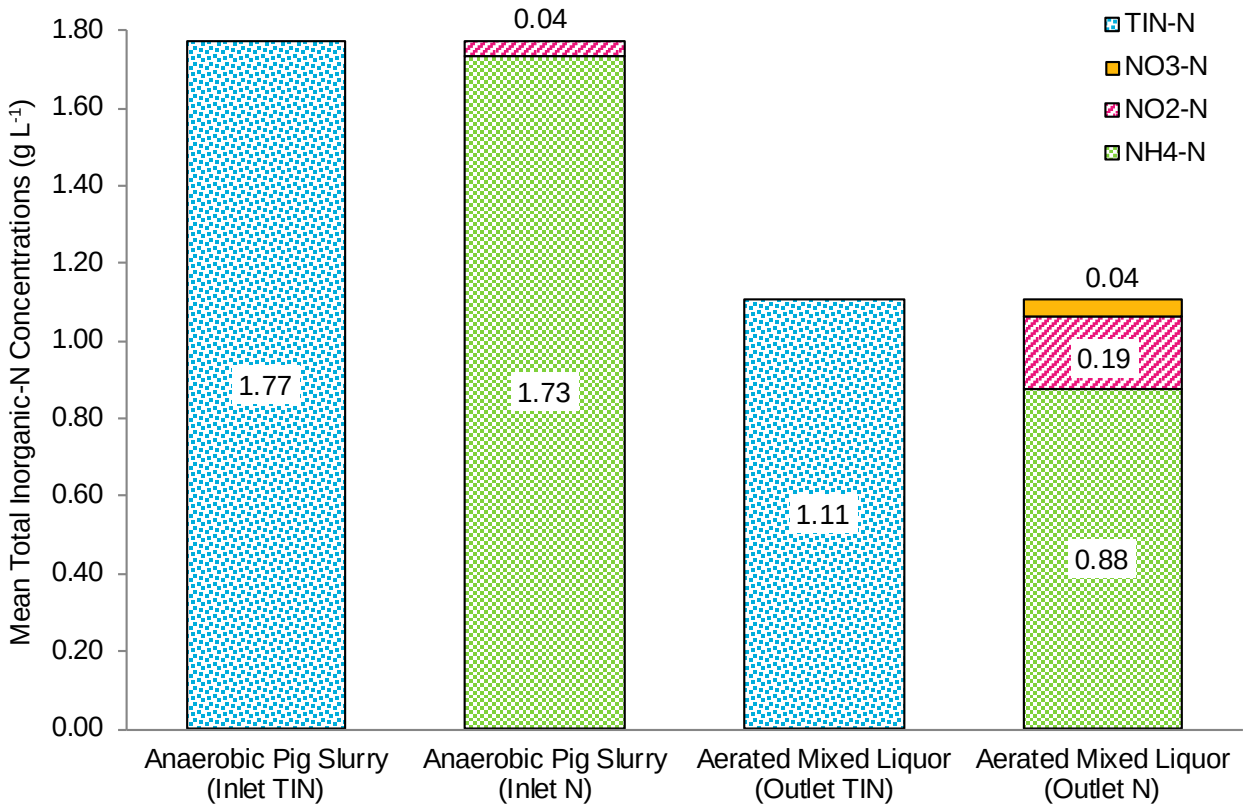

Figure 4.8: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment (outlet) at a DO (*in situ*) concentration of 1.3± 0.2 mg O_2 L^{-1} at 21.4 ± 0.5°C (approx.17-20% saturation) and a 5 d THRT during ST3. A pH of 8.6± 0.0 maintained.

Operation of the AWTS towards the high end of the air saturation scale; 6.3 ± 0.8 mg O_2 L^{-1} at 20.0 ± 1.1°C (no DO set point; ~100% saturation; ST4) also generated an oxidation trend similar to those observed at the 0.8 mg O_2 L^{-1}(~10% saturation; ST2) and 1.5 mg O_2 L^{-1} (20% saturation; ST3) set points. THRT remained at 5 d with a mean pH of 8.4 ± 0.7.

Figures 4.9 and 4.10 shows that an increase in DO up to 6.3 ± 0.8 mg O_2 L^{-1} in an uncontrolled DO environment, yielded removal of 53% NH_4^+-N; 30% greater than those achieved at the lower saturation set points (F (2, 41) = 7.61; $p \leq 0.001$). On average NH_4^+-N concentrations measured 0.71 g L^{-1} lower in the treated outlet, post treatment than the inlet pre-aeration, the difference statistically significant (W = 131 $p \leq 0.001$) (Figure 4.9). Correspondingly, an increase in NO_2^--N accumulation from <BLOD (inlet) to 0.32 ± 0.23 g L^{-1} was also observed post-aeration, with 46% of the 0.71 g L^{-1} NH_4^+-N oxidised to NO_2^--N in the outlet (Figure 4.9).

Despite a clear raise in nitritation, NO_3^--N remained <BLOD, an indication that not the entire oxidised N had nitrified. This coincided with the results of the previous two set points as shown in Figure 4.10. Figure 4.10 presents the comparison of inlet and outlet TIN concentrations for the three configurations operated with a 5 d THRT. Inlet and outlet TN and TIN concentrations measured lower in ST4 at 6.3 ± 0.8 mg O_2 L^{-1} than those detected in either ST2 or ST3 by 1.0 - 2.5 times. Organic-N was detected in ST1 only (inlet 0.73 ± 0.78 g L^{-1}, outlet; 0.01 ± 0.64 g L^{-1}) <BLOD for ST2, 3 and 4. Analysis of the data demonstrated the difference in inorganic-N fractions to be statistically significant between those obtained without DO control (~up to 100%) and those at 10% and at 20% saturation for NH_4^+-N (outlet only), NO_2^--N ($p \leq 0.05$) but not for NH_4^+-N (inlet only), NO_3^--N and estimated organic-N ($p \geq 0.05$) when compared to one another.

The results signify a lack of active nitrifiers within the mixed liquor, an indication that the THRT may not be long enough to enable a suffice population to establish and low nutrient availability, possibly the result of an additional demand within the system. One way to improve this was to increase the THRT from 5 d to 10 d as shown in Figure 4.11.

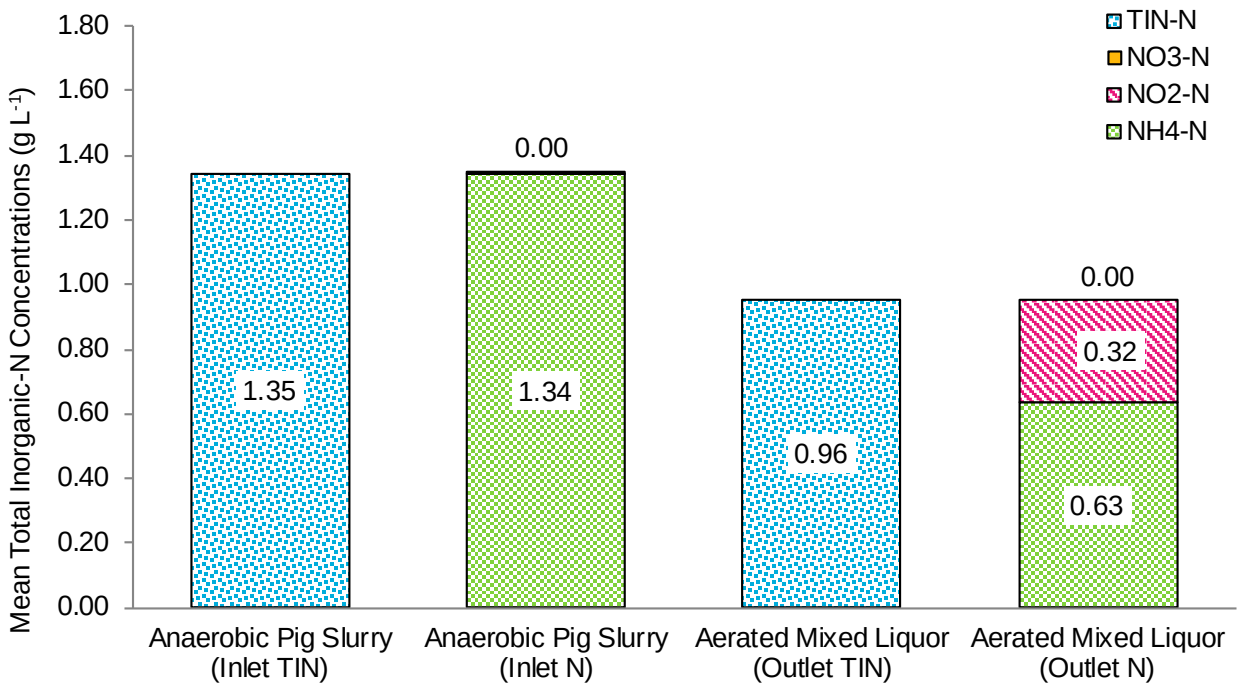

Figure 4.9: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment in an uncontrolled DO environment and THRT of 5 d during ST4. A mean DO (*in situ)* concentration of 6.3 ± 0.8 mg O_2 L^{-1} at 20.0 ± 1.1°C (No DO set point; ~ 100% saturation) and pH 8.4 ± 0.7 achieved over a 46 d treatment period.

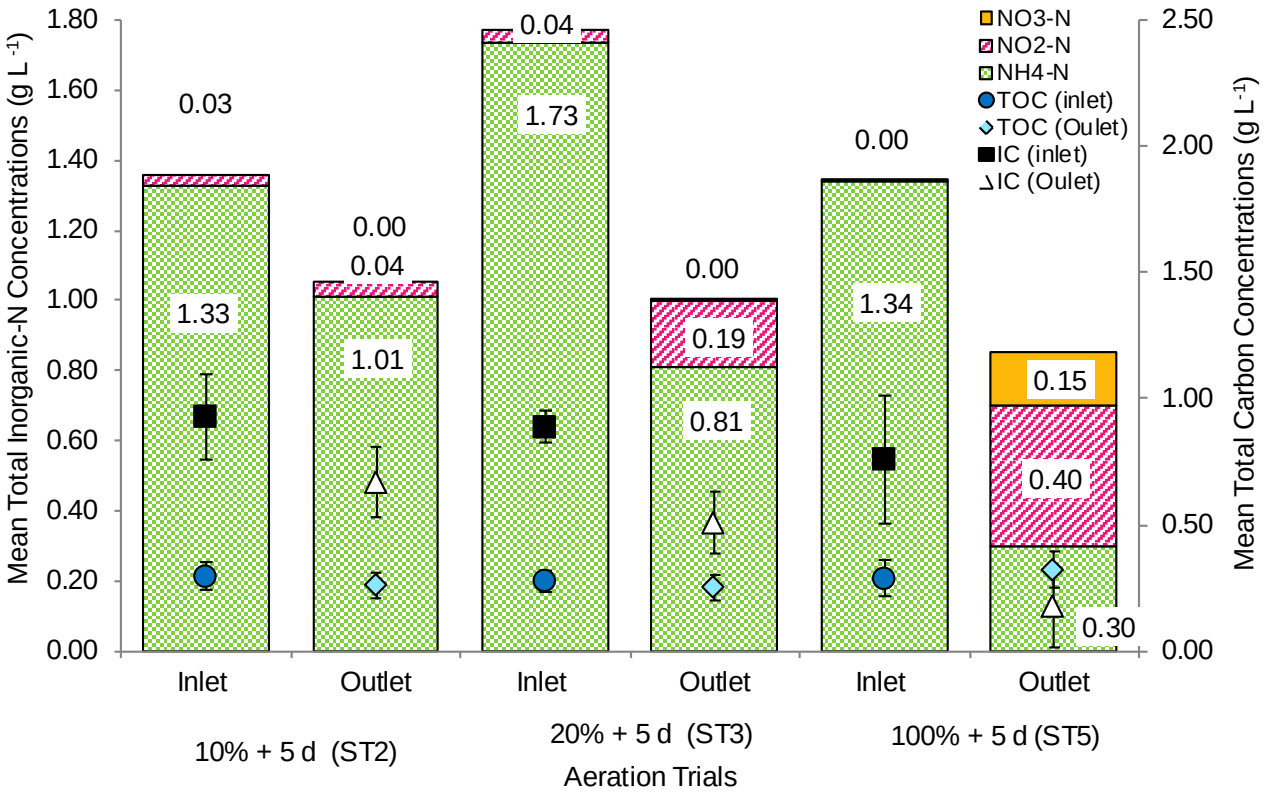

Figure 4.10: A comparison of outlet N and C levels analysed during trials ST2, ST3 and ST4 operated with a 5 d THRT and DO saturation set points of 10%, 20% or no DO control (100%) set points .

The effect of THRT on ammonia oxidation

In Figure 4.11, an increase in THRT from 5 d to 10 d demonstrated a significant improvement in nitrification levels post treatment during ST5 (no DO set point; ~100%). DO (uncontrolled) averaged 5.1 ± 1.7 mg O_2 L^{-1} at 20.9 ± 0.8°C during the trial period. At this configuration, 0.38 and 0.20 g NH_4^+-N L^{-1} was nitrified to NO_2^--N (44%; 0.39 ± 0.04 g L^{-1}) and NO_3^--N (23%; 0.20 g L^{-1}) respectively, whilst 0.28 ± 0.12 g L^{-1} remained as NH_4^+-N (24%). An average reduction in NH_4^+-N of up to 76% from 1.17 ± 0.09 in the inlet to 0.28 ± 0.12 g L^{-1} in the outlet was obtained; the highest oxidation rate achieved across the five runs (Figure 4.11).

Evaluation of the analysed nitrogen levels before and after treatment at the longer THRT identified the difference to be statistically significant for each of the quantifiable-N fractions measured during ST5 ($p \leq 0.05$). Inorganic-N content in the outlet found to be 4.2 times lower for NH_4^+-N (t (15) = 13.02, $p \leq 0.001$) and 32.6–67.3 times greater for NO_2^--N (t (14) = 33.70, $p \leq 0.001$), and NO_3^--N (W = 7, p = 0.04 ≤ 0.05) than those of the inlet, respectively (Figure 4.11).

A comparison of mean inlet and outlet nitrogen concentrations at both THRTs found nitrification to be at its greatest at the longer THRT (DO levels over 5.1 ± 1.7 mg O_2 L^{-1} (Figure 4.12). This was denoted by a significant increase in NO_3^--N accumulation. NO_3^--N levels measured 0.20 g L^{-1} greater at 10 d than at 5 d (W = 24, $p \leq 0.001$) with an uncontrolled DO set point (set at 100% saturation). An indication of an increased nitrifying biomass within the mixed liquor occurred; a result of the longer THRT,

The findings indicate an association between changes in THRT and nitrification. Unfortunately, the association may have been confounded by the inlet characteristics (more notably in TN, organic-N, TOC, and IC concentrations). Inlet N levels detected higher in ST5 for TN (1.6), NO_2^--N (0.01 g L^{-1}) and organic-N (11.0 times) than ST4 but had a lower TIN (1.1) and NH_4^+-N (1.2 times) content; NO_3^--N no difference. The difference between the two inlets were significant for TN (p = 0.01 ≤ 0.05) and organic-N ($p \leq 0.05$). TIN (p = 0.30 ≥ 0.05), NH_4^+-N (p = 0.30 ≥ 0.05), NO_2^--N (p = 0.20 ≥ 0.05) and NO_3^--N (p = 0.18 ≥ 0.05) however, showed no statistical difference, accordingly. The results suggest nitrification may have been confounded by this variation. These increases could be

the result of organic-N volatilisation in ST5 but not ST6. As a result, there is not enough substantial evidence to reject the null hypothesis that a change in THRT would have no significant effect on nitrification.

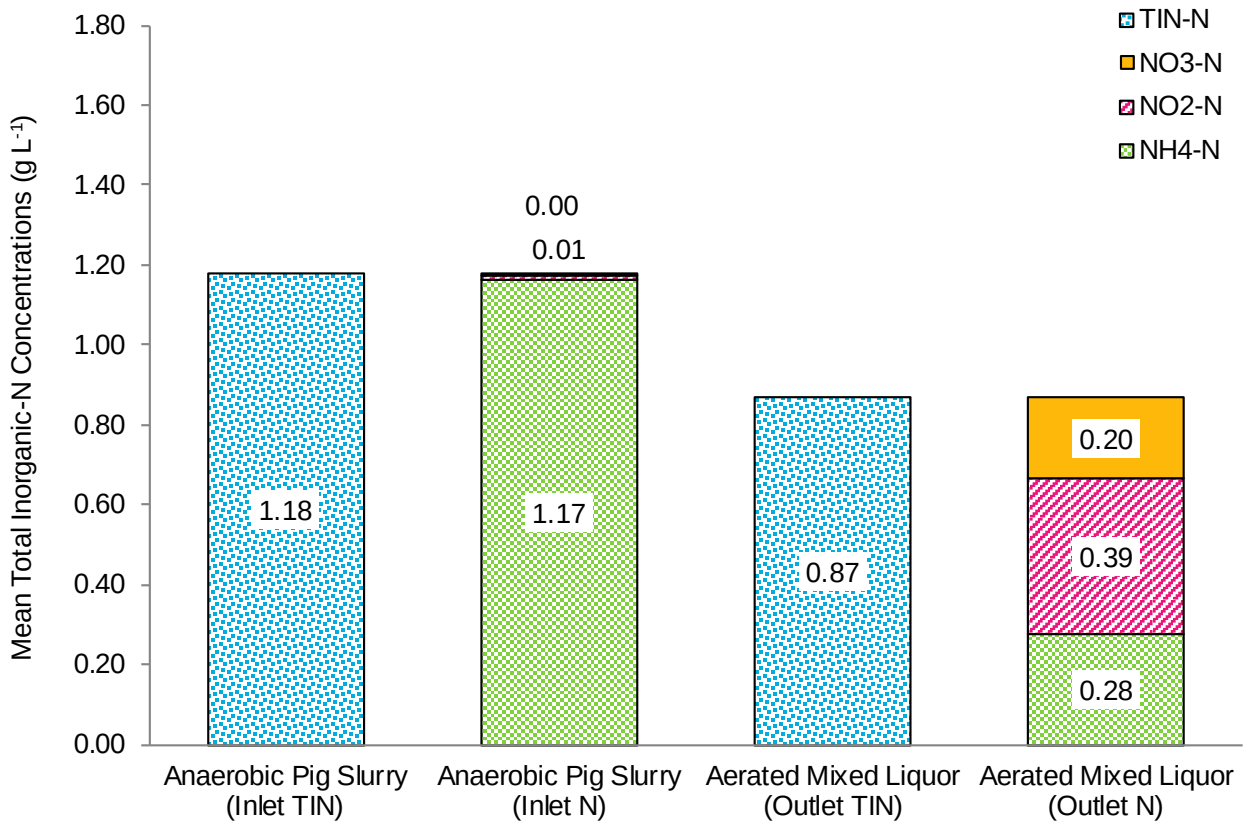

Figure 4.11: An inorganic-N mass account (g L^{-1}) of ANPS produced before and after undergoing aerobic treatment in an uncontrolled DO environment and 10 d THRT during ST5. A mean DO (*in situ*) concentration of 5.1 ± 1.7 mg O_2 L^{-1} at 20.9 ± 0.8°C (up to 100% saturation) and pH 8.2± 0.2 achieved over a 41 d treatment period.

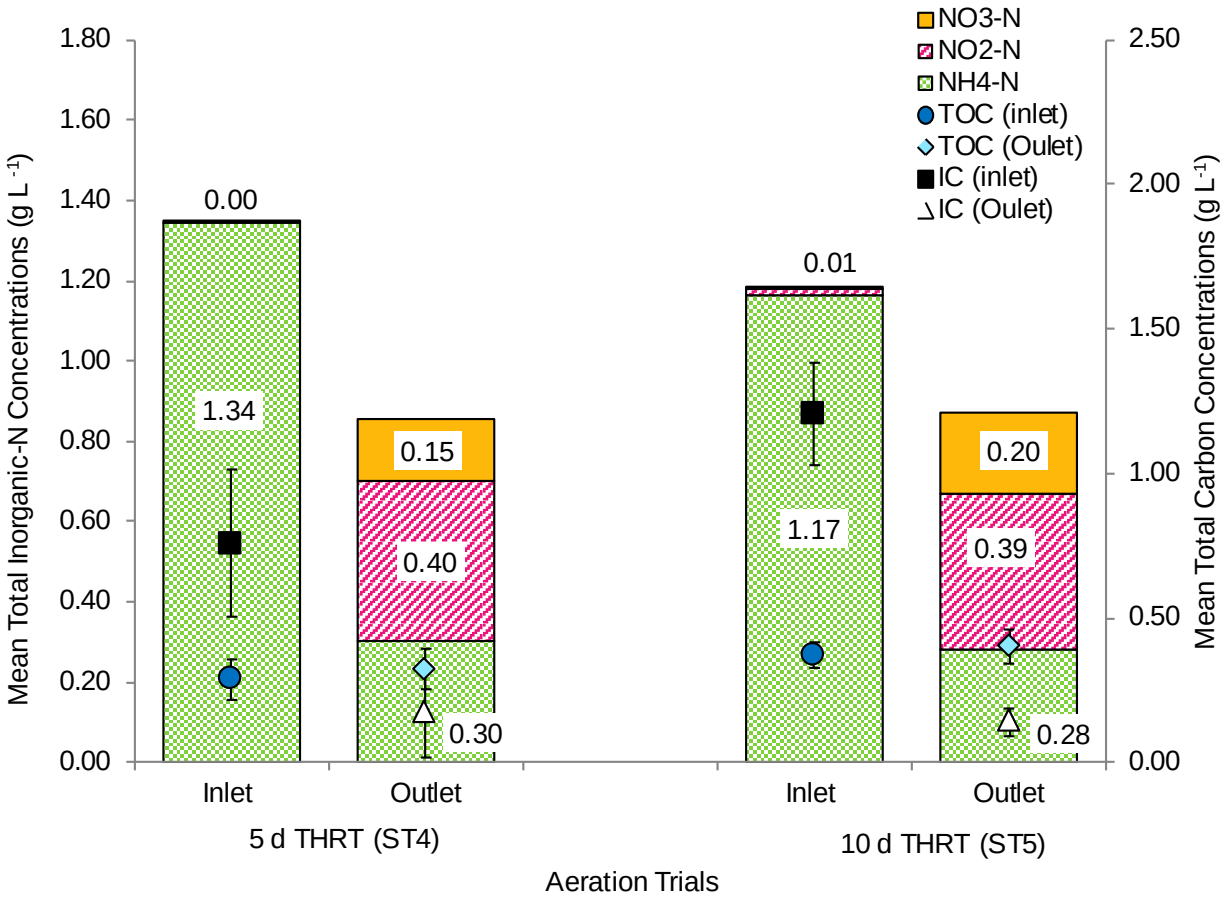

Figure 4.12: A comparison of TIN and TC levels in both the inlet and mixed liquor outlet analysed during trialsST4 and ST4 when aerated with an uncontrolled DO set point (up to 100%) and either a 5 or 10 d THRT operation configuration.

Nitrogen mass balance

Concentrations of inorganic-N were monitored regularly throughout the five aeration trials, to compile a nitrogen transformation profile for the operating conditions assessed in the modelled AWTS. A stoichiometric nitrogen mass balance was produced accordingly, by comparing inlet and outlet TIN fractions per trial (Figures 4.5–4.12). As seen in Figures 4.5-4.12 mean inlet TIN concentrations ranged from 0.96 ± 0.07–1.77 ± 0.52 g L^{-1} pre-treatment and consisted primarily of NH_4^+-N, which accounted for between 98-100% of the TN content. Under aerobic conditions, TIN levels were on average 1.1-1.7 times lower in the treated outlet slurry (0.87 ± 0.87 to 1.33 ± 0.52 g L^{-1}), for all operational parameters (Welch-two sample t-tests; p≤ 0.05 and Wilcoxon rank sum tests; p ≤ 0.05).

Shown in Figures 4.5-4.12, 39% of N lost occurred post treatment. This was most likely through

ammonium volatilisation associated with the high pH (8.2–8.6) and aeration of the mixed liquor during treatment. Of the remaining N, 32-100% was made up of NH_4^+-N, 0.04-45% NO_2^--N and 0-23% NO_3^--N. The presence of NO_2^--N and NO_3^--N particularly during ST5 at 5.1 ± 1.7 mg O_2 L^{-1} (~up to 100% air saturation) and a 10 d THRT suggested nitrification had occurred. This was supported by the significant decrease in IC concentrations observed across the five trials (from 0.76-1.90 g IC L^{-1} in the inlet to 0.14–0.91 g IC L^{-1} in the outlet (Figures 4.13- 4.15). However, low NH_4^+-N to NO_2^--N and NO_2^--N to NO_3^--N conversions were observed across the five trials with nitrification shown to have stopped at NO_2^--N. NO_2^--N levels were detected 3.0 times higher in the outlet than NO_3^--N; an indication that incomplete or partial nitrification had occurred (Figures 4.5-4.12).

A notable colour change was observed in the collected slurry following aerobic treatment from dark taupe (greyish brown) in the inlet to near black in the aerated mixed liquor. Interestingly, as nitrification progressed the treated slurry adopted a more orange-brown hue. This was more prevalent in trials ST4 (towards the end) and ST5.

4.3.4 Carbon

Concentrations of inorganic and organic carbon recorded over the 25–49 d aeration periods identified a total carbon mass balance for each trial run following aerobic treatment under the four operating parameters examined (Figures 4.13-15).

Mean inlet TC concentrations ranged from; 2.65 ± 0.41 (ST1), 1.23 ± 0.08 (ST2), 1.17 ± 0.10 (ST3), 1.04 ± 0.32 (ST4) and 1.58 ± 0.22 g L^{-1} (ST5). The TOC and IC content equated to the sum of TC. The addition of aerobic treatment stimulated reduction yields up to 26-66% TC, 10-35% TOC and 29-88% IC post-treatment across the five trials with the exception of ST5 where a slight increase in TOC was detected (3-8%, respectively). The difference between inlet and outlet C levels were found statistically significant for each of the analysed carbon factor (TC, TOC (ST1, only) and IC) per trial. This provided evidence that carbon oxidation had occurred during the

nitrification process.

Of particular interest was the gradual reduction in IC over the different treatments. Mean IC concentrations were consistently lower in the treated outlet than in the inlet for all operating parameters tested (Figures 4.13-15). ST5 was notable in this regard with a mean outlet IC concentrations 1.06 g L^{-1} lower post treatment; from 1.20 ± 0.18 to 0.14 ± 0.05 g L^{-1}. Over the five trials, IC concentrations were observed to have reduced in connection with an increase in both NO_2^--N and NO_3^--N concentrations and a decrease in NH_4^+-N. This is likely due to the consumption of inorganic carbon (CO_2 and $CaCO_3$) as a carbon source for nitrifiers during nitrification.

Aeration at 10% DO with a 5 d THRT reduced the inlet C content by 14- 35% TOC and 29–51% IC with carbon oxidation noted higher during ST1 than ST2 (Figure 4.13). TOC and IC concentrations were reported 1.5-2.5 times higher in both the inlet ($W = 35$, $p \leq 0.001$) and outlet mixed liquor ($W = 123$, $p \leq 0.001$ and $t\ (21) = 3.56$, $p \leq 0.001$), respectively for ST1 than ST2, attributing to the higher nitrification rates observed in ST1.

An increase in DO saturation levels from 10% to 20% and up to 100% saturation was shown to have had a significant effect on the mean outlet IC concentrations across the 5 d THRT trials with IC levels detected up to 1.2-6.6 times lower in the more oxygenated slurry, particularly those of ST4 (0.27 ± 0.16 g L^{-1}) (Figure 4.14). The difference between the three outlets significant at the 95% confidence interval ($F\ (2, 42) = 36.24$, $p \leq 0.001$) providing evidence that aeration level indirectly effects carbon oxidation during nitrification. No statistical significance was observed in the mean inlet IC concentrations across the three saturation levels concentrations ranged from 0.76 ± 0.25 g L^{-1} to 0.93 ± 0.17, measured highest in ST2 (1.04-1.22 times) (Kruskal Wallis Chi square (2) = 1.04, $p = 0.59 \geq 0.05$) (Figure 4. 14),

An increase in THRT from 5 d (ST4) to 10 d (ST5) during continuous aeration (no DO control) was shown to have also had a similar effect on IC consumption, with concentrations found up to1.06 g L^{-1} lower post aeration ($t\ (15) = 20.71$, $p \leq 0.001$). Figure 4.15 presents the mean TC data obtained before and after aeration with no set DO control point and either with a 5 d or 10 d THRT. Mean IC

concentrations were 1.6 times higher in the inlet but 1.9 times lower in the aerated mixed liquor for the longer THRT (ST5; 10 d THRT) than those at 5 d for ST4 (t (11) = -3.17, $p \leq 0.001$ and t (18) = 3.07, $p \leq 0.001$). Similarly, the TOC content of ST5 was 1.3-1.6 times higher in ST5 than ST4 (inlet; t (11) = -2.10, $p = 0.06 \geq 0.05$, outlet; $W = 14$, $p \leq 0.001$), This combined with the nitrification data obtained in Section 4.3.3 suggests low CO_2 availability during ST2, possibly due to competition from an additional oxygen demand within the mixed liquor.

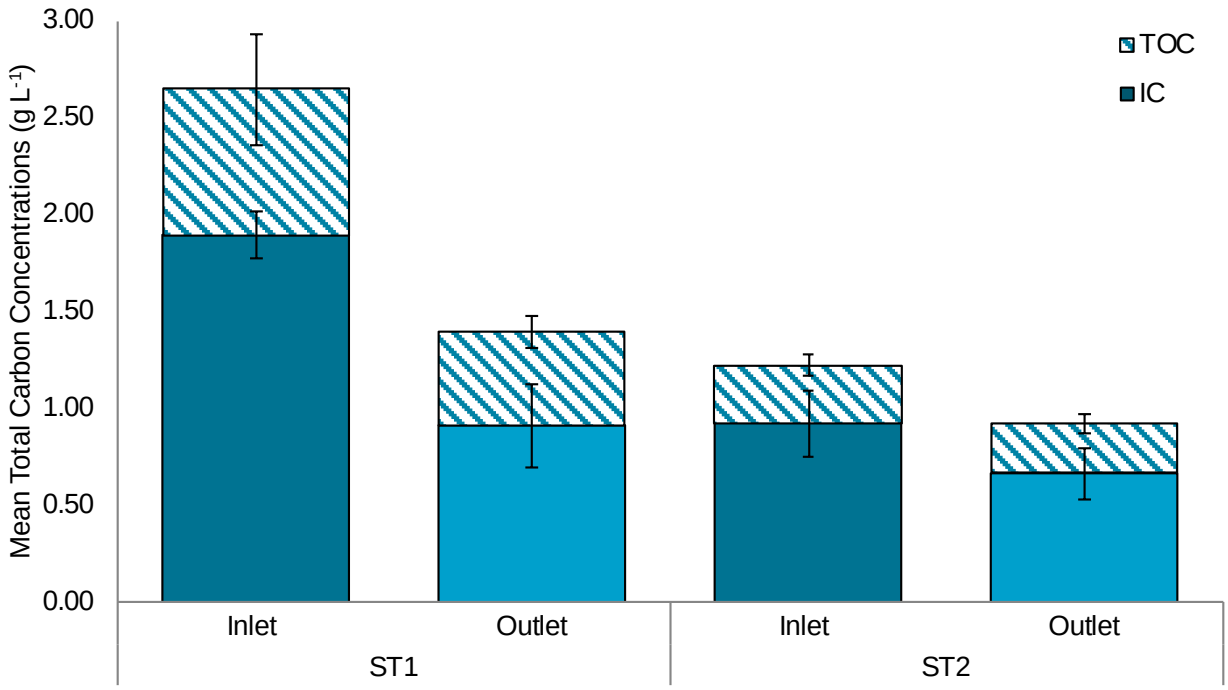

Figure 4.13: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS within the ANPS before (inlet) and after (outlet) aerobic treatment with a 0.8 mg O_2 L^{-1} (10% saturation) set point and 5 d THRT for trials ST1 and ST2.

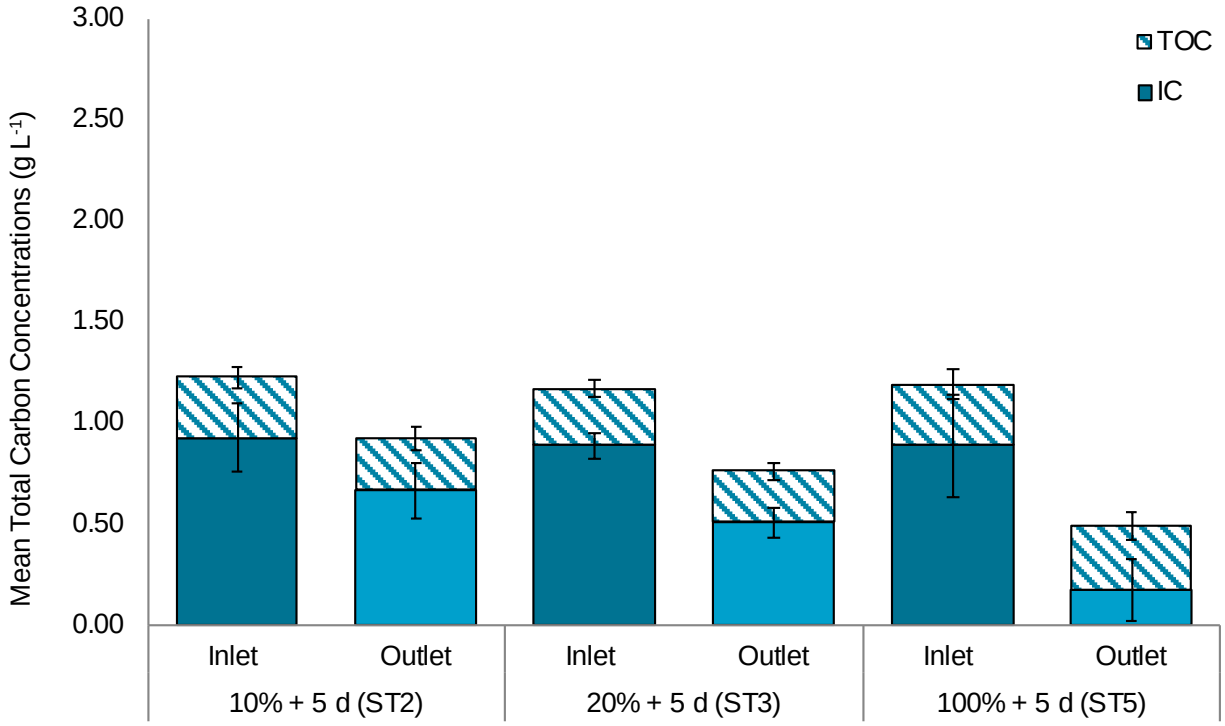

Figure 4.14: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before (inlet) and after (outlet) aerobic treatment with a 5 d THRT and DO set points of 10%, 20% or no DO control (100% DO) (ST2-4).

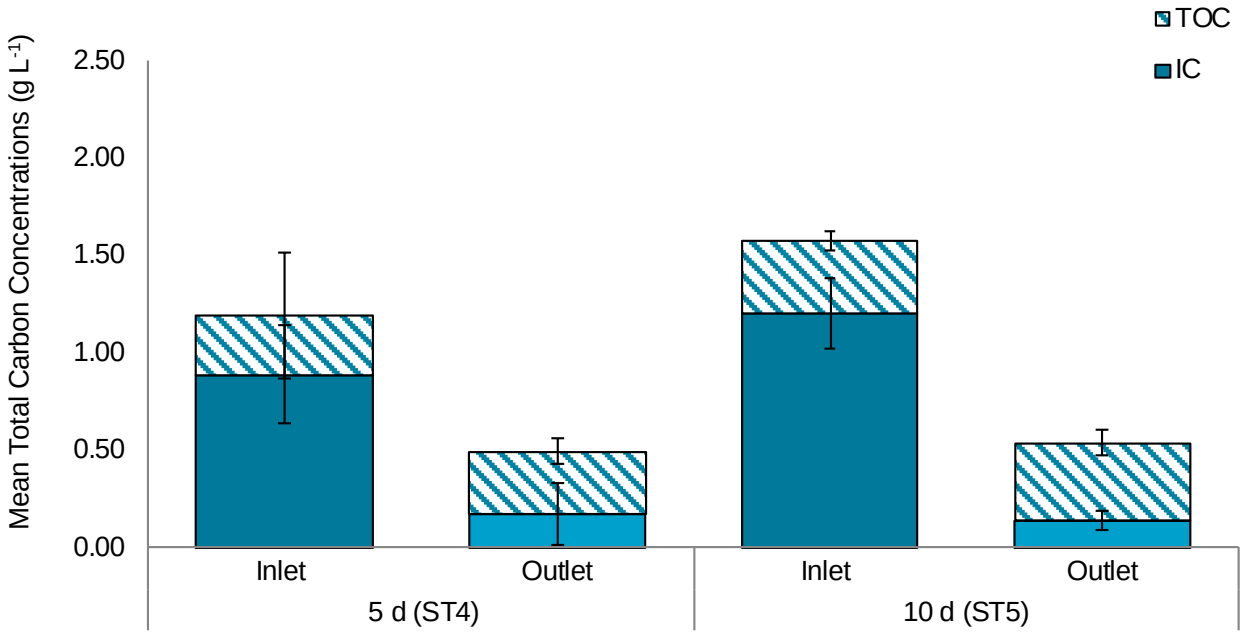

Figure 4.15: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before (inlet) and after (outlet) aerobic treatment at no DO set point (100%) with either a 5 d or 10 d THRT and for trials ST4 and ST5.

4.4 Discussion

The use of inadequately treated wastewater for shed flushing, wash down and irrigation exposes both pigs and humans to elevated ammonia and potential pathogen levels (Buchanan *et al.*, 2013, Murphy, 2011, Petersen *et al.*, 2007). Algal growth on the anaerobically digested effluent is a potential strategy for improved wastewater quality, reduced greenhouse gas emissions, and alternative feed and energy sources (Aguirre *et al.*, 2011, Buchanan *et al.*, 2013, Kebede-Westhead *et al.*, 2006). The high concentration of ammonia and SS present in the anaerobically digested effluent is of particular concern for algal growth. The combination of low light availability and ammonia toxicity are a major limitation, which must be overcome to achieve the promised high yields of algae growth on pig slurry (Buchanan *et al.*, 2013). On a laboratory scale, this project investigated a unique integration of wastewater treatment technologies, with the potential to overcome these limitations, predominately, through an intermediate aeration step between the existing anaerobic digestion and intended HRAPs. As a novel concept, optimisation is key to achieving high nitrification rates.

To optimise the system and enable on farm use, two operating parameters; DO saturation and THRT were assessed across a set of five trials performed in the modelled AWTS (described in Chapter 2); based on the requirements needed for nitrification (Evans *et al.*, 1986). Alterations to air saturation and THRT can affect the speciation of inorganic-N present during nitrification (Béline and Martinez, 2002, Béline *et al.*, 1999, Buchanan *et al.*, 2013, Evans *et al.*, 1979, Evans *et al.*, 1983, Evans *et al.*, 1986). For instance at DO concentrations below 1%, only organic-N and $NH_3$are present according to Evans *et al.* (1986). As concentrations exceed 10%, NH_4^+ is oxidised to NO_2^- and NO_3^-it's less toxic state (Béline and Martinez, 2002, Béline *et al.*, 1999, Buchanan *et al.*, 2013, Evans *et al.*, 1979, Evans *et al.*, 1983, Evans *et al.*, 1986). Control of DO concentrations and THRT are considered a practical and economical way to best operate an aeration system according to a study by Guo *et al.* (2009), with low DO concentrations and equivalent treatment time recommended. Thus for aeration to be considered feasible, the minimum requirements for NO_3^- production to occur need to be identified, including a treatment time (2.5-14 days) and a DO

concentration above>10% (Evans *et al.*, 1979, Evans *et al.*, 1983, Evans *et al.*, 1986, Svoboda *et al.*, 2013). Characterisation in the past was predominately performed using whole or diluted raw slurry, with DO consumed not only for N oxidation but also, also for a large amount of carbon oxidation (Buchanan *et al.*, 2013, Evans *et al.*, 1979, Evans *et al.*, 1983, Evans *et al.*, 1986, Gerardi, 2002, Svoboda, 1995, Svoboda and Fallowfield, 1989). However, there is little to no documentation available on the aeration and operation characterisation of ANPS in which a large amount of organic-C would have been removed during anaerobic digestions and thus highlights the need for an optimized operating regime and the main focus of this adaptive investigation (Buchanan *et al.*, 2013, Svoboda *et al.*, 2013).

In lieu of this, control parameters were selected from within the range identified for raw slurry; DO at 10%, 20% and up to 100% saturation (uncontrolled) and a THRT equivalent to 5 and 10 d.

4.4.1 Operation performance

Factors such as DO, pH and temperature affect the rate of nitrification during aerobic treatment (Prinčič *et al.*, 1998). The experiments conducted here support this, with a variation in, *in situ* characteristics observed throughout the experimentation period (Table 4.3). Aeration of the AWTS was controlled at three air saturation levels; 10%, 20% and up to 100% (uncontrolled). Laboratory based monitoring found the actual DO concentrations (mean) were lower on average than those of the set control values. Regular dosages of the anaerobic inlet were identified as the most likely cause. As such, a fluctuation in DO occurred during aerobic treatment despite operation at these set saturation values. Fluctuations in DO were more noticeable after an influx of ANPS during the feeding periods (every 4h), where a corresponding rapid decline in DO was observed. This was as expected. The feeding regime and high oxygen demand of the ANPS feedsource was the cause of this observation (Bernet *et al.*, 2000). These results were similar to those reported by Bernet *et al.* (2000) where a decline in DO concentrations (to 0.6–0.8 mg O_2 L^{-1}) was detected post anaerobic effluent addition. Equally, a concentration spike was observed as the oxygen demand of the injected ANPS was satisfied.

Unfortunately, faults with both the DO sensor and transmitter rendered the control of DO in the

reactor problematic. As such, system operation was forced to take place in an uncontrolled DO environment during ST4 and ST5, until the issue could be resolved and a replacement found. Nevertheless, the results from this would provide a realistic idea of the environmental conditions expected in the field should DO control not be available.

Research has demonstrated a correlation between a decrease in pH and reduced nitrification (Shammas, 1986, Svoboda *et al.*, 2013). The optimal pH required for nitrification is widely disputed within the literature. However, pH within the range of 7.2 and 9.0 is generally recommended (Alleman, 1985, Gerardi, 2002, Kutty *et al.*, 2011a, Prakasam and Loehr, 1972, Shammas, 1986, Svoboda *et al.*, 2013). During the aeration trials pH ranged between 8.2 and 8.6, this fell within the optimal range.

Interestingly, a notable colour change was observed between the inlet ANPS and outlet mixed liquor (APS) post treatment. Colour ranged from dark taupe (greyish brown) in the ANPS to near black when aerated. Willers *et al.* (1998) noted a similar colour change in digested raw pig slurry following liquid separation and aerobic treatment. The rich colouration was thought to be the reason for the erratic NO_3^--N results produced according to Willers *et al.* (1998). Similar observations were found during this study. However, upon investigation, no abnormalities were detected in slurry absorbance across the wavelengths required to carry out nitrification analyses; NH_4^+-N; M = 590 nm, R = 720 nm; NO_2^--N and NO_3^--N; M = 540 nm, R = 720 nm. Interference was tested for 190–1000nm. Once a relatively active nitrifying population was present, the APS took on a more orange-brown hue. This distinct colour change (more significant post filtration) became indicative of an active nitrifying population and subsequent nitrification processes had taken place. This was more noticeable in trials 4 and 5 at high saturation (62-100% DO).

4.4.2 Effect of aeration level and residence time on suspended solid removal

Inlet SS content ranged between 0.81 and 1.15 g L^{-1}. The anaerobic inlet wastewater appeared murky as a result. This would not only restrict light penetration required for algal growth and treatment, but could cause blockages in equipment and machinery (Tchobanoglous *et al.*, 2003). High suspended solids are also associated with high nutrient and pathogen levels (Zhang *et al.*,

2011). Strategies to rectify this are highly sought after.

Svoboda and Fallowfield (1989) identified the inclusion of an effluent pre-treatment step prior to algal growth in a HRAP. This pre-treatment step has to have the potential to both reduce the solid content and subsequently improve light penetration to enable algal growth(Buchanan *et al.*, 2013, Svoboda, 1989). Approximately 60-90% of biodegradable organic matter are removed during anaerobic digestion and sedimentation (APL, 2004, 2015b). However, for these processes to occur, a long THRT is required (~20d) (Buchanan *et al.*, 2013, Cheng and Liu, 2002). Large investment costs can incur as a result; a disadvantage for continuous operation and rapid reuse (Buchanan *et al.*, 2013, Cheng and Liu, 2002). The addition of aerobic treatment post anaerobic digestion was predicted to stimulate a further reduction in solids (Svoboda *et al.*, 2013). A correlation between solid removal and aeration conditions (i.e. air saturation and aeration residence time) has been documented within the literature (Buchanan *et al.*, 2013, Evans *et al.*, 1979, Evans *et al.*, 1983, Evans *et al.*, 1986). As such, the adoption of aerobic treatment into the integrated treatment regime was used during this investigation.

Passing the ANPS through an aerobic reactor yielded a reduction in SS by a further (>) 50%, additional to the 60-90% reduction estimated during anaerobic digestion (APL, 2004, 2015b). This was apparent in each of the five trials run during this investigation. Removal increased in correlation to an increase in DO as indicated by the comparison of trials operated with a 5 d THRT; ST2 (at a 10% aeration level), ST3 (20%) and ST4 (100% uncontrolled) (Figure 4.10). Removal detected greatest at the higher DO concentrations (5.1 ± 1.7 mg O_2 L^{-1}).

A significant improvement in SS removal occurred when THRT was increased from 5 d to 10 d ($p \leq 0.01$); with 71%, SS removed during aeration at 5.1 ± 1.7 mg O_2 L^{-1} (ST5). This yielded the greatest removal of the five trials run. Levels measured 1.3 times lower ($p \leq 0.01$) than those of the shorter THRT (5 d; ST4) when aerated continuously (no DO set point). These results were consistent with those of Evans *et al.* (1983), who detected a gradual decline in TSS when THRT was increased from 1 to 8 days.

Whilst, changes in parameters applied (DO and THRT) influenced SS removal; It should also be noted that as two farms are identical, and therefore subject to slurry variations both within and across farms (lagoons), owing to differences in diet, handling (i.e. shed flushing), and treatment practices adopted (Burton, 1992, Burton and Turner, 2003). This can alter the nutrient load within pig waste and the subsequent removal rate making it difficult to maintain a consistent slurry composition (APL, 2015b) and could explain the diversity in inlet ANPS characteristics noted within and across the trials run during the investigation and could subsequently account for the low nitrification observed (below). Particularly, in that although stock slurries were collected from the same lagoon, slurry compositions varied (periodically). This disparity could be attributed to the varied lagoon collection times (subject to requirement) and that the lagoon from which the slurry was collected from was attached to a working farm, which constantly adapted its practices according to its needs. Both of these factors can have an effect on slurry composition (APL, 2015b). This was more noticeable between trials ST1 - ST2 and ST4 -ST5 when similar operating conditions were maintained (10% saturation set point with a 5 d THRT and an uncontrolled DO set point with either a 5 d or 10 d THRT, respectively). As a result, the findings from this adaptive design study are to be taken with care.

4.4.3 Effect of aeration level and residence time on ammonia oxidation

Along with the high SS loads, elevated concentrations of NH_3 in reuse water have been shown to have a negative effect on algal growth, and pig and worker health (Chynoweth *et al.*, 1999, Dosman *et al.*, 2000, Mobin and Alam, 2014). The literature reports the maximum exposure level to aerial NH_3 deemed safe is 10 mg L^{-1}; 7 mg L^{-1} for humans and 11 mg L^{-1} for pig health within pig sheds (Banhazi and Cargill, 1996, Cargill *et al.*, 2002, Cargill and Skirrow, 1997, Murphy, 2011). Whilst, NH_3 concentrations in excess of 42 mg L^{-1} were reported as toxic to numerous algal species (Chaiklahan *et al.*, 2010, Matsudo *et al.*, 2009). Inlet NH_4^+-N ranged from 0.94 to 1.73 g L^{-1} (Figures 4.5-4.12) in this study, which is greater than the threshold tolerated (Banhazi and Cargill, 1996, Cargill *et al.*, 2002, Cargill and Skirrow, 1997, Murphy, 2011). To reduce potential risks from exposure to these elevated levels, a decrease is required.

ANPS has been reported to have a higher NH_4^+ content than that of raw effluent (Möller and Müller, 2012, Svoboda, 1995). This is largely due to the degradation of organic-N to NH_4^+ during anaerobic digestion (Robertson and Groffman, 2007, Svoboda, 1995).

Up to 0.73 g L^{-1} of inorganic-N (41%) was lost during aerobic treatment, most likely due to the high pH (8.2-8.5) recorded during treatment. NH_3 volatilisation considered and presumed the most likely cause for N loss in the system (Evans *et al.*, 1986). These findings were consistent with those of Evans *et al.* (1986) who identified N losses of 40%, when raw slurry was aerated above 15^oC, and DO saturation >15% (pH 7).

During anaerobic digestion, majority of TIN was made up of NH_4^+-N. The AWTS oxidised 24% - 76% of NH_4^+-N post aeration, as seen in Figures 4.5-4.12. Findings were slightly lower to those observed for aerated raw slurry by Béline *et al.* (1999) where 69-95% of total NH_4^+-N (g N kg^{-1}) was oxidised under aeration conditions of DO above 1-2% saturation and THRT >3 days. However, in the study by Béline *et al.* (1999) there was evidence that denitrification had occurred, which could account for the higher nitrogen losses detected. A study by Islam *et al.* (2011) achieved an NH_4^+ reduction efficiency of 31% following treatment in an SBR composed of an oxic-anoxic process; aeration conditions; DO 5.1 – 6.9 mg O_2 L^{-1} and a 5 d THRT which were similar to those of ST4. Islam *et al.* (2011) suggested that the conversion of organic-N to NH_4^+-N is a continuous occurrence during the aerobic treatment phase and offers a potential explanation for the low reduction rate. This same logic could offer an explanation as to the fluctuations of NH_4^+-N and TN concentrations during aeration and feed periods (every 4 h) observed in this study, particularly with the low oxidation efficiency of ST1 and ST2. Regardless the results of this investigation are indicative that the AWTS was a successful treatment strategy for NH_4^+oxidation.

4.4.3.1 Comparison of ammonia oxidation (or nitrogen mass balance) for the different air saturation levels

Operational parameters and their effect on NH_3 oxidation was first assessed at low DO concentrations set at ~ 0.8 mg O_2 L^{-1} (10% saturation) with a 5 d THRT; the minimum requirement needed to convert NH_4^+ to NO_3 according to Evans *et al.* (1986) and Svoboda (1995).

A reduction in NH_4^+-N concentrations of 29% - 20% (68% outliers included) (p 0.05) (Figures 4.5, and4.6) was observed when the system was run at 10% of the saturated value (DO) at a set point value of 0.8 mg O_2 L^{-1} and 5 d THRT. Of the oxidised NH_4^+-N, 16 - 20% was, converted to NO_2^--N but only <BLOD- 3% to NO_3^--N (Figure 4.5 and 4.6). This is indicative that some nitrification (albeit partial) had taken place. Interestingly, that whilst both ST1 and ST2 were operated using the same set aeration parameters (0.8 mg O_2 L^{-1} and 5 d THRT set point) nitrification was shown to have occurred in the exploratory trial ST1 only owing to the presence of NO_2^--N and NO_3^--N in the outlet mixed liquor. Inspection of the two inlet compositions before and after treatment identified the nitrogen content of ST2 to be half that of ST1 (Figure 4.7). The variable nature of the ANPS used deemed the most probable cause (as described in Section 4.4.2 above). Whilst, this variation was not ideal, it provided a realistic representation of what can occur in practice and is something that will need to be taken into consideration when implementing the system out in the field. It is possible that ammonification of organic-N had also occurred. Both of which could explain the greater TN content detect in ST1 but not ST2 and why the oxidation of NO_2^--N to NO_3^--N was low in both cases (Gerardi, 2002, Strauss and Lamberti, 2000, van Haandel and van der Lubbe, 2012).

An increase in DO to 1.3 mg O_2 L^{-1} (20% saturation 1.5 mg O_2 L^{-1} set point; ST3), exhibited mean NH_4^+-N concentrations up to 1.6 times lower than those obtained at 0.74 ± 0.21 mg O_2 L^{-1} (10%saturation; ST2), a 30% improvement in NH_4^+-N removal (50% removed) achieved (Figure 4.10). The observed reduction in NH_4^+-N suggests that an increase in DO concentration would lead to greater oxidisation. However, this was not the case, the conversion to NO_3^--N was again minimal (3%), suggesting incomplete nitrification had occurred. Béline *et al.* (1999) found at DO concentrations of up to 4 mg O_2 L^{-1} and a residence time of up to 7 days also resulted in incomplete nitrification.

Factors such as environmental stress; DO availability, pH and temperature, residence time, alkalinity, toxicity, inlet variation, and the high NH_4^+-N concentrations were considered responsible for the low NH_4^+ and NO_2^- oxidation according to Béline *et al.* (1999) and Gerardi (2002). Significant changes to any or all of these factors have been proven to have inhibitory effects on the nitrification

process. For example, Gerardi (2002) suggests that if the system is poorly buffered (alkalinity), the pH of the mixed liquor will drop and become more acidic and with it, a significant increase in NH_4^+ levels could occur.

Competition for available oxygen, NH_4^+-N and organic-C by heterotrophic bacteria particularly when the C:N (TOC:TN) is high was also found to reduce nitrification according to Strauss and Lamberti (2000). This could explain why both the *in situ* DO concentrations and active nitrifying bacteria populations within the mixed liquor were low for the trials operated at 10% and 20% air saturation. One strategy was to increase the amount of oxygen entering the system to maximum (100%) to ensure available oxygen is suffice to carry out the required processes and that the system could in fact perform theses oxidations.

Trial ST4 was operated continuously with an uncontrolled DO set point (up to 100% saturation), correspondingly. An increase in DO to 6.3 ± 1.1 mg O_2 L^{-1} (ST4) improved NH_4^+-N oxidation significantly with NH_4^+-N concentrations detected 1.3-1.6 times lower post-aeration than those at 0.7 ± 0.2 mg O_2 L^{-1} (10%; ST2) and 1.3 ± 0.2 mg O_2 L^{-1} (20%; ST3) DO. Outlet NH_4^+-N concentrations was found to have decreased by 53%, under this configuration during ST4. Of notable interest was the slight increases in NO_2^--N content recorded during ST4 compared to those at the lower DO saturations of ST2 and ST3. An indication that NH_4^+-N increases with increasing DO saturation and THRT (Figure 4.10). Unfortunately, no real improvement in NO_3^--N occurred, confounded by an insufficient nitrifying population. Extension to the THRT was one solution recommended to improve population density (Buchanan *et al.*, 2013, Svoboda, 1995)

4.4.3.2 Comparison of ammonia oxidation (or nitrogen mass balance) at different slurry THRT

Nitrifying bacteria are slow growing organisms with a generation time of 7 to 13 hours (Belser, 1979, Gerardi, 2002, Svoboda *et al.*, 2013). Therefore; THRT plays an important role during nitrification. For instance, if the THRT was too short, growth would be insufficient to match the dilution rate and thus running the risk of population washout, alternatively if the THRT were too long, operation would become too costly (inefficient) (Belser, 1979, Burton, 1992, Svoboda *et al.*,

2013). A residence time of 2-14 d was identified as most practical according to Evans *et al.* (1986) and Svoboda *et al.* (2013). Two THRT were, examined during this experiment set, 5 and 10 d.

A significant improvement in both NH_4^+-N oxidation and NO_2^--N and NO_3^--N accumulation occurred when THRT was increased to 10 d. At a THRT of 5 d, NH_4^+-N removal averaged 53%; at 6.3 ± 1.1 mg O_2 L^{-1}. Extending the THRT to 10 d,76%of the NH_4^+-N content was removed when an uncontrolled DO set point of up to 100% saturation was used (averaged 5.1 ± 1.7 mg O_2 L^{-1}). It should be noted however, that due to time restraints, only one trial was carried out under this operation configuration. Nevertheless, this was the greatest removal rate observed not only across the two THRTs (set at 100% air saturation (uncontrolled)) but across all five of the trials run using the AWTS, an improvement of 23-56%. Yet, NO_3^--N accumulation remained low.

An insufficient nitrifying population, a plausible explanation for the low NO_3^- accumulation seen across the five trials (Buchanan *et al.*, 2013).Increasing feed times to every 4h, demonstrated a slight improvement in the nitrification results of this study, as did a raise in DO saturation and THRT. Still low NO_2^- oxidation persisted. This poses a potential problem for the growth of microalgae on the treated waste, which is one of the end goals for this research. One strategy to overcome this is to include a returned activated slurry (RAS) feedback step into the matrix post aeration (Buchanan *et al.*, 2013). This will be explained in detail in Chapter 5.

Regardless, the findings of this study show that when a higher amount of DO (up to 100% saturation) was available, greater NH_4^+-N and NO_2^-N oxidations was achieved regardless of THRT. By increasing the THRT to 10 d, a slight improvement in nitrification activity resulted compared to when a 5 d THRT was used. It is likely, that this increase in THRT provided a time sufficient for a nitrifying population to establish (Evans *et al.*, 1986, Svoboda *et al.*, 2013). However, as indicated in Section 4.4.3 anaerobic pig slurry is a very variable resource, the effect of which could have had an influence on the nitrification results exhibited in the two trials operated without DO control (ST4 and 6) and could also offer an explanation as to why nitrification was detected higher in ST5 (Burton, 1992, Burton and Turner, 2003). For instance it is possible that like that of ST1 there may have been another organic-N source present within the slurry of ST5 (but not in ST4) which could

explain the higher TN levels at the longer THRT despite there being not much difference in TIN and NH_4^+-N. This suggests that organic-N from the atmosphere was volatilised, which may have contributed to the higher nitrification rates observed.

Whilst aeration at 100% saturation (uncontrolled) provided a slight improvement in nitrification, this would be offset by the higher additional running cost of the system to operate under these conditions. Cost of operation, whilst outside the scope of this investigation, would need to be considered for implementation in the field on a full-scale system. It must be noted, that the purpose of running the aeration system continuously at such a high DO concentration in this investigation was simply to pump in as much air into the system as possible to facilitate nitrification and establish whether nitrification could in fact take place in the system and not a realistic option when applied into the field.

4.4.4 Carbon

The removal of nitrogen during aerobic treatment is a two-step process; NH_4^+ and NO_2^- oxidation via two distinct autotrophic oxidising bacterial groups that derives carbon from inorganic-C compounds such as CO_2 (Buchanan *et al.*, 2013, Guisasola *et al.*, 2007). As NH_4^+ is oxidised to NO_2^- and NO_3^- during nitrification, IC as CO_2 is consumed as a carbon source by nitrifying bacteria (Gerardi, 2002, 2011, Sherrard, 1976). A link has been identified in the literature between carbon deficiency, alkalinity, and a reduced nitrification rate (Gerardi, 2002, Guisasola *et al.*, 2007, Sherrard, 1976, Wett and Rauch, 2003). Therefore, to ensure that ample growth and reproduction occurs adequate IC sources are required (Gerardi, 2002, Wett and Rauch, 2003). Carbon deficiencies or limitations have been shown to affect nitrification; either by reducing or reversing this process, such that inadequate carbon levels can result in incomplete nitrification (Buchanan *et al.*, 2013, Gerardi, 2002, 2011, Guisasola *et al.*, 2007).

In theory, as IC is consumed, an increase in NO_2^--N and NO_3^--N is expected to occur (Buchanan *et al.*, 2013, Burton, 1992, Gerardi, 2002, Guisasola *et al.*, 2007). Evidence of this was observed during this investigation during ST5 at 5.3 ± 1.7 mg O_2 L^{-1} (>100% saturation) and a 10 d THRT. Up to 1.06 g L^{-1} of IC was reduced following an increase in NO_2^--N (from 0.01 ± 0.01 g L^{-1} to 0.39 ±

0.04 g L^{-1}) and NO_3^--N (from <BLOD to 0.20 ± 0.11 g L^{-1}) concentrations (Figure 4.12). However, low nitrification rates were observed during trials ST1-ST4, despite a decrease in IC post-aeration of up to 28-81% (Figure 4.8) This suggests that there was inadequate CO_2 levels available for nitrification, and offers a plausible explanation as to why NO_2^--N accumulation and little to no NO_3^--N was detected throughout the trials run,

To compensate for this it has been recommended that an addition of chemicals containing, bicarbonate, hydroxide or carbonate could help to raise the IC content (Gerardi, 2002). However, due to the intended farm application upon up-scaling, addition of chemicals was not favourable for this system, the investigation focused on keeping the treatment as natural as possible to prevent further exposure risks.

4.4.5 General observations and future research

ANPS characteristics are not always considered suitable for algal biomass production or shed wash down, aeration is a potential approach to improve the quality of wastewater for reuse (Buchanan *et al.*, 2013). Filling this knowledge gap involves characterising the most suited DO saturation level and THRT for nitrification to occur as well as to provide water fit for reuse and algal growth. In this study, a significant reduction in TSS and NH_4^+-N content of ANPS was achieved when DO concentrations exceeded 10% - 100% saturation at a THRT of 5 d or 10 d.

While NH_4^+ removal was a success, the conversion to NO_3^--N was not. Nitrification struggled to reach completion within the system. This is not uncommon. Studies identified a correlation between fluctuation in slurry characteristics, environmental conditions, population maintenance, and a lack in nitrification performance (Gerardi, 2002, Willers *et al.*, 1998). The most predominant problem encountered was the struggle to ascertain and maintain an active nitrifying population. Since nitrification is dependent upon a thriving population, techniques to ensure this, requires investigation in the AWTS. Recycled activated slurry (RAS) feedback is a technique proven relatively successful for raw slurry (Burton, 1992). There is a gap in the literature as to whether inclusion of a clarifier and RAS feedback step, to the AWTS examined, results in improved nitrification performance. Examination of this inclusion is carried out in Chapter 5.

4.5 Conclusion

Aeration of ANPS in an AWTS has the potential to alter the way piggery waste is both treated and viewed as a sustainable resource, in the future. Implementation would offer greater benefits to both industry and producer (Buchanan *et al.*, 2013). Including, a decrease in pollutants, nutrient manipulation, odour elimination, energy production, quality reuse water, heat production and a subsequent improvement in pig and worker health (Burton, 1992, Choi, 2007, Svoboda *et al.*, 2013). However, properties within the ANPS are not suited for reuse or algal biomass production (Buchanan *et al.*, 2013).

This, study clearly identifies aeration conditions as important factors that affect nitrogen manipulation, and water quality, providing a number of combinations to optimise an AWTS on a laboratory scale.

It is clear that aeration parameters need to be managed at conditions that are both economical and output beneficial. A good operating regime includes the ability to convert NH_4^+ to NO_3^-, improve light penetration, and enhance reuse-water output for algal growth and pig health. This study was unique, in that it was able to demonstrate a successful aeration of ANPS in an AWTS on a laboratory scale. In particular, the study was able to identify, DO concentration greater than 10% of the saturated value and a residence time of 5 d or more, enables the reduction of both SS and NH_4^+ levels.

While, removal was successful at each parameter combination, the conversion of NO_2^--N to NO_3^--N was less so. From the experiments carried out, it can be concluded that although a slight improvement at relative complete saturation (high DO, ~100%) occurred, nitrification was still considered poor regardless of operating parameters.

Nevertheless, a DO concentration of greater than 5 mg O_2 L^{-1} and a saturation level of 68-100% saturation when combined with a THRT of 10 d is recommended for both NH_4^+-N and SS removal based on the findings derived during this work, noting that there was limitations due to slurry variations within the ANPS. Conversely If the main treatment objective of interest is solely SS removal, then aeration at any DO concentration over 0.8 mg O_2 L^{-1} (10% - 100% saturation) and a

THRT 5 d is suffice to yield a reduction of over 50-77%.

However, cost of operation for the recommended parameters, whilst, this might be of no significance on the laboratory scale, it may be considered not viable in the field. Thus, further assessment into strategies to reduce operation set points and maintain an active nitrifying population is recommended.

All the same, the outcomes of these experiments could be used to assist in the upscale from bench-top to pilot scale. More research is required to fully understand the limitations of nitrification in this particular treatment system and examine techniques that would optimise operating performance.

5. RECYCLING AERATED ANPS BACK THROUGH AN AEROBIC REACTOR TO DRIVE AMMONIA OXIDATION AND SUSPENDED SOLID REMOVAL; IMPROVING PIGGERY SLURRY REUSE QUALITY

5.1 Introduction

In Chapter 4, the inclusion of an AWTS to the existing anaerobic digestion regime currently in place throughout Australian piggeries was found successful at laboratory scale for the reduction of both SS and NH_4^+-N oxidation. Optimisation of the system conditions was the overall aim. Reduction yields of greater than 52% for TSS and 20-76% for NH_4^+-N were obtained across the five trials run. Conditions were found slightly more favourable when DO concentrations measured above 5.1 ± 1.7 mg O_2 L^{-1} (approximately 68-100% saturation) and a THRT equivalent to 10 d.

However, nitrification was relatively poor for the conversion of NO_2^--NO_3^-. In each case, partial or incomplete nitrification resulted (Figure 4.14). A deficient nitrifier population, variation in ANPS characteristics or insufficient DO were identified as probable causes. This denotes the importance of an adequate aeration regime, particularly when the objective of the end product is reuse (Buchanan *et al.*, 2013, Matsudo *et al.*, 2009, Murphy, 2011, Svoboda *et al.*, 2013, Svoboda, 1995, Svoboda and Fallowfield, 1989). Techniques to stimulate nitrifier population, and enhance NO_2^- oxidation whilst lowering operational cost are of interest. Particularly, as operation at this high level of DO may not be considered an economically viable option by producers associated with the likely high-energy consumption required to operate this system over long periods (Buchanan *et al.*, 2013, Burton, 1992, Burton and Farrent, 1998, Evans *et al.*, 1986, Zhang and Zhu, 2005).

One strategy is to operate a return activated sludge (RAS) feedback step into the process post-aeration (Burton and Turner, 2003, Gerardi, 2002). This is a novel approach in the treatment of aerated ANPS; numerous successes have been documented for the treatment of domestic and industrial wastewaters, sewage, and animal slurries (Burton, 1992, Downing and Nere, 1964, Muller *et al.*, 1995, Seviour and Nielsen, 2010, Surmacz-Gorska *et al.*, 1996, Wittmann *et al.*, 1990).

RAS addition could be predicted to enhance nitrification, through active biomass feedback, by way of re-introducing RAS into the aeration vessel and preventing biomass washout from occurring as an active biomass is regularly circulated throughout the system (Buchanan *et al.*, 2013, Downing and Nere, 1964, Seviour and Nielsen, 2010). This would enable the active biomass (enriched with high density microbial population, (which according to the previous study's findings is considered low at present), to mix with the incoming inlet and mixed liquor supply (Downing and Nere, 1964, Gerardi, 2002, Wittmann *et al.*, 1990). Here it will remain in constant suspension (through aeration) gaining access to the available nutrients within the mixed liquor, nutrients that in the presence of suffice DO, enables microbial cell growth and reproduction (Gerardi, 2002, Seviour and Nielsen, 2010, Wittmann *et al.*, 1990). Thereby, reducing treatment time by inciting the existing population and speeding up metabolism and growth processes as the population is already in an active state (Blok, 1976, Gerardi, 2002, Seviour and Nielsen, 2010).

The RAS process is a two-phase treatment regime; aerobic treatment and SS removal via clarification and sedimentation (Burton, 1992, Kutty *et al.*, 2011b, Seviour and Nielsen, 2010). The second phase of particular interest, in which a portion of the enriched (with a high density of microbial populations) activated sludge, is either wasted (removed from the system) or recirculated back through the aeration tank to assist treatment efficiency (Burton, 1992, Gerardi, 2002, Seviour and Nielsen, 2010).

Clarifiers or 'secondary settling tanks' reduce suspended solids and microorganisms within the mixed liquor through gravity sedimentation (Gerardi, 2002, Rieger *et al.*, 2012, Wittmann *et al.*, 1990). In an activated sludge, process a fraction of the settled sludge enriched with a high density of activated microbial population is returned to the aeration tank to ensure an activate population is always present within the aeration tank (Gerardi, 2002, Rieger *et al.*, 2012, Seviour and Nielsen, 2010, Wittmann *et al.*, 1990). The influent slurry (ANPS) provides nutrients for the incoming activated population. Application of a returned activated sludge feedback step was included to enhance the nitrification within the AWTS by stimulating and enhance the existing bacterial population needed to drive the processes.

This chapter reports the results of a continuation study of the trials performed in Chapter 4 with the adaptation of a RAS feedback step. The objective of this research was to study the effect of RAS recirculation during aerobic treatment of ANPS on inorganic-N transformation concerning the oxidation of NH_4^+ to NO_3^- and TSS removal and provide clarified effluent with greater optical properties required for algal growth. The aim of these trials was to

- Assess whether RAS inclusion had an effect on TSS removal and nitrification when incorporated into the AWTS matrix.
- To identify optimal aeration conditions to achieve this at a laboratory scale.
- To conserve the N in the slurry as NO_3^--N for algal growth

It was hypothesised that the inclusion of RAS feedback step would have no significant effect on the treatment of ANPS in the AWTS, with particular emphasis on the removal of SS and nitrification.

5.2 Materials and methods

5.2.1 Equipment set up and operational configuration

An incorporation of a RAS feedback step to the AWTS was examined at a laboratory scale over a set of six trials to stimulate the growth of nitrating bacteria within the mixed liquor and establish suitable operating conditions for the AWTS. For the purpose of this investigation, the RAS trials are referred to as “R” with corresponding trials number (i.e. R1).

Operation of the laboratory-scaled AWTS was carried out as described in Chapter 4.2.1 with modifications. Modifications included the incorporation of a 1 L conical, glass Imhoff settling solid cone (clarifier) to the outlet discharge vessel coupled with a recycled inlet pipe (35 mm L x 6 mm – internal diameter from clarifier (3 cm from bottom) to reactor. Inlet pipe was set at the same height as that of the slurry inlet pipe described in Chapter 2.1.1 (Figure 5.1).

Aeration was DO dependant, controlled according to pre-set concentrations at 20% (1.5 mg O_2 L^{-1}), 50% (3.8 mg O_2 L^{-1}), 70% (5.3 mg O_2 L^{-1}), and 100% saturation. A THRT of 5, 7, or 10 d was maintained. Operational parameters for each individual trial are outlined in Table 5.1.

It must be noted that as this was an adaptive design study, changes in saturation levels and THRT were adjusted depending on the outcome of the trial previous.

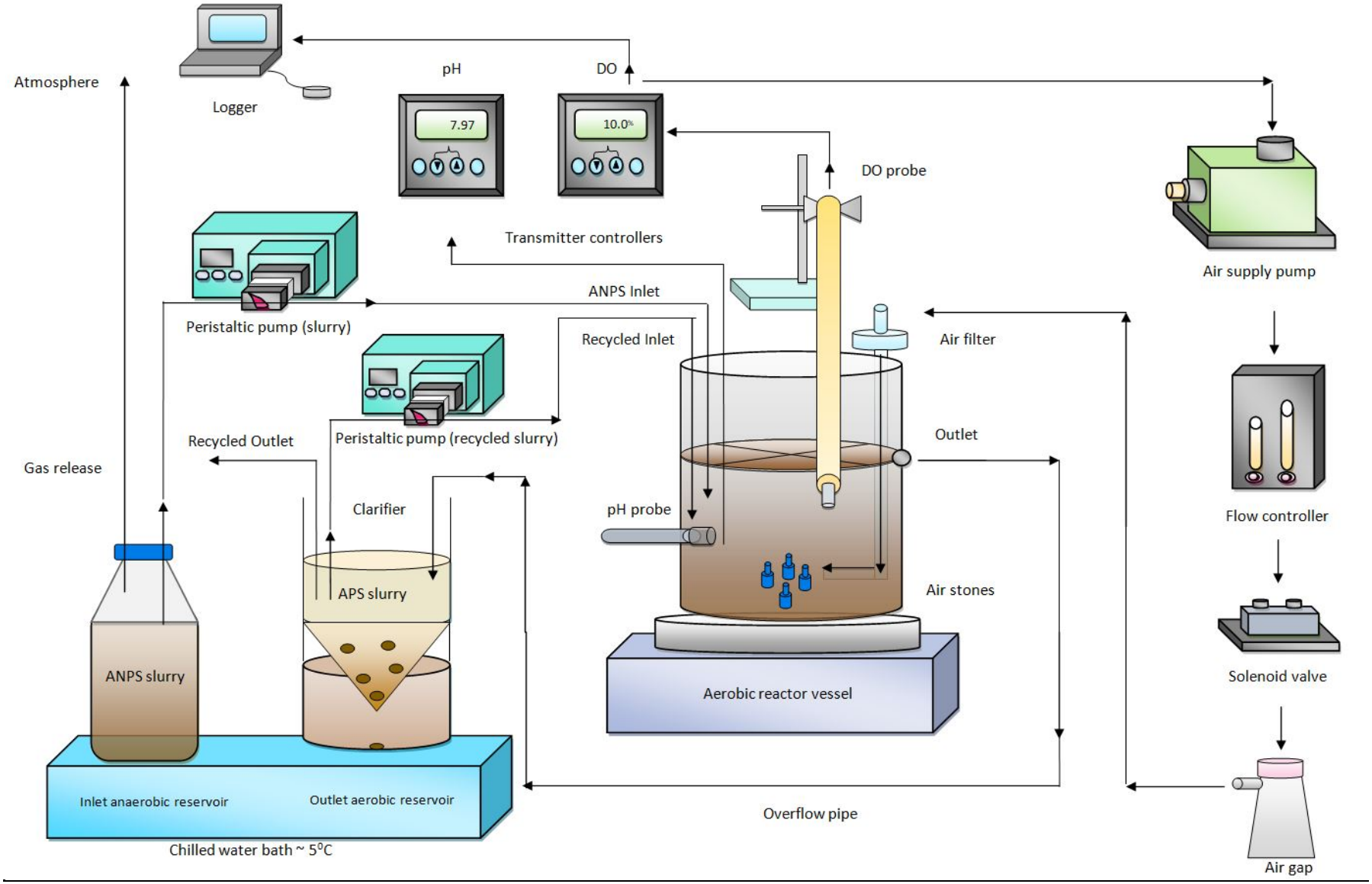

Figure 5.1: A schematic diagram of the aeration of ANPS in a modelled AWTS with the inclusion of a returned activated effluent feedback step

5.2.2 Returned activated slurry (RAS) feedback – seeding the reactor

The reactor was seeded with RAS every 4 h with either 40 (R1-2 and R5-6), 80 (R3) or 57 mL (R4) in 3 minutes of stock (ANPS (stored in the "inlet") and 10 (R1-2 and R5-6), 20 (R3) or 14 mL (R4) in 0.2 minutes of the treated RAS (stored in the "outlet") in an 80:20 percent ratio. Volumes and pumping times per trial were dependent on THRT. Two peristaltic pumps; Watson Marlow, variable speed peristaltic pump, model 503s R/L (Pump 1) and a Master flex L/S Variable-Speed Modular Drive Model 7553-73 with a High performance pump head Model L/S 77250-62 (Pump 2) were used respectively.

Pump run times were controlled via an Arlec Compact Digital Time Switch, PC697 (Chapter 2) and pump timer connected to the Arlec Compact Digital Time Switch, respectively.

Table 5:1: Operating conditions per recycled slurry trials

Trial number	1	2	3	4	5	6
THRT (days)	10	10	5	7	10	10
Controlled Aeration level (% saturation)	100%	70%	100%	70%	20%	50%
Controlled Aeration set-point (DO mg L^{-1})	>7.5	5.3	>7.5	5.3	1.5	3.8
Running time (days)	66	56	43	58	44	46
RAS feedback (%)	20%	20%	20%	20%	20%	20%

5.2.3 Sampling and Water quality analysis

160 mL of treated and inlet slurry was collected every 2-4 and 5-20 d, respectively as outlined in Chapter 2.5 with modification. Collection times were subject to variation depending on both THRT and volume of slurry required per feed (with only 80% sourced from the inlet). Outlet samples (RAS) were acquired via siphon in 40 mL aliquots, from the clarifying cone into 50 mL plastic tubes. An outlet collection pipe was placed into the clarifier approximately 8 cm from the bottom. This was to enable collection of APS without disruptions to the settled solids at the bottom of the cone, required for slurry feedback. All samples were stored frozen (4°C) until required for analysis.

5.2.4 Water Quality analysis

5.2.4.1 Environmental conditions

In situ environmental conditions were monitored in the mixed liquor several times a week (multiple times per day) for DO, pH and temperature as described in Chapter 2.5 along with daily electronic measurements of DO recorded in five - minute intervals via T-tech data loggers (Chapter 2.5.1.2 and 2.5.1.3). All values represent the mean (± SD) values per day, unless stated otherwise.

5.2.4.2 Water quality parameters

Water quality parameters (TSS, NH_4^+-N, NO_2^--N, NO_3^--N, TN, TC, TOC and IC) were analysed as described in Chapter 2.5.

5.2.5 Statistical Analysis

Data were analysed for statistical significance as described in Chapter 2.6.

5.3 Results

5.3.1 Performance of the reactor

Environmental conditions in the mixed liquor were monitored, several times a week, multiple times per day for DO, pH and temperature. Collated results are presented in Table 5.2. Results are reported as the mean ± SD and (n) number of samples analysed per trial, unless stated otherwise. Mixed liquor pH during treatment ranged from 7.0–8.2 at a mean temperature range of 20.2–22.2°C (Table 5.2). These values are comparable to both, the optimum nitrification range of 7.1-9.0 and similar to those reported in Chapter 4.3.1 (8.2–8.6 at 20.0–21.4°C). Statistical differences were determined in pH between trials R1-R6 and those without the RAS step (ST1-6) ($p \leq 0.05$).

Mixed liquor DO's were set to maintain theoretical values of up to 1.5 (20%; R5), 3.8 (50%, R6), 5.3 (70%, R2 and R4) and up to 7.5 mg O_2 L^{-1} (~100% saturation; R1 and R3), respectively throughout treatment as instigated in Chapter 4.3.1. Although similar to the set point values described above, mixed liquor DO were significantly lower on average (1.12–2.31 times) in comparison as shown in Table 5.3. This indicates there was a greater oxygen demand within the mixed liquor, as reflected in the analyses of slurry compositions before and after aeration (below). Regular dosages of anaerobic effluent and RAS every 4 hr a probable cause for the lower values recorded *in situ*.

Table 5:2: Values of measured operating parameters and *In situ* environmental conditions collated during the RAS feedback trials

Trial no.	THRT (d)	Experiment Duration (d)	DO (mg O_2 L^{-1})			Temperature (°C)		pH	
			Set point	Mean	n	Mean	n	Mean	n
R1	10	66	7.5	6.4 ± 2.6	30	21.8 ± 0.6	42	7.5 ± 0.5	41
R2	10	56	5.3	3.4 ± 1.5	67	20.9 ± 0.8	32	7.0 ± 0.7	32
R3	5	43	7.5	4.1 ± 1.9	44	21.9 ± 2.9	23	8.2 ± 0.4	25
R4	7	58	5.3	2.3 ± 2.0	59	21.1 ± 0.8	26	7.6 ± 0.8	26
R5	10	44	1.5	1.3 ± 0.5	57	22.2 ± 0.9	24	8.2 ± 0.4	24
R6	10	46	3.8	2.8 ± 1.1	47	20.2 ± 1.4	24	7.4 ± 0.8	24

5.3.2 Total Suspended solid

5.3.2.1 The effect of RAS operational parameters on SS removal

Incorporation of RAS feedback (20%) to the AWTS depicted a reduction trend in TSS similar to that of the non-recycled trials of Chapter 4.3.2. Under each condition assessed, the mean TSS loads were generally observed to be lower (by 2.0 – 3.2 times) in the treated outlet than in the inlet, these differences were statistically significant in each case (Welch two-sample t-test, $p \leq 0.05$; Wilcoxon rank sum test, $p \leq 0.05$). Inlet SS (mean ± SD) varied (but, not significantly) between 1.50 ± 0.62 and 2.82 ± 0.75 g SS L^{-1} across the trials, attributed to the variable nature of ANPS ($F(5, 26) = 1.34$, $p = 0.29 > 0.05$). Inlet TSS measured highest in R6 (2.82 ± 0.75 g SS L^{-1}). Outlet concentrations varied between 0.51 ± 0.18 and 1.12 ± 0.43 g SS L^{-1}. Reduction in SS in the treated outlet compared with the inlet values were between 50-70%. This is evident in Figure 5.2.

Figure 5.2 presents the average TSS concentrations before and after treatment with 20% RAS feedback. Removal was found greatest during R1 at a mean DO concentration of 6.4 ± 2 mg O_2 L^{-1} (approx. 86–100% saturation) and a 10 d THRT; 79% of TSS removed over a 66 d aeration period. SS concentrations dropped from 2.05 ± 1.80 to 0.64 ± 0.64 g SS L^{-1} (Figure 5.2f). The difference in

TSS before and after RAS feedback significant (W = 120, p = ≤ 0.001). Conversely, at a DO set point of 3.8 mg O_2 L^{-1} (50% saturation; maintained 2.8 ± 1.1 mg O_2 L^{-1}) and a 10 d THRT (R6), up to 50% of TSS were removed post treatment from 2.35 ± 1.10 to 1.17 ± 0.48 g L^{-1} and thus yielded the lowest removal rate detected across the 6 trials (Figure 5.2d). The difference in inlet and outlet TSS concentrations was statistically significant (t (16) = 3.13, p = 0.01 ≤ 0.05). The null hypothesis that aeration under RAS conditions would not have a significant effect on the SS load after treatment was thus rejected.

Assessment of the effect of aeration configuration parameters on SS removal was divided into two sections; first by DO set points and then by THRT over a series of aeration trials. Data obtained during these assessments is presented in Figures 5.3 – 5.5.

A comparison of the mean outlet TSS concentrations for each trial performed with RAS feedback and a 10 d THRT as shown in Figure 5.3, demonstrated when operation took place under different aeration regimes (DO set points) statistical significances existed in SS levels post treatment. This was evident between trials R1, R2, R5 and R6 when compared to one another (Kruskal Wallis Chi square (3) = 38.98, p ≤ 0.001). Aeration levels were set at 7.5 (100% saturation, uncontrolled), 5.25 (70%), 3.8 (50%) and 1.5 mg O_2 L^{-1} (20%). A pair wise comparison identified the difference in outlet SS to be significant between trials R1 - R5 (p ≤ 0.001), R1 - R6 (p ≤ 0.001), R2 - R5 (p ≤ 0.001) and R2 - R6 (p ≤ 0.001), but not between R1 - R2 (high DO levels; p = 1.00 ≥ 0.05) or R5 - R6 (low DO levels; p = 1.00 ≥ 0.05). These findings suggest DO may influence TSS reduction in the system in spite of inlet variation (p ≥ 0.05).Removal shown to have decreased (slightly) with reduced DO; R5 at 1.3 ± 0.5 mg O_2 L^{-1} (set at 20% saturation) the exception exhibiting the second highest removal (67%) behind R1 (79%) at 6.4 ± 2.6 mg O_2 L^{-1} (no DO control) (Figure 5.3).

Reducing the THRT from 10 d to 5 d (at a 100% DO set point) in R3 and then from 10 d to 7 d (70% DO set point) in R4, was shown to have also removed > 50% of TSS post aeration, respectively (Figure 5.2 and 5.4). Such that halving the THRT from 10 d to 5 d during R3, resulted in a 62% reduction of SS (from 2.26 ± 0.58 to 0.85 ± 0.35 g L^{-1}; Figure 5.2a) when set at a 100%

DO control point (4.1 ± 1.9 mg O_2 L^{-1} averaged) (Figure 5.2a). 17% less than that achieved at the longer THRT (R1; 79% removed at 6.4 ± 2.6 mg O_2 L^{-1}). TSS measured on average 1.1 times lower in R3 than R1 before (t (9) = 0.33, p = 0.75 ≥ 0.05) and 1.7 times higher after (W = 51, p ≤ 0.001) treatment (Figure 5.4a). This suggests R1 had a higher nitrifying biomass compared to R3.

This was also evident during R4 when the THRT was shortened from 10 d to 7 d and aeration was set at 70% saturation (2.3 ± 2.0 mg O_2 L^{-1} averaged) (Figure 5.4b). 56% of SS removed at this parameter set (2.21 ± 0.81 to 0.97 ± 0.53 g L^{-1}); 6% lower than that achieved at the longer THRT (R2; 62% removed at 3.4 ± 1.6 mg O_2 L^{-1}) (Figure 5.4b). A significant difference was identified in the outlet SS concentrations of the two THRT when compared to one another; but not in the inlet; concentrations reported 1.7 times lower and 1.5 times higher at the longer THRT (10 d; R2) (W = 24, p ≤ 0.001 and t (9) = -1.734, p = 0.117 ≥ 0.05), respectively.

These findings suggest that removal was influenced not only by changes to the applied parameters (DO, THRT) but from the inclusion of a RAS feedback step and influent characteristics. Confounding due to inlet characteristics was considered unlikely, with no statistical significances identified between the SS, N, and C concentrations across the corresponding trials.

As such, there is not enough conclusive evidence to reject the null hypothesis that a change in THRT and air saturation level would have no significant effect on TSS removal.

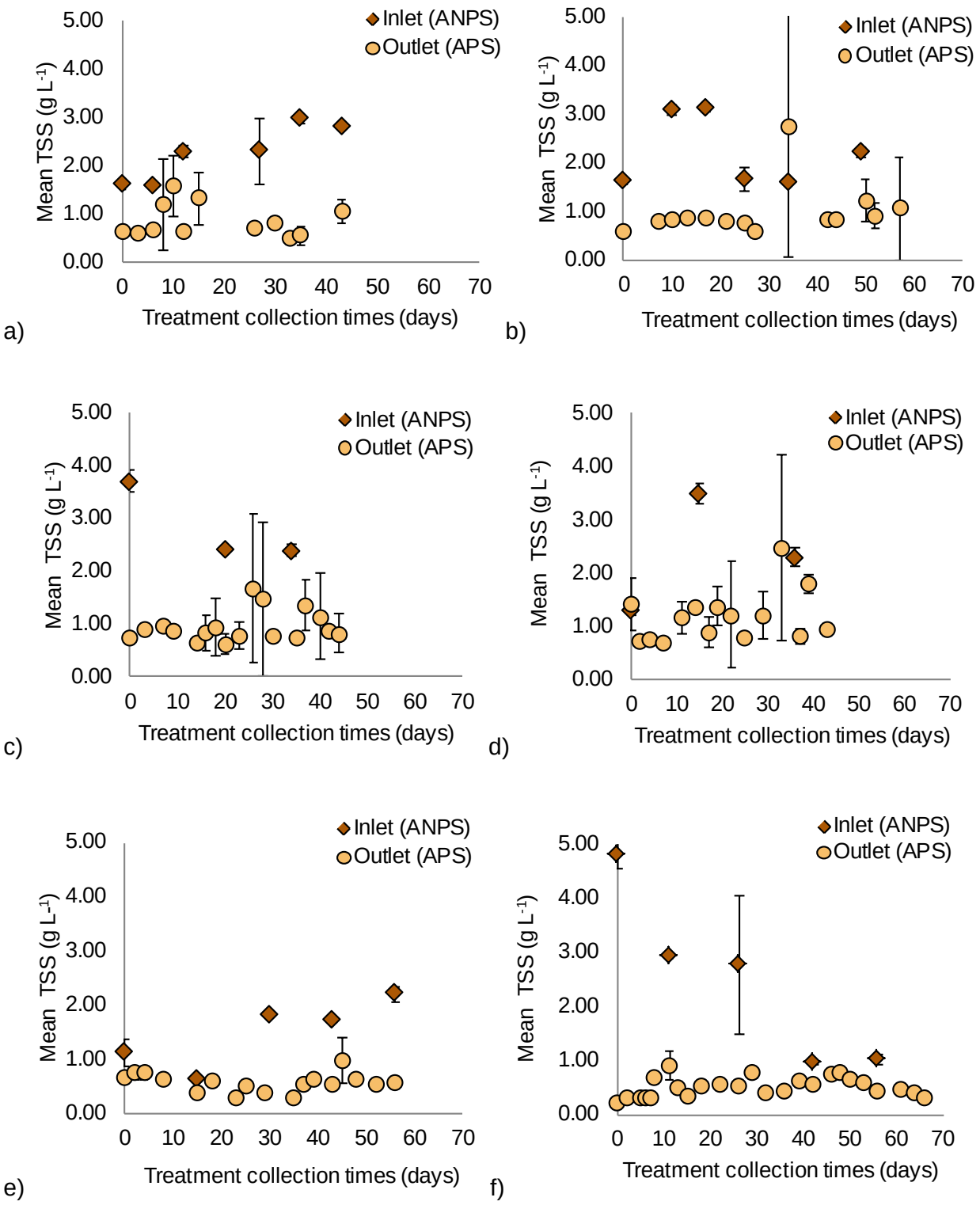

Figure 5.2: Average TSS concentrations (g L^{-1}) before (inlet) and after (outlet) aeration in the AWTS with 20% RAS feedback under various operating regimes: a) THRT 5 d at 4.1 ± 1.9 mg O_2 L^{-1} (~up to 100% air saturation, uncontrolled), 21.8 ± 2.9°C (R3); b) THRT 7 d, 2.3 ± 2.0 mg O_2 L^{-1} (60-70% air saturation), 22.1 ± 0.8°C (R4), c) THRT 10 d, 1.3 ± 0.5 mg O_2 L^{-1} (20% air saturation), 22.2 ± 0.9°C (R5), d) THRT 10 d, 2.8 ± 1.1 mg O_2 L^{-1} (42-50% air saturation), 20.2 ± 1.4°C (R6), e) THRT 10 d, 3.4 ± 1.5 mg O_2 L^{-1} (66-70%), 20.9 ± 0.8°C (R2) and f) THRT 10 d, 6.4 ± 2.6 mg O_2 L^{-1} (86-100% air saturation) at 21.8 ± 0.6°C (R1).

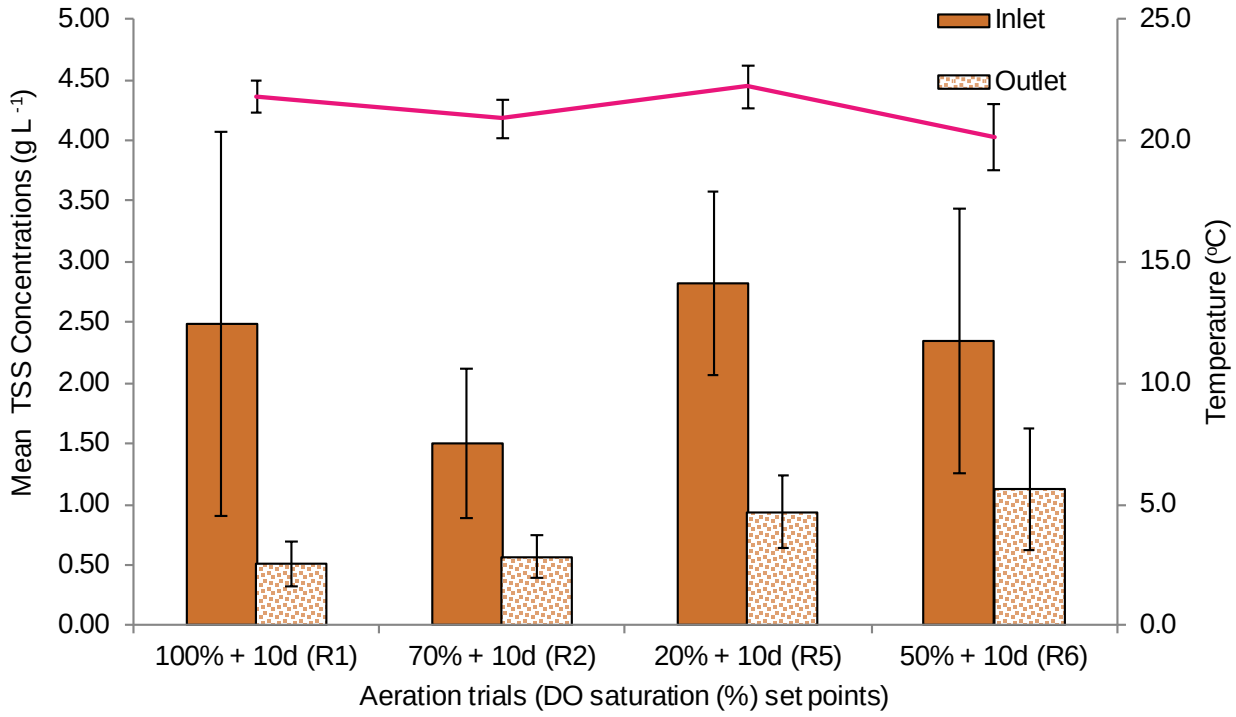

Figure 5.3: Comparison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before and after aeration with RAS feedback when operated with a 10 d THRT and the mixed liquor set to maintain DO concentrations of 20%, 50%, 70% or ~100% saturation over a series of four experiments.

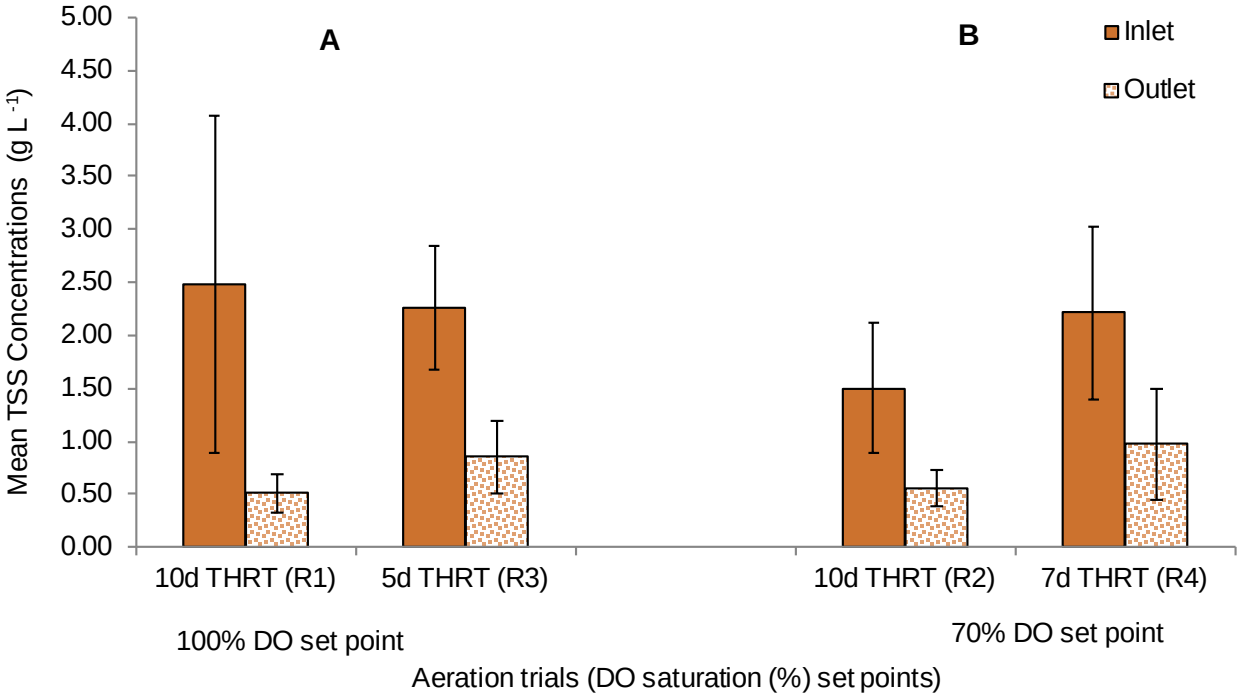

Figure 5.4: Comparison of mean (± SD) SS concentrations (g L^{-1}) in ANPS before and after aeration with RAS feedback when operated at a) an uncontrolled DO set point up to 100% saturation with either a 10 d (R1) or 5 d (R3) THRT and b) at 70% saturation with either a 10 d (R2) or 7 d (R4) THRT.

Comparison of TSS removal in the AWTS operated with and without inclusion of RAS

In Figure 4.2e (Chapter 4.3.2), 71% of SS on average were removed when DO maintained 5.1 ± 1.7 mg O_2 L^{-1} (set at 100% DO saturation (uncontrolled)) with a 10 d THRT. Under similar set operating parameters (100% air saturation set point (6.4 ± 2.6 mg O_2 L^{-1} averaged) and a 10 d THRT), inclusion of a RAS feedback step offered a slight improvement in SS removal with 79% removed during R1 over a 66d period (Figure 5.2f). 8% more than that achieved in ST5 without the additional treatment step (Figure 5.5a). Interestingly, outlet TSS measured significantly lower (1.8 times) in ST5 without RAS (0.28 ± 0.06 g L^{-1}) than R1 with it (0.51 ± 0.18 g SS L^{-1}) (W = 51, $p \leq$ 0.001). This was probably, attributed to the re-introduction of SS via RAS feedback and the high (2.58 times) inlet TSS content of R1 (2.48 ± 1.59 g SS L^{-1}) compared to ST5 (0.96 ± 0.40 g SS L^{-1}) and from. However, according to an independent two-sample t-test with equal variance the difference in inlet SS contents were not statistically significant (t (7) = 1.85; p = 0.11 ≥ 0.05) and therefore unlikely to have had a significant influence over the results.

This same pattern however, was not observed between R3 and ST4 when the THRT was set to 5 d (uncontrolled DO set point up to 100% saturation). The inclusion of a RAS feedback step (R3 at 4.1 ± 1.9 mg O_2 L^{-1}) yielded TSS removal 6% lower than when no RAS was used (ST4 at 6.3 ± 2.6 mg O_2 L^{-1}). Mean TSS concentrations were found to be significantly higher in R3 with RAS inclusion than in ST4 without it, by up to 2.0 times in the inlet (W = 48, p = 0.01 ≤ 0.05) and 1.4 times in the outlet (W = 192, $p \leq$ 0.001) (Figure 5.5b). Re-introduction of solids through return of the activated slurry deemed the most probable cause for the higher outlet concentrations. Nevertheless, a reduction of 62% and 68% was obtained in each case, respectively (Figures 5.5b).

The differences in inlet concentrations could be attributed to the variations in slurry compositions despite being collected from the same lagoon. Whereas the higher outlet TSS is most likely due to the re-introduction of SS into the mixed liquor, a result of the incorporated activated slurry return step and from the longer THRT, enabling longer growth periods within the system.

Keeping in mind the limitation of lagoon slurry variation, there was not enough conclusive evidence to reject the null hypothesis that the inclusion of a RAS feedback step to the treatment matrix

would have no significant effect on the treatment of ANPS in the AWTS, with particular emphasis on the removal of SS.

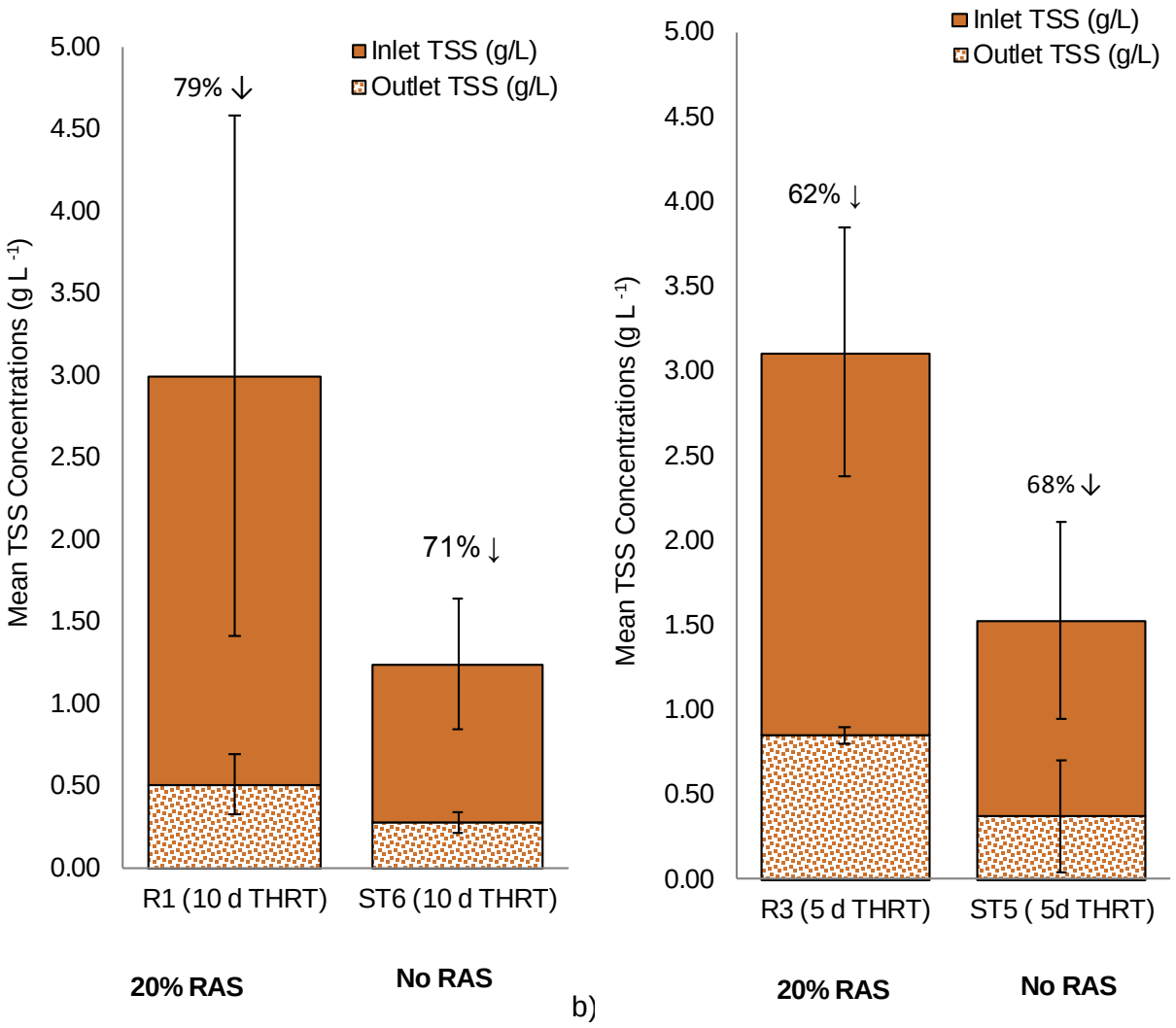

Figure 5.5: A comparison of the mean TSS load when RAS feedback was present to those of the non-recycled trials of Chapter 5 under the same operating parameters; set at 100% DO saturation (uncontrolled) and either a) a 10 d (R1 and ST5) or b) a 5 d (R3 and ST4) THRT.

5.3.3 Effect of RAS feedback on ammonium oxidation and nitrification

Inorganic-N data was collected for both the inlet ANPS and aerated mixed liquor to assess the inter-conversion of inorganic-N fractions following treatment in the AWTS described in Chapter 2 coupled with a RAS feedback step. Data was collected at regular intervals over a series of six experiments; the duration of each experiment ranged from 43–66 d.

TN, concentrations ranged from 1.01 ± 0.38 – 1.39 ± 0.18 g L^{-1} on average in the non-aerated ANPS (inlet) prior to aeration. Data analysis identified that whilst there was a slight fluctuation in TN concentrations, across the inlet samples, these fluctuations, were not statistically significant when compared to one another irrespective of trial and the intended treatment parameters (except between trials R1-R3) ($p \geq 0.05$). The different inorganic-N fractions (TIN, NH_4^+-N, NO_2^--N, NO_3^--N) measured during the investigation yielded similar results. No statistically significant differences were detected in any of the inlet samples across the four trials investigated with a 10 d THRT (R1, R2, R5, and R6) ($p \geq 0.05$*).*Confounding due to inlet variation was unlikely.

Figures 5.6-5.12 summarises the results of a TIN mass balance for each of the recycled aeration trials (RAS feedback) by comparing the measured N (NH_4^+-N, NO_2^--N and NO_3^--N) content of both the inlet and treated mixed liquor accordingly. A slight variation in inorganic-N transformation from NH_4^+-N to NO_3^--N was observed across the trials depending on operating parameters.

Non-aerated ANPS (inlet) were generally observed to have a higher TIN content (0.96–1.77 g L^{-1}), predominately in the form of NH_4^+-N (99-100%) than that of the aerated mixed liquor (outlet; 0.76 - 1.15 g L^{-1}) by up to 1.06–2.32 times (Figures 5.6-5.16). R1 was the only exception, with inlet (0.96 ± 0.27 g L^{-1}) TIN and TN (1.01 ± 0.38 g L^{-1}) detected up to1.09 times lower than outlet (1.05 ± 0.14 g L^{-1}) TIN and TN (1.07 ± 0.09 g L^{-1}) content. An indication that ammonification of organic-N may have taken place. Data analysis identified that the lower TIN in the outlet was significantly different from the inlet TIN, for three out of the six trials run; R3, R5, and R6 ($p \leq 0.05$) (Figure 5.6-16).

Ammonia removal was quantified as the difference between inlet and outlet NH_4^+-N (g L^{-1}) concentrations. On average up to 70-90% of the inlet NH_4^+-N content was removed during aerobic treatment. A concentration decrease from 0.96 ± 0.26 - 1.77 ± 0.90 g NH_4^+-N L^{-1} to 0.17 ± 0.10 –

0.42 ± 0.09 g NH_4^+-N in the mixed liquor was observed at 20.2 ± 1.4 – 22.2 ± 0.9°C. A corresponding increase in NO_2^--N and NO_3^--N was observed for each trial. Changes in THRT and DO operating concentrations were found to effect nitrogen transformation.

At a mean DO concentration of 6.4 ± 2.6 mg O_2 L^{-1} at 21.8 ± 0.6°C and a 10 d THRT in an uncontrolled DO environment, inclusion of 20% RAS feedback yielded a reduction in NH_4^+-N concentrations of up to 82%, from 0.95 ± 0.26 g L^{-1} to 0.17 ± 0.10 g L^{-1} (W = 120; $p \leq 0.001$). Of which 45% and 47% were transformed into NO_2^--N and NO_3^--N, respectively. A subsequent increase in NO_2^--N and NO_3^--N levels from <BLOD to 0.43 ± 0.06 and 0.45 ± 0.13 g L^{-1} occurred, respectively. This was evident in the inorganic-N transformation plot shown in Figure 5.6. Data analysis identified that the lower NH_4^+-N and higher NO_2^--N and NO_3^--N concentrations recorded in the outlet differed significantly from those measured in the inlet ($p \leq 0.05$). Interestingly, evaluation of the data shows that all of the inorganic-N content in the inlet could be accounted for in the outlet-N post treatment (Figure 5.6). This however, was not the case for the remaining five trials, with up to 6-32% of N unaccounted for (Figures 5.7-5.12), due either to organic-N or nitrogen loss, through NH_4^+ volatilisation.

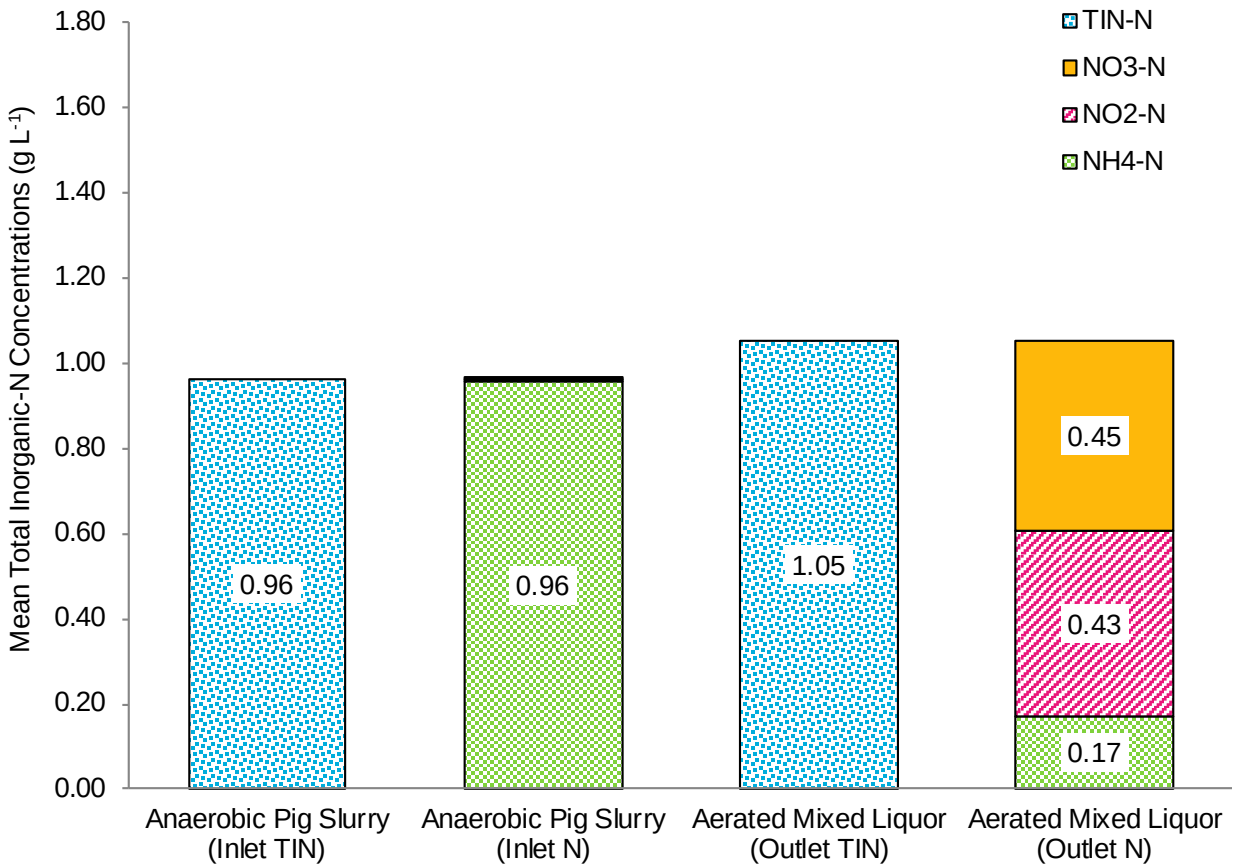

Figure 5.6: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback in an uncontrolled DO environment set to maintain 7.5 mg O_2 L^{-1} and 10 d THRT during R1. A mean DO (*in situ*) concentration of 6.4 ± 2.6 mg O_2 L^{-1} at 21.8 ± 0.6°C (up to 100% saturation) and pH 7.5 ± 0.5 achieved over a 41 d treatment period.

At DO concentrations of 3.4 ± 1.5 mg O_2 L^{-1} (set at~70% air saturation, 5.3 mg O_2 L^{-1} set point), an oxidation trend similar to that at 100% was observed during R2 over a 56 d treatment period (Figure 5.7). TIN and NH_4^+-N were found to be significantly lower in the treated mixed liquor than in the inlet by up to1.09 - 3.90 times (W = 50, p = 0.56 ≥ 0.05 and W = 85, p ≤ 0.001, respectively) yielding an average reduction of 8% and 75% post treatment (Figure 5.7). This was similar to the 82% (NH_4^+-N) achieved at 6.4 ± 2.6 mg O_2 L^{-1} in R1. The difference in NH_4^+-N was shown to be statistically significant between R1 and R2, for outlet concentrations (t (39), p = 0.02 ≤ 0.05), but not inlets, with R11.10 times lower at the higher DO concentration of R1 (t (8) = 0.63, p = 0.55 ≥ 0.05). Of the 75% of NH_4^+-N reduced, 67% was oxidised to NO_2^--N (1.1%) and NO_3^--N (66%) (Figure 5.7). R2 displayed the greatest nitrification rate of all the trials examined in both this study and that of Chapter 4.3.3, with particular emphasis on NO_3^--N accumulation. An average of 0.70 ±

0.20 g NO_3^--N L^{-1} was achieved in the mixed liquor post treatment, a statistically significant increase from the <BLOD g NO_3^--N L^{-1} detected pre-treatment (inlet) (W = 4.0, p ≤.0.001). Interestingly, NO_3^--N accumulation was noted significantly higher at a set point value of 5.3 mg O_2 L^{-1} (3.4 ± 1.6 mg O_2 L^{-1} averaged) than at any of the other experimental runs examined with a 10 d THRT (R1, R5 and R6) by up to 0.19–0.33g NO_3^--N L^{-1}, an improvement of 27-47% (F (3, 70) = 15.37, p ≤ 0.001) (Figure 5.12).

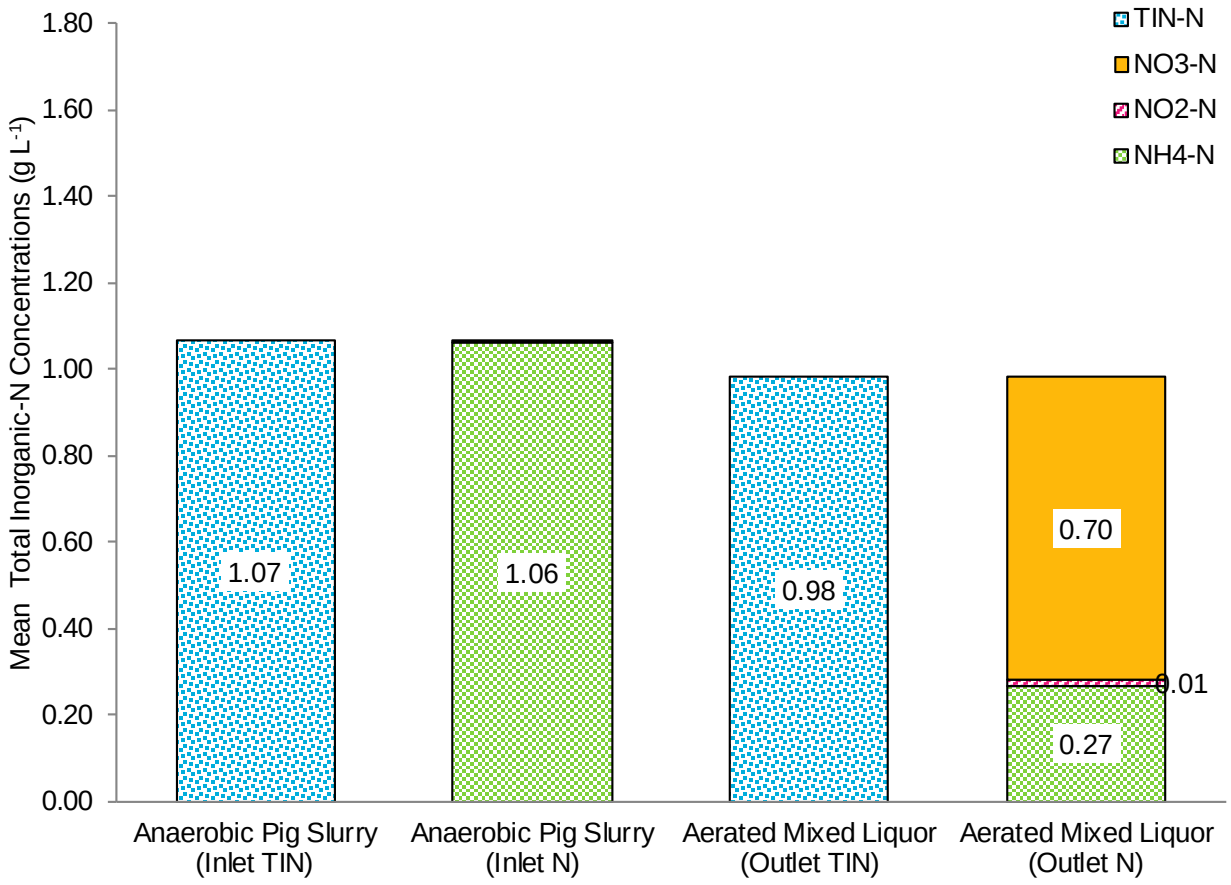

Figure 5.7: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 5.3 mg O_2 L^{-1} (~70% air saturation) and 10 d THRT during R2. A mean DO concentration of 3.4 ± 1.5 mg O_2 L^{-1} at 20.9 ± 0.8°C and pH 7.0 ± 0.7 achieved over a 56 d treatment period.

Reducing DO to a 50%, air saturation set point value of 3.8 mg O_2 L^{-1} (~2.8 ± 1.5 mg O_2 L^{-1} averaged; R6), with 20% RAS feedback and THRT of 10 d, mean inlet NH_4^+-N concentrations averaged 1.39 ± 0.25 g L^{-1} (Figure 5.8). The mixed liquor NH_4^+-N concentrations was only 0.18 ± 0.10 g L^{-1}, and noted significantly lower than inlet NH_4^+-N (W = 48, $p \leq 0.001$) (Figure 5.8). This shows, that most of the NH_4^+-N was removed during the 43 d aeration period of R6 (Figure 5.8). It was calculated that about 1.13 g NH_4^+-N L^{-1}, approximately 86% was removed, on average. R6 had the greatest NH_4^+-N removal yields (86%) of all operating parameters examined both in this study and in those of chapter 4.3.3 (No-RAS). The difference in mixed liquor NH_4^+-N of R6 was observed to be not significant compared to those of the other trials operated with a 10 d THRT (R1, R2 and R5) according to a pair wise comparison (Kruskal-Wallis Chi square (3) = 8.91; p = 0.03 ≤ 0.05; pair wise comparison $p \geq 0.05$) (Figure 5.10). Inlet NH_4^+-N concentrations were also noted 0.04-0.81 g L^{-1} higher in R6 than those of R1, R2 and R5, but not significantly, (F (3, 12) = 1.41, p = 0.29 ≥ 0.05). This suggests that NH_4^+-N oxidation was not confounded by inlet variations.

A TIN mass account at the 3.8 mg O_2 L^{-1} DO set point presented in Figure 5.8 shows that of the 1.13 g NH_4^+-N L^{-1} reduced, 0.07 ± 0.03 g L^{-1} and 0.51 ± 0.16 g L^{-1} was oxidised to NO_2^--N (6.2%) and NO_3^--N (45%), respectively). Comparing mean NO_3^--N values of R1 (100% air saturation set point) and R2 (70% air saturation set point) with R6, a significant difference was identified between R6 and R2, only (F (3, 70) = 15.37, $p \leq 0.001$; Tukey post hoc multi-comparison; lower = -0.33, upper = -0.06, $p \leq 0.001$); concentrations were noted 1.37 times higher in R2 than R6 (Figure 5.10). Significant differences in mean outlet NO_2^--N values were also identified between R1, R2 and R6 (Kruskal-Wallis Chi square (3) = 63.97, $p \leq 0.001$, post hoc pairwise comparisons; $p \leq$ 0.001 for all comparisons) (Figure 5.10). As mentioned above no significant differences were detected in inlet NO_3^--N concentrations across the four 10 d THRT with 20% RAS feedback trials (Kruskal-Wallis Chi square (3) = 6.94, p = 0.07 ≥ 0.05).

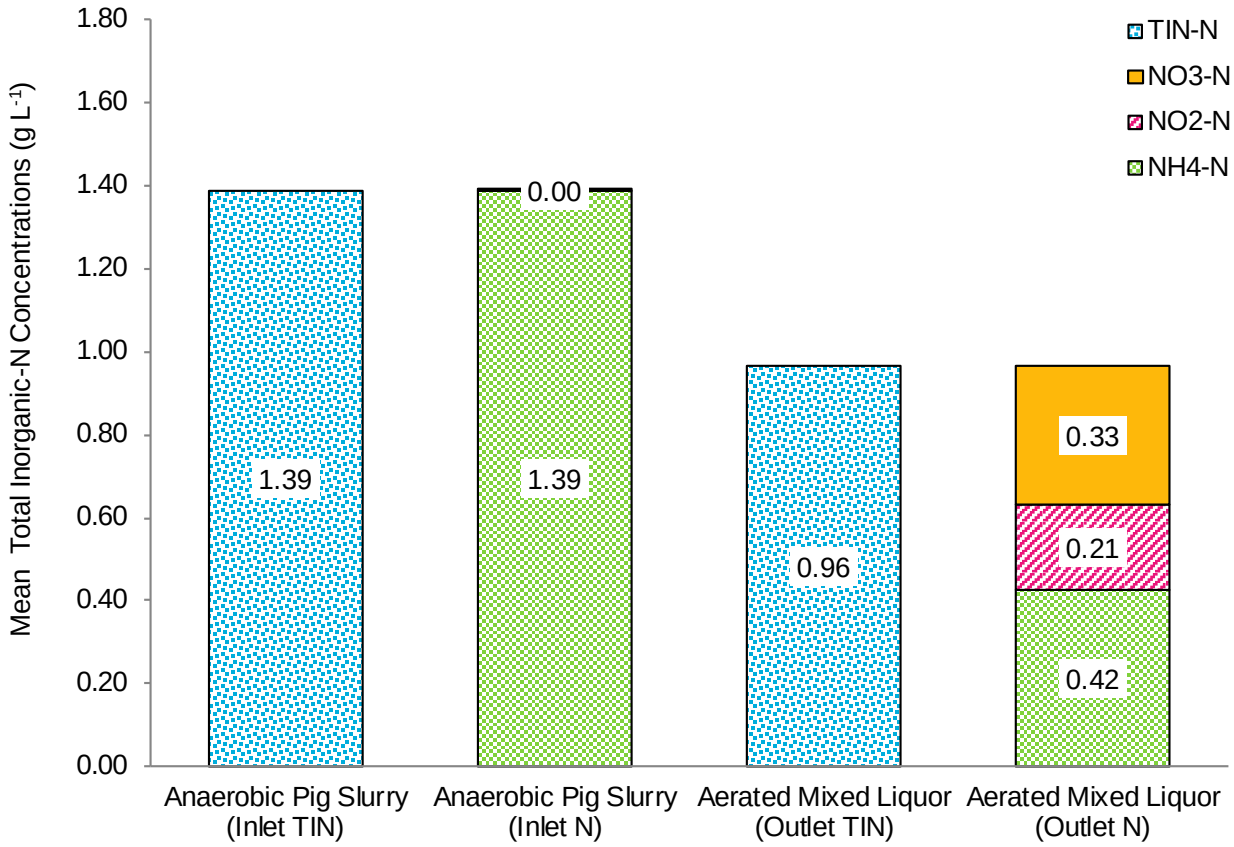

Figure 5.8: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 3.8 mg O_2 L^{-1} (~50% air saturation) and 10 d THRT during R6. A mean DO concentration of 2.8 ± 1.1 mg O_2 L^{-1} at 20.2 ± 1.4°C and pH 7.4 ± 0.8 achieved over a 24 d treatment period.

Figure 5.9 presents the results of a total inorganic-N transformation when air saturation was sustained towards the lower end of the saturation scale; set at ~20% saturation with a 10 d THRT over a 44 d treatment period and 20% RAS feedback during R5. Inlet TIN averaged 1.17 ± 0.07 g L^{-1} pre-treatment, primarily as NH_4^+-N; 1.11 ± 0.07 g L^{-1} (99%). A drop in mean mixed liquor DO to 1.3 ± 0.5 mg O_2 L^{-1}, yielded a 23% reduction in TIN, and 80% NH_4^+-N post treatment (Figure 5.8). Of the 1.11 ± 0.07 g NH_4^+-N L^{-1}detected in the inlet, 20% remained as NH_4^+-N, while 24% and 34% was oxidised to NO_2^--N and NO_3^--N in the mixed liquor post treatment, respectively (Figure 5.9). The remaining 22% as organic-N or lost through NH_4^+-N volatilisation. This oxidation trend was comparable to those at the high saturation concentrations of experimental runs R1, R2, and R6. A significant difference in outlet NO_2^--N and NO_3^--N concentrations was identified between R1, R2 and R6 when compared to R5 (Kruskal- Wallis Chi square (3) = 63.97, $p \leq 0.001$ and F (3, 70) = 15.37, $p \leq 0.001$ (R2-R5 only; $p \leq 0.001$)).

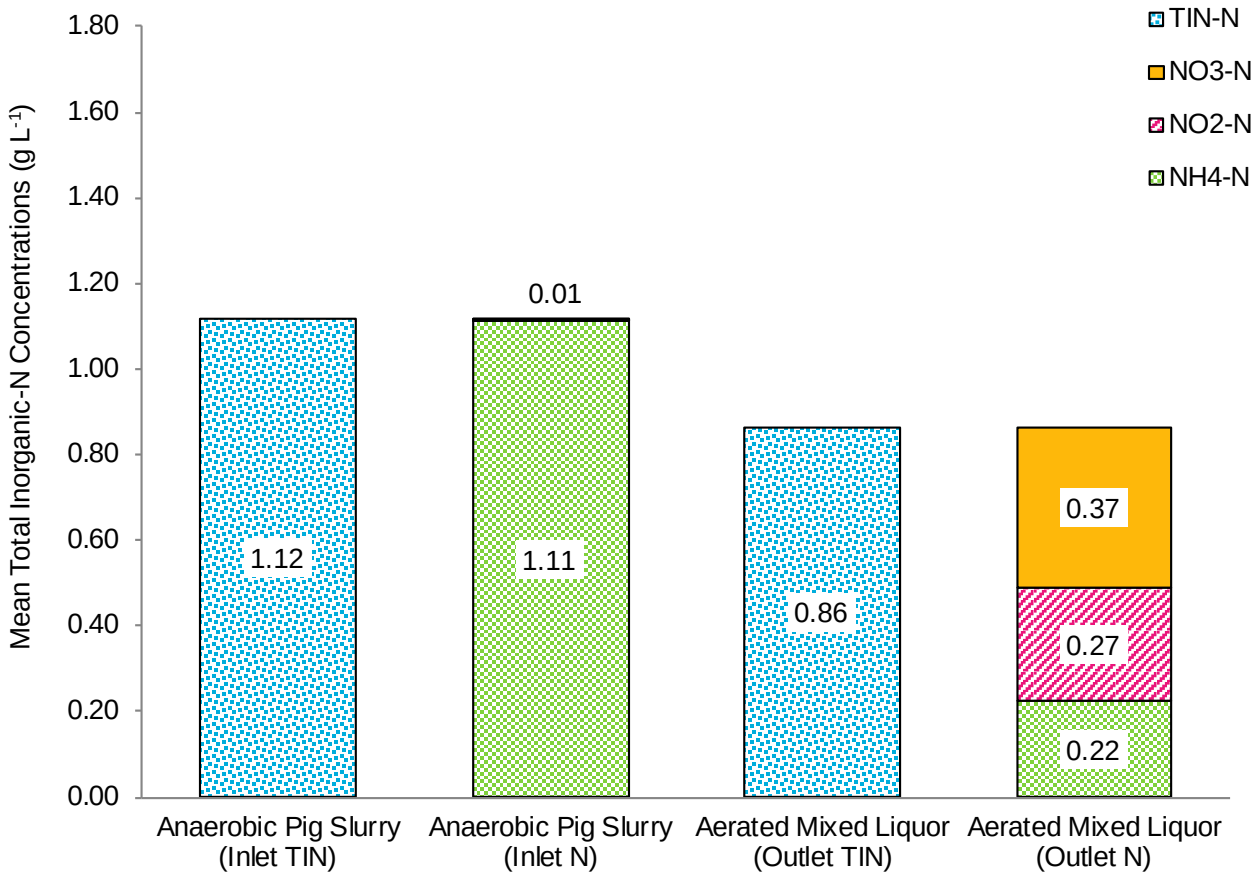

Figure 5.9: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 1.5 mg O_2 L^{-1} (~20% air saturation) and 10 d THRT during R5. A mean DO concentration of 1.3 ± 0.5 mg O_2 L^{-1} at 22.2 ± 0.9°C and pH 8.2 ± 0.4 achieved over a 44 d treatment period.

A comparison of mean outlet inorganic-N concentrations for each trial performed with 20% RAS feedback and a 10 d THRT as shown in Figure 5.10, demonstrated a clear transition in N fractions from NH_4^+-N to NO_3^--N in each of the 4 trials. Each trial, under aerobic conditions removed over 75% of NH_4^+-N, on average (Figure 5.10).

The presence of NO_2^--N and NO_3^--N in the outlet signified that nitrification had gone through to nitration, with NO_3^--N concentrations measuring greater than NO_2^--N (Figure 5.10). This was supported by the significant drop in IC levels post treatment; ranging from 0.89-1.07 g L^{-1} (inlet) to 0.03–0.20 g L^{-1} (outlet) across the four trials (Figures 5.14 and 5.17). Whilst the trials showed nitrification to have occurred in each of the four trials, the process was still incomplete with not all of the oxidised NH_4^+-N converted to NO_3^--N most probably volatilised or stayed as NO_2^--N.

Nevertheless, evaluation of the collated data identified there to have also been a significant improvement in NO_3^--N accumulation post-aeration from the <BLOD g L^{-1} detected in the ANPS inlet to the mean 0.37-0.70 g L^{-1} detected in the outlet. This was consistent across the four treatments and suggested that with the aid of RAS any aeration regime is likely to increase NO_3^--N production based on the findings of the four trials (Figure 5.10). Nitrification was shown most proficient when aeration maintained 3.4 ± 1.6 mg O_2 L^{-1} at a DO set point value of 5.3 mg O_2 L^{-1} (~70% saturation), with an average NO_3^--N concentration of 0.70 ± 0.20 g NO_3^--N L^{-1} and 0.01 ± 0.02 g NO_2 L^{-1} in R2. In fact, NO_3^--N levels were significantly higher in R2 than in any of the treatment conditions tested during this study by up to 1.4 - 2.1 times ($p \leq 0.05$) (Figure 5.10). The difference in outlet NO_2^--N and NO_3^--N statistically significant between the different aeration levels examined with the 10 d THRT and RAS feedback (20%) (Kruskal-Wallis Chi-square (3) = 63.97, $p \leq 0.001$ and ANOVA; F (3, 70) = $p \leq 0.001$, respectively) (Figure 5.10). More precisely, significance existed between all four trials for NO_2^--N ($p \leq 0.001$) and between trials R1-R2 ($p \leq 0.001$), R5-R2 ($p \leq 0.001$), and R6-R2 ($p \leq 0.001$) for NO_3^--N according to post hoc pairwise comparisons.

These findings suggest nitrification to have been influenced by a change in DO set points when operated with a 10 d THRT and 20% RAS in spite of the variable inlet characteristics and environmental conditions. As signified by the improved nitrification rates observed over the four saturation set points. RAS feedback a probable cause, and may have contributed to the lower DO levels detected during treatment (greater biomass population) (Table 5.2).

Thus, there is enough evidence to reject the null the hypothesis that a change in DO saturation set points would have no significant effect on nitrification and 20% RAS following the inclusion of RAS feedback to the AWTS when operated with a 10 d THRT. The next step was to determine if reducing THRT had a significant effect on nitrification when aerated with a DO set point of (up to) 100% and 70% saturation with 20% RAS feedback.

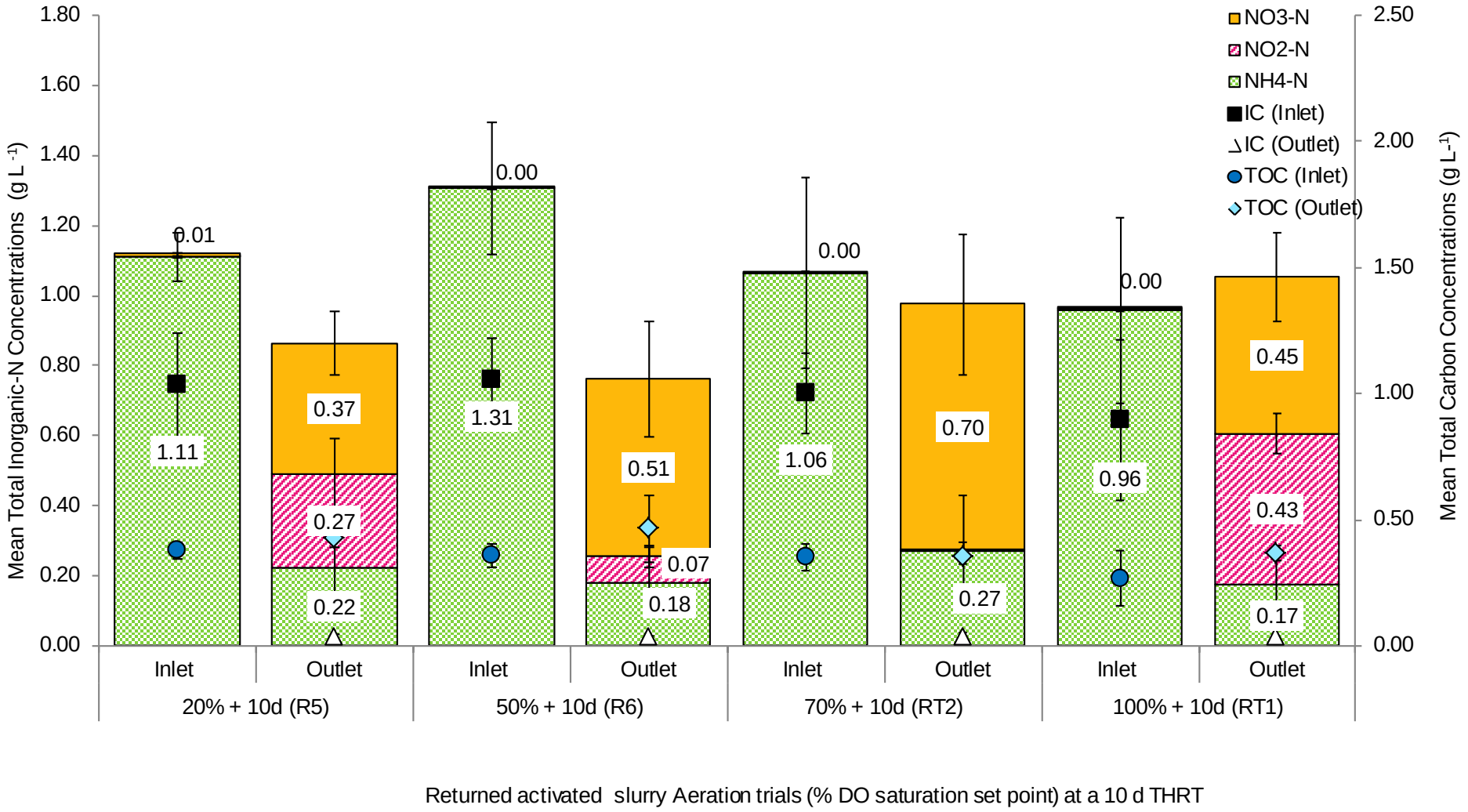

Figure 5.10: A comparison of inorganic-N levels analysed during the aeration of ANPS equipped with 20% RAS feedback and a 10 d THRT during trials R1, R2, R5, and R6, set at 20%, 50%, 70%, and 100% DO saturation set points, respectively.

As seen with the SS concentrations; reducing the THRT from 10 d to 5 d (at a 100% DO set point) in R3 and then from 10 d to 7 d (70% DO set point) in R4, > 70-75% of NH_4^+-N was removed post treatment with RAS feedback included.

In Figure 5.11, a reduction in THRT to 5 d (R3) demonstrated a similar transformation trend as observed at the longer THRT of 10 d (R1), post treatment. DO was set to maintain a set point value of 7.5 mg O_2 L^{-1} (uncontrolled) with an average of 4.1 ± 1.9 mg O_2 L^{-1} sustained at 21.9 ± 2.9℃ during this period. Treatment time proceeded over a 43 d period. Under this condition 0.21 and 0.33 g NH_4^+-N L^{-1} was nitrified to NO_2^--N and NO_3^--N, respectively. 0.42 ± 0.09 g L^{-1} remained as NH_4^+-N. A removal of up to 70% NH_4^+-N was observed (Figure 5.11). The lowest removal (NH_4^+-N) detected across the six RAS trials (Figures 5.6-5.13). The difference between inlet and outlet N levels of R3 identified as significant for each fraction examined ($p \leq 0.05$).

In addition to the lowest NH_4^+-N oxidation, R3 (5 d THRT) also exhibited the lowest nitrification rate of all trials tested with RAS feedback. This was observed in the inorganic-N content of the mixed liquor post treatment (Figures 5.11 and 5.13). Such that, a comparison of mean outlet inorganic-N levels at a DO (uncontrolled) set point of ~7.49 mg O_2 L^{-1} found nitrification to be significantly lower when operated at a shorter THRT of 5 d (R3) than at 10 d (R1). This was denoted by a higher NH_4^+-N but lower TIN, NO_3^--N and NO_2^--N outlet content, of R3 compared to R1 by up to 1.09-2.47 times (Figure 5.13). The difference in outlet N levels between the two THRT were statistically significant for NH_4^+-N (t (34) = -7.31, p ≤ 0.001) and NO_2^--N (W = 284, $p \leq 0.001$), and not statistically significant for TN (t (34) = 1.96, $p = 0.06 \geq 0.05$), TIN (t (34) = 1.82, $p = 0.08 \geq 0.05$) and NO_3^--N (W = 199, $p = 0.07 \geq 0.05$). A significant reduction in NO_3^--N accumulation was also observed. NO_3^--N levels measured on average 0.12 g NO_3^--N L^{-1} lower at 5 d than at 10 d under this saturation set point (W =199, p= 0.07 ≥0.05).

These findings indicate a reduction in THRT from 10 d to 5 d at an aeration level set to 100% saturation, resulted in reduced NH_4^+-N and NO_2^--N oxidation of more than 12%. Whilst the findings show a change in THRT to have had an effect on nitrification (greater at the longer THRT of 10 d),

RAS incorporation and potential confounding due to inlet characteristic variation may have also had an effect with inlet N levels found to be 1.38-1.45 times higher in R3 ($p \leq 0.05$). Hence, there is not enough evidence to reject the null hypothesis that a change (reduction) in THRT from 10 d to 5 d would not have a significant effect on nitrification when operated at a DO set point of up to 100% saturation and 20% RAS feedback.

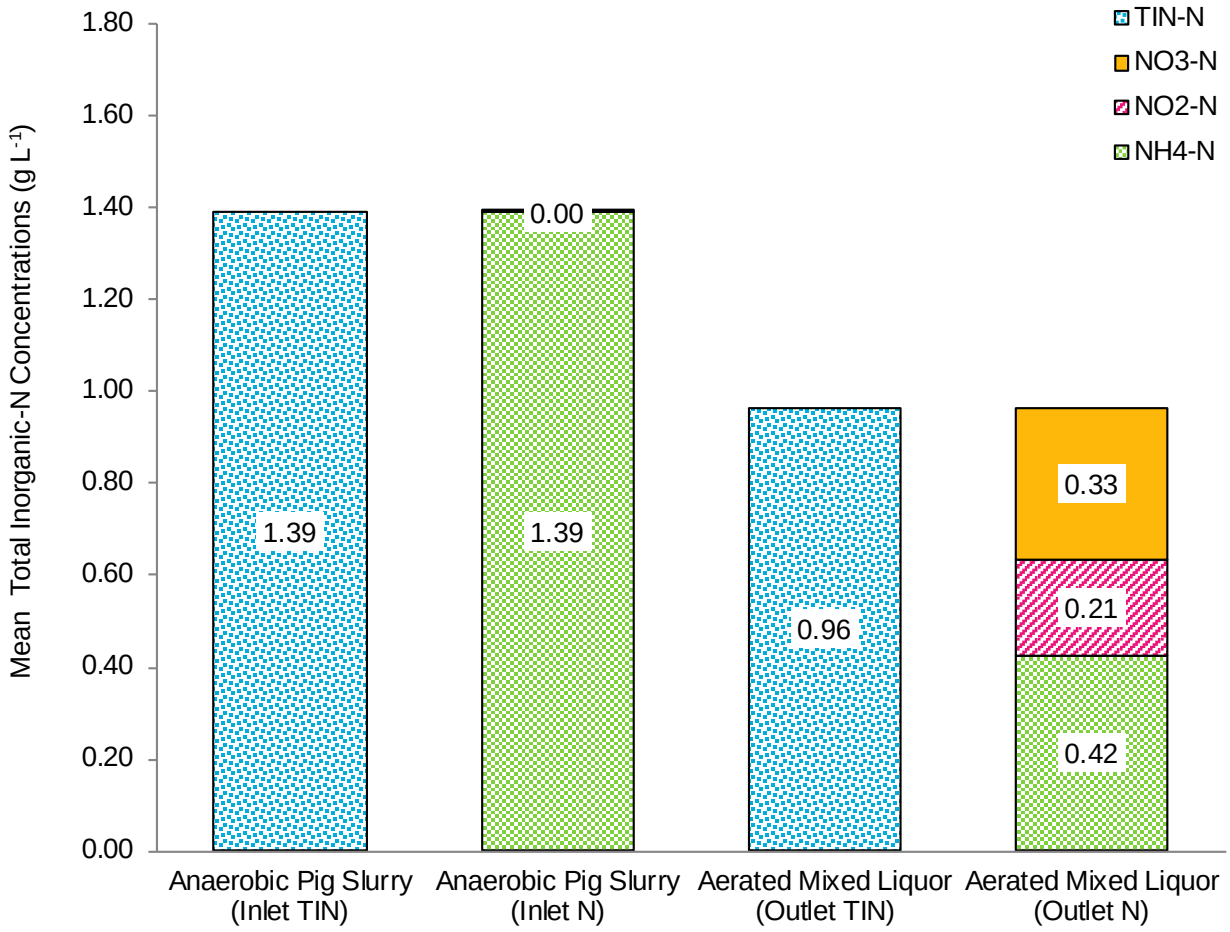

Figure 5.11: A comparison of inorganic-N transformation during the aeration of ANPS following the inclusion of 20% RAS feedback set to maintain 7.5 mg O_2 L^{-1} (~100% air saturation) and 5 d THRT during R3. A mean DO concentration of 4.1 ± 1.9 mg O_2 L^{-1} at 21.9 ± 2.9°C and pH 8.2 ± 0.4 achieved over a 43 d treatment period.

Similar results were illustrated in Figure 5.12 for R4 when operated at a 7 d THRT and a 70% saturation set point value of 5.3 mg O_2 L^{-1} (2.3 ± 2.0 mg O_2 L^{-1}, averaged). Over the 57 d treatment period, up to 6.9% of TIN was removed (Figure 5.12). A subsequent reduction in mean NH_4^+-N content up to 75% was detected post treatment also. These were comparable to the 7.8% (TIN) and 75% (NH_4^+-N) reduction yields of R2 at a 10 d THRT. No statistical significance was evident in reduction yields between the two treatment types even though the TN, and NH_4^+-N concentrations of R4 measured 1.15 times higher than R2 in both the inlet and outlet slurries ($p \geq 0.05$) (Figure 5.13b).

Likewise, no real improvement in NO_3^--N accumulation was evident in R4 following a reduction in THRT to 7 d than that observed in R2 (Figure 5.13b). In fact, NO_3^--N accumulation was significantly lower (32%) at 7 d (R4; 0.48 ± 0.21 g NO_3^--N L^{-1}) than 10 d THRTs (R2; 0.70 ± 0.20 g NO_3^--N L^{-1}) ($t(20) = 2.92$, $p \leq 0.001$) (Figure 5.13b). Nevertheless, NO_3^--N was detected significantly greater post treatment on average than in the inlet; an increase of 0.48 ± 0.21 g NO_3^--N L^{-1} (Figure 5.12).

Consequently, a reduction in THRT from 10 d to 7 d when operated with a DO set point of 70% saturation and 20% RAS did not significantly improve NH_4-N oxidation and nitration, with nitrification found greater at 10 d THRT. Thus, the null hypothesis that a reduction in THRT from 10 d to 7 d would significantly improve NH_4^+-N oxidation and nitrification when aeration was set to maintain 70% DO saturation and in combination with RAS feedback was thus rejected.

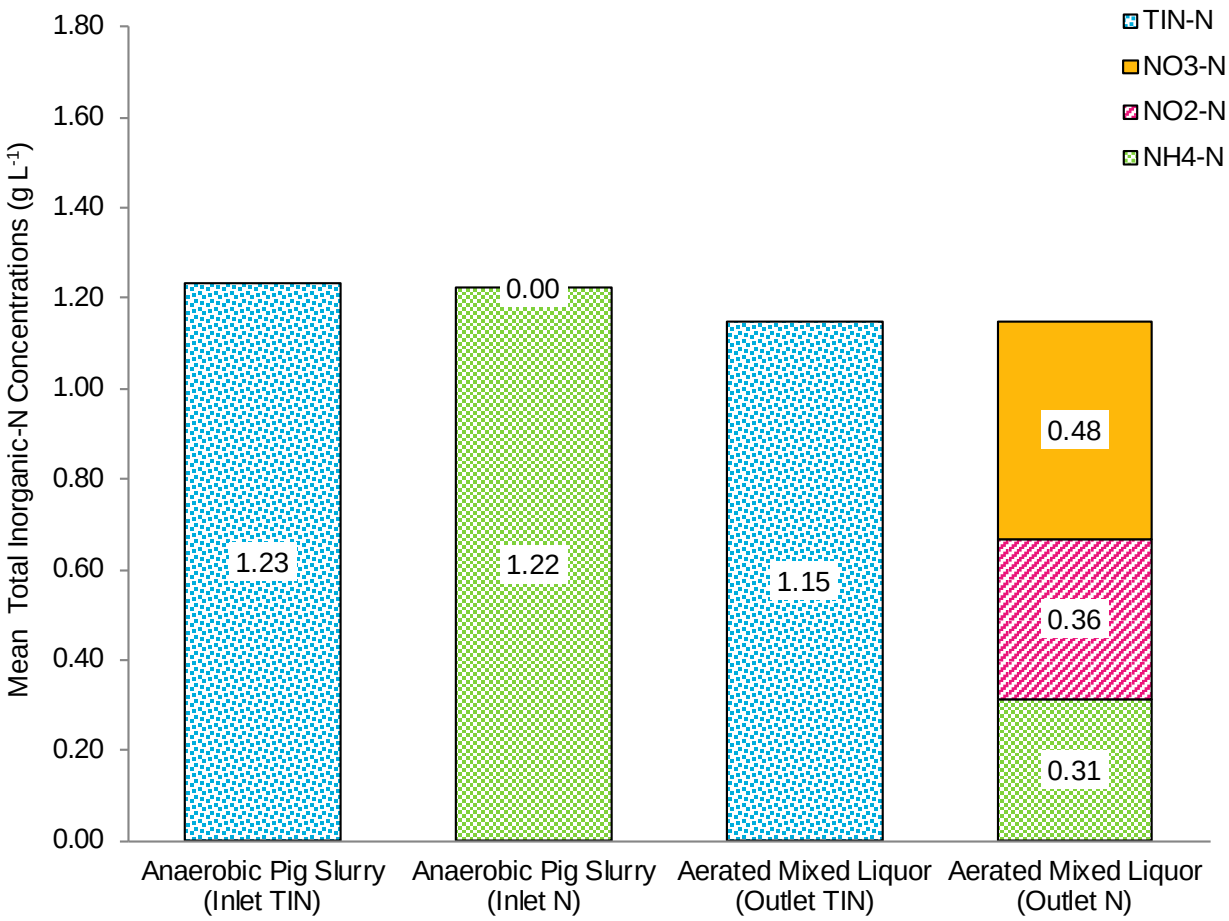

Figure 5.12: A total inorganic-N mass account acquired during the aeration of ANPS equipped with 20% RAS feedback for R4. DO was set to maintain 5.3 mg O_2 L^{-1} (~70% air saturation) and 7 d THRT. A mean DO concentration of 2.3 ± 2.0 mg O_2 L^{-1} and pH 7.6 ± 0.8 at 21.1 ± 0.8°C was observed over a 43 d treatment period.

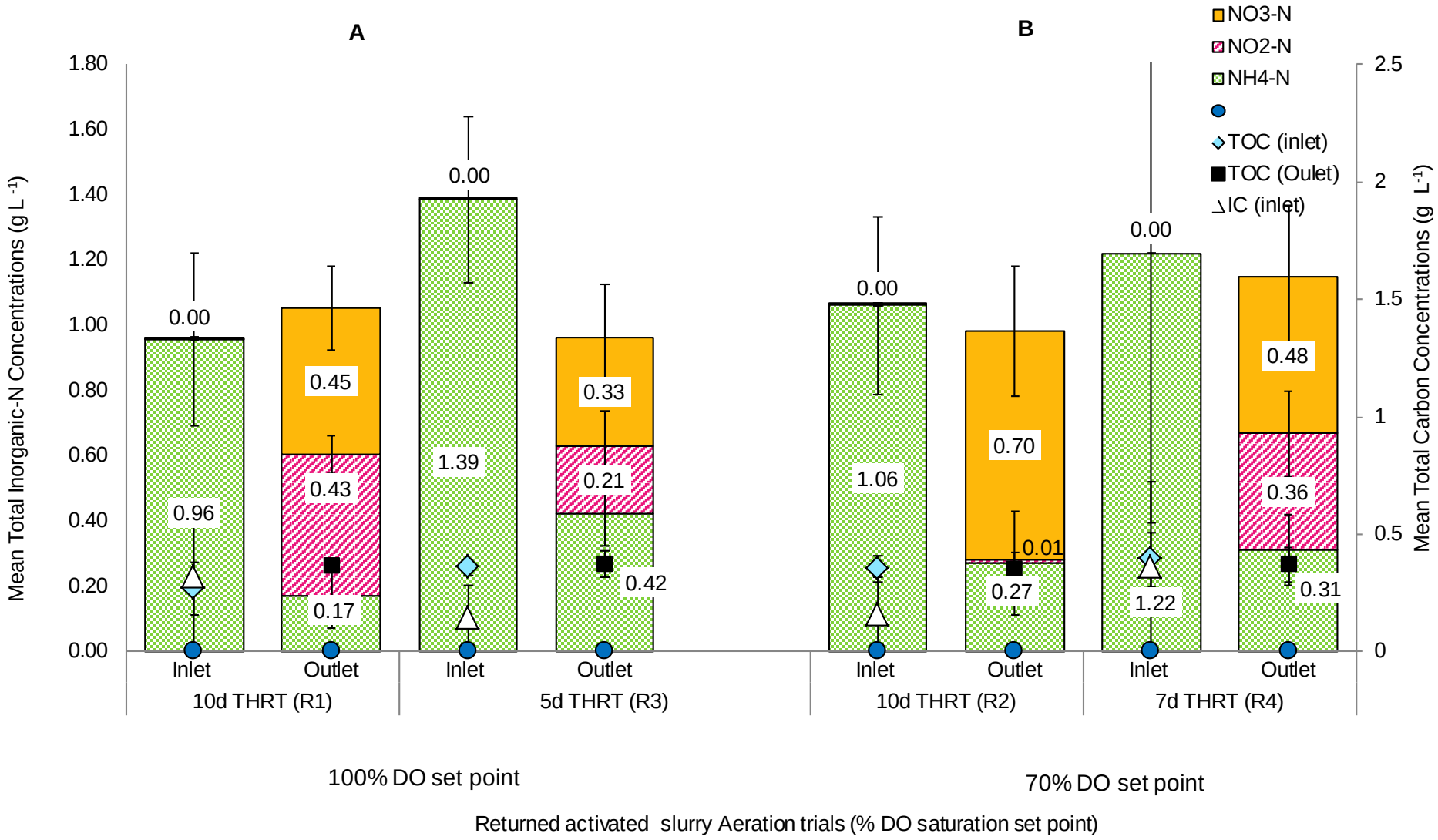

Figure 5.13: A comparison of inorganic-N and carbon concentrations measured in the ANPS before and after aeration with RAS feedback when operated at a) an uncontrolled DO set point up to 100% saturation with either a 10 d (R1) or 5 d (R3) THRT and b) at 70% saturation with either a 10 d (R2) or 7 d (R4) THRT

Nitrogen transformation – a comparison of RAS inclusion vs. no-RAS

Inclusion of RAS feedback and secondary clarification step yielded a significant improvement in nitrification with particular emphasis on NH_4^+-N removal and NO_3^--N accumulation compared to when no recycling was used (Figure 5.14). On average reduction was detected 6-17% greater in the trials with RAS feedback (R1 and R3) than without (ST4-ST5; Chapter 4.3.3) when the AWTS was set to aerate at an air saturation set point of 100%.

For instance, Figure 5.14a presents a comparison between trials R1 (at 6.4 ± 2.6 mg O_2 L^{-1}) and ST5 (at 5.1 ± 1.7 mg O_2 L^{-1}; Chapter 4.3.3) when run under the same operating parameters; an uncontrolled DO set point of 7.5 mg O_2 L^{-1} and 10 d THRT. Up to 76% NH_4^+-N was removed from 1.17 ± 0.09 to 0.28 ± 0.12 g L^{-1}, 44% and 23% oxidised to NO_2^--N (0.39 ± 0.04 g L^{-1}) and NO_3^--N (0.20 ± 0.11 g L^{-1}) during non-recycled treatment in ST5 (Figure 5.14a). Inclusion of a RAS feedback and secondary clarification increased this value by up to 6% in NH_4^+-N removal (82%) from 0.96 ± 0.26 to 0.17 ± 0.10 g L^{-1}) and 10% NO_3^--N accumulation (from <BLOD to 0.45 ± 0.13 g L^{-1}) (R1). The difference between R1and ST5 outlet N content was statistically significant for all quantifiable TIN fractions in the aerated mixed liquor ($p \leq 0.05$) (Figure 5.14a). The improved nitrification results seen in R1 were probably attributed to a greater nutrient load and nitrifying biomass density within the mixed liquor, a result of RAS. A comparison of the two inlet characteristics identified that although N levels were on average higher (1.22–1.38 times) in ST5 than R1, the difference in DO and quantifiable TIN fractions was not significant ($p \geq 0.05$).

A similar finding was evident in the comparisons between R3 (4.1 ± 1.9 mg O_2 L^{-1}) and ST4 (6.3 ± 1.1 mg O_2 L^{-1}) at a 5 d THRT when DO was set at 7.5 mg O_2 L^{-1} (100% saturation; uncontrolled). A 17% and 0.33 g L^{-1} difference in NH_4^+-N removal and NO_3^--N accumulation yields were noted between the two trials, with outlet NO_3^--N concentrations significantly higher in R3 ($p \leq 0.05$) (Figure 5.14b). Like those with a 10 d THRT, analyses of the two inlets characteristics found the differences between them to be not statistically significant, also despite R3 having a slightly higher TIN (1.03 times) and NH_4^+-N (1.04 times) content compared to ST4 (t (13) = 0.21, p = 0.84 and t (13) = 0.22, p = 0.83 ≥ 0.05, respectively).

Consequently, inclusion of a RAS feedback step imposed an improvement in NO_3^--N accumulation than when RAS was absent. Thus, there is sufficient evidence to reject the null hypothesis that incorporation of a recycled feedback step to the AWTS matrix would have no significant effect on inorganic-N transformations during nitrification to those without this addition when operated with a 10 d THRT and an uncontrolled DO set point of up to 100% saturation was thus rejected.

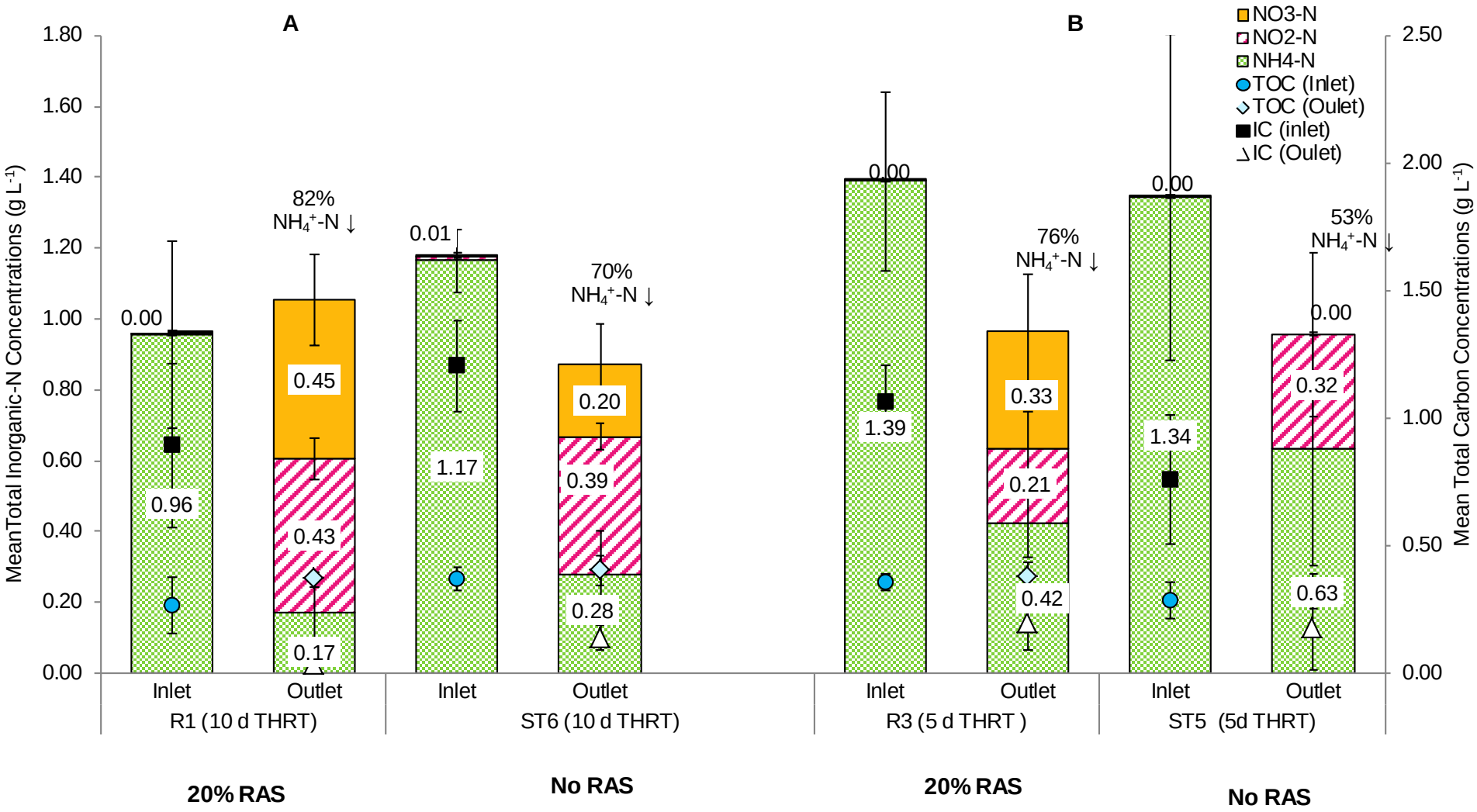

Figure 5.14; Effect of 20% RAS vs. No-RAS on mixed liquor inorganic N transformation (mean ±SD) before and after aeration at an air saturation level set to 100% and THRT of either a) 10 d (ST5 and R1 or b) 5 d (ST4 and R3). A significant difference in all inorganic-N fractions was detected before and after aeration.

The effect of RAS and operational parameters on slurry appearance

Aeration equipped with RAS feedback was shown to have also had a positive impact on slurry appearance, with particular emphasis on pigmentation and consistency. Of notable interest was the distinct colour change in mixed liquor pigmentation, which transitioned from dark taupe to orange following treatment. The orange pigmentation was observed more predominant in the slurries exhibiting greater nitrification, particularly in trials R1, R2, and R6. This coincided with the finding exhibited in ST4 and ST5 of Chapter 4.

5.3.4 Carbon

Mean inlet TC concentrations ranged between 1.16 ± 0.43 and 1.43 ± 0.47 g TC L^{-1}, comprised of 23-27% and 73-77% TOC and IC (Figure 5.15), respectively. Aerobic treatment coupled with RAS feedback and secondary clarification generated removals of up to 65-72% TC, 5.8% TOC (R4 only) and 82-91% IC. Statistical differences were identified between inlet and outlet C fractions for all trials ($p \leq 0.05$). TOC content of R2, R3, and R4 an exemption ($p \geq 0.05$) (Figure 5.15). This signifies that positive carbon oxidation had taken place under these treatments. Trends were similar to those derived in Chapter 4.3.4.

A reduction in mixed liquor NH_4^+-N coupled with the increase in NO_3^--N accumulation was apparent, a reduction of up to 97% IC was found for each treatment operated with a 10 d THRT (R1, R2, R5 and R6) , consumed as a consequence of nitrification) (Figure 5.15). Unlike results reported in Chapter 4.3.4 changes in DO set point values (1.5, 3.8, 5.3 and up to 7.5 mg O_2 L^{-1}) were shown to have no major impact on the amount of IC consumed following clarification when operated at a 10 d THRT. This was evident in trials R1, R2, R5, and R6 (Figure 5.14). No statistical significance was detected in the mean outlet IC content between the four trials at the 95% confidence interval (Kruskal Wallis Chi square (3) = 7.41, $p = 0.06 \geq 0.05$).

However, an increase (0.2-27%) in TOC post treatment was also noted in the four trials, and suggests that not all of the carbon oxidised was consumed by the nitrifiers. An additional oxygen demand was most likely present. The difference in outlet TOC across the trials was significant (Kruskal-Wallis chi square (3) = 36.50, $p \leq 0.001$). A post hoc pairwise comparison identified the

differences to be significant between trials; R1-R5 ($p \leq 0.001$), R1-R6 ($p \leq 0.001$), R2-R5 ($p \leq 0.001$) and R2-R6 ($p \leq 0.001$).

High IC consumption was also evident in the trials when the THRT was reduced from 10 d to either 5 d (R3; set to 100% air saturation) or 7 d (R4; set to 70% air saturation) (Figure 5.16).

Shortening the THRT from 10 d to 5 d, yielded IC consumption of up to 82% in R3 compared to the 96% achieved at the 10 d THRT (R1) as demonstrated in Figure 5.16a. A difference of 14%. Both trials were set at the 7.5 mg O_2 L^{-1} set point. This coincided with the slightly lower NH_4^+-N and NO_2^--N oxidation rates of R3 (Figure 13a). Carbon concentrations (before and after) were noted 1.20-6.57 times higher in R3 than R1 for each of the carbon fractions quantified. The higher carbon content significant for inlet TOC ($W = 3$, $p = 0.03$) and outlet TC ($W = 29$, $p \leq 0.001$) and IC ($W = 11$, $p \leq 0.001$).

Similar results were observed between trials R2 and R4 following a decrease in THRT from 10 d to 7 d (Figure 5.16b). Comparing the two carbon contents (before and after treatment at an air saturation level of 70%) found statistically, there was little difference between the two THRT despite concentrations measuring 0.96–2.67 times higher in R4 (10 d) ($p \geq 0.05$). Outlet IC the exception, with significantly less IC consumed when aerated with a 7 d THRT (R4; 93% from 1.04 ± 0.36 to 0.08 ± 0.06 g L^{-1}) than a 10 d THRT (R2; 97% from 1.00 ± 0.16 – 0.03 ± 0.02 g L^{-1}) ($W = 60$, $p = 0.02 \leq 0.05$). This was also shown to coincide with the lower (slightly) nitrification detected in R4 (Figure 16b). The findings suggests that THRT had a significant effect on IC,

These IC consumption trends were found consistent with that of the non-recycled trials of Chapter 4.3.3.2. Inclusion of a RAS feedback step imposed an improvement in IC consumption of 8-17% more with IC concentrations significantly lower in the trials with secondary clarification (R3 and R1) than those without (ST4 and ST5). Particularly when configured with similar operating parameters; DO set at 7.5 mg O_2 L^{-1} (uncontrolled) and a THRT of either 5 or 10 d, respectively ($p \leq 0.05$) (Figure 5.17).

This finding was also reflected in the higher nitrification rates observed (Figure 5.14). The

difference in mean carbon before and after aeration was shown to be significantly different for each of the quantified carbon fractions measured in the outlet between R1 and ST6 (10 d THRT) ($p \leq 0.05$), but not in the inlet concentrations ($p \geq 0.05$) according to an unpaired two sample t-test and Wilcoxon rank sum test. Significant differences were also noted in the outlet TC, IC and inlet TOC concentrations between R3 and ST5 when operated with a shorter THRT ($p \leq 0.05$).

The results presented in Figures 5.15-16 show that the TOC was recalcitrant and not readily degraded in any of the treatments. This suggests that the driver for nitrification was the IC initially present within the feed and any CO_2 dissolved in the slurry during aeration.

For both sets of experiments IC consumption was shown to increase both with increasing THRT and DO concentrations, as well as NO_3^--N accumulation. This suggests that there is a relationship between nitrification and IC consumption, i.e. higher NO_3^--N production resulted in more IC consumed.

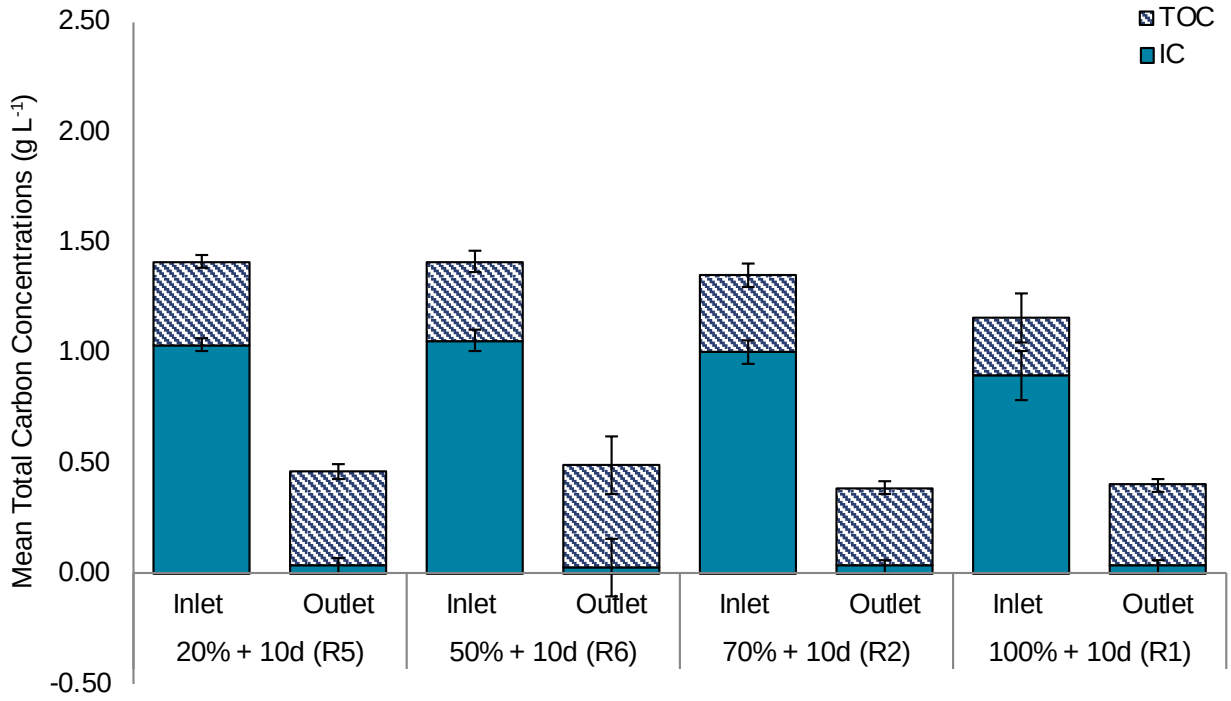

Figure 5.15: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before and after aerobic treatment with 20% RAS feedback for each of the four air saturation set points examined (20%, 50%, 70% and 100%) with a 10 d THRT (R1, R2, R5 and R6).

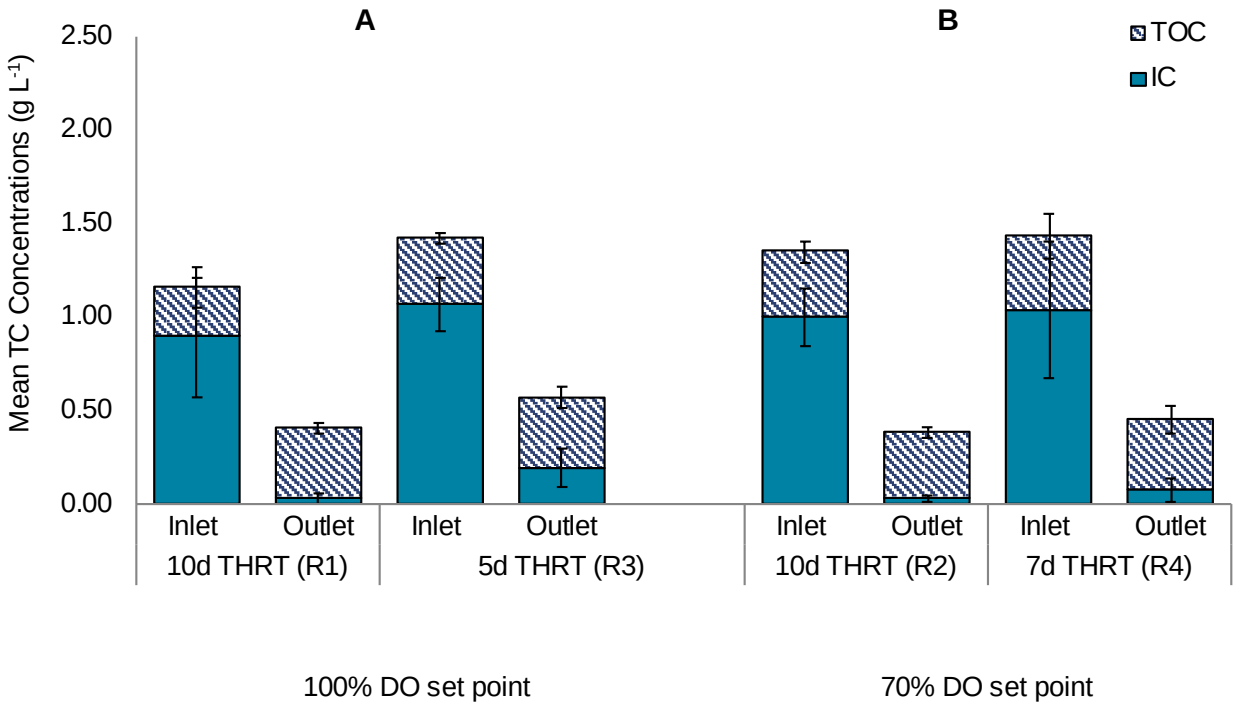

Figure 5.16: A balanced mass account of the total carbon levels (g L^{-1}) within the ANPS before and after aerobic treatment with 20% RAS feedback following a reduction in THRT from 10 d to either 5 d or 7 d at an aeration level set at a) 100% saturation (R1, R3) and b) 70% saturation (R2, R4) DO set points.

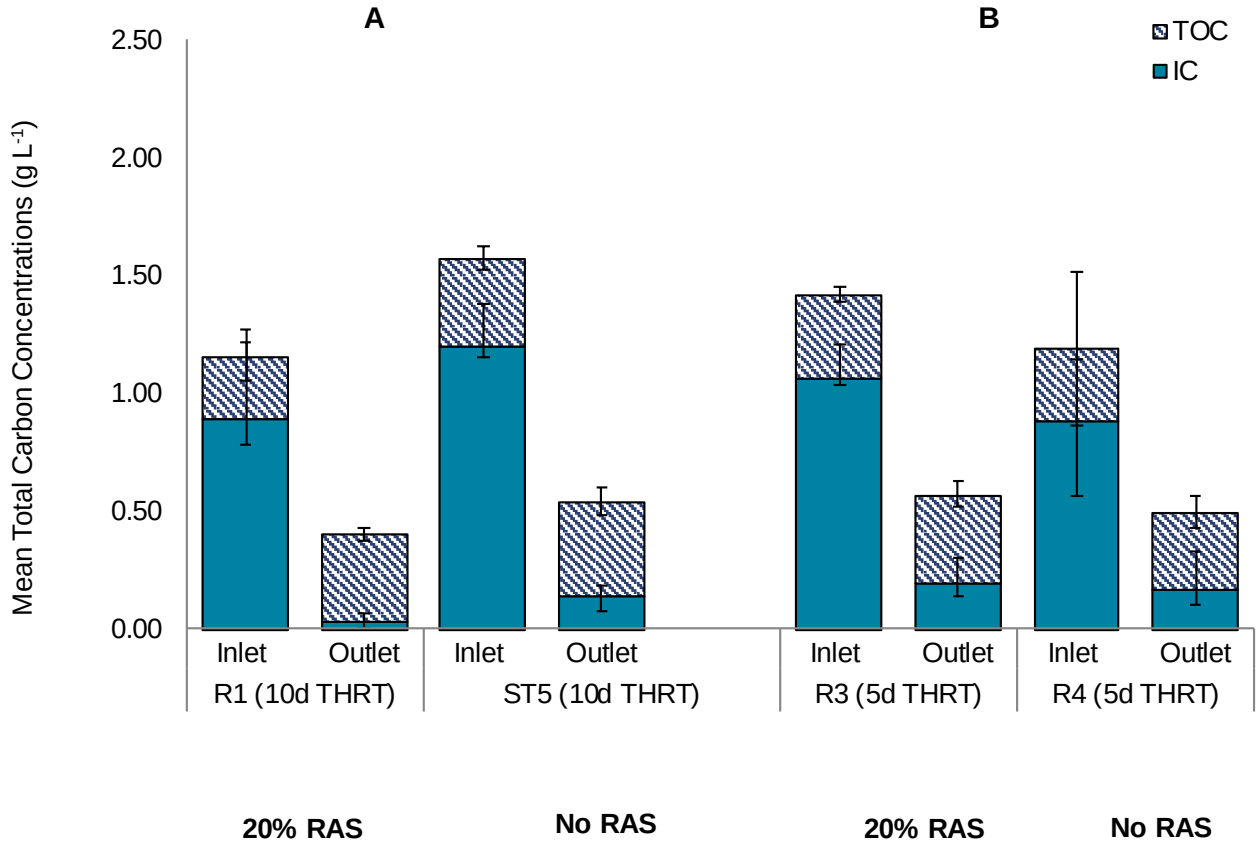

Figure 5.17: A comparison of the total carbon levels (g L^{-1}) within the ANPS before and after aerobic treatment with an aeration level set to a 100% saturation set point (7.5 mg O_2 L^{-1}) with or without RAS (20%) feedback and a THRT of a) 10 d (R1 and ST5) and b) 5 d (R3 and ST4).

5.4 Discussion

A clarified, recycled sludge feedback step was incorporated into the AWTS to further reduce the large nutrient load present in the mixed liquor and enhance the nitrification process. This chapter aimed to identify operating parameters best suited for nutrient removal and nitrification.

In Chapter 4, aeration of ANPS collected from the treatment lagoons, yielded an additional removal in TSS and NH_4^+ loads by a further (>) 50% and 20-76%, respectively under each of the operating parameters assessed (THRT and aeration levels). Operation found best when DO of 5.1 mg O_2 L^{-1} or more at a saturation level of 68-100% was maintained in combination with a 10 d THRT (ST5). Although a vast improvement in nutrient removal, nitrogen transformation struggled with partial or incomplete nitrification observed in each of the assessed treatment parameters (Figures 4.5-4.12).

5.4.1 Effect of RAS inclusion on suspended solid removal

Inlet (ANPS) SS concentrations ranged between 1.50 ± 0.62 to 2.82 ± 0.75 g L^{-1}. These concentrations were 1.04 to 3.48 times greater than the inlet TSS load reported in Chapter 4.3.2. Although collections were from the same site, variation in management strategies may have occurred between collection periods and could explain the slight deviation in slurry characteristics, as farms constantly look to update and improve practices (APL, 2015b, Buchanan *et al.*, 2013, Burton, 1992, Burton and Turner, 2003).

Moderate to high removal (TSS) rates (52-79%) were observed for all six operating regimes examined across the six RAS trials (R1-R6) performed; the highest (79%) reported for R1 (Section 5.3.2). These were remarkably similar to the removal rates (52-79% TSS) achieved in trials ST1-ST5 without the RAS treatment step (Chapter 4.3.2). Statistically, there was a significant improvement in TSS removal following incorporation of RAS (R1) when aerated at the same set parameters as those without for a 7.5 mg O_2 L^{-1} set point and a 10 d THRT (ST5) ($p > 0.05$), however this was not the case when a 5 d THRT was used). This could be in part due to a slight build-up of suspended solids in the ARV caused by the recirculation of up to 10% of the clarified solids back into the system (Gerardi, 2002).

Regardless, a distinctive change in slurry appearance was noted in each of the six RAS trials. This

was denoted both by a distinct colour change (more significant post filtration) from grey-brown to orange post aeration and by an increase in light transmission. Interestingly, as nitrification increased (See below 5.4.2) the treated mixed liquor took on a more distinct orange hue, more so than that observed in Chapter 4.4.1.

As noted by Tchobanoglous *et al.* (2003) and Bilotta and Brazier (2008) a high SS load would not only cause blockages in equipment and machinery but would inhibit necessary light penetration, giving the slurry a turbid appearance; a major issue when using ANPS for algal growth and disinfection (Buchanan *et al.*, 2013). The results from this study showed aeration with RAS reduced more than half of the TSS load present in the ANPS, in doing so improving the optical density of the mixed liquor. This should allow greater light penetration to occur, increasing the disinfection and growth capability (Bilotta and Brazier, 2008). The next phase of the integrated treatment would be to determine whether the mixed liquor now enables suffice light penetration and nutrient load for algal growth. This will be addressed in Chapter 6.

5.4.2 Effect of RAS inclusion on ammonia oxidation

Low nitrification rates were observed in all six aeration trials outlined in Chapter 4.3.3 (highest 0.20 g NO_3^--N L^{-1} accumulated, ST5). This could be in part due to denitrification processes or an insufficient nitrifying population in the mixed liquor, contributing to the partial or incomplete mass balance detected in at least five of the six trials (ST1-5) (Gerardi, 2002, Svoboda *et al.*, 2013, Svoboda and Evans, 1987). NH_4^+-N conditions, whilst improved may still be unfavourable for growth and reuse (Buchanan *et al.*, 2013, Matsudo *et al.*, 2009, Murphy, 2011, Svoboda *et al.*, 2013, Svoboda, 1995, Svoboda and Fallowfield, 1989). This highlights the importance of an adequate aeration regime. A RAS feedback process was included to the AWTS post aeration in the expectation it will increase the numbers and activity of nitrifiers within the mixed liquor and aid nitrification (Burton and Turner, 2003, Gerardi, 2002). Whilst, novel in the use of aerated ANPS, RAS processes have been shown successful for the treatment of domestic and industrial wastewaters, sewage, and animal slurries (Burton, 1992, Downing and Nere, 1964, Muller *et al.*, 1995, Seviour and Nielsen, 2010, Surmacz-Gorska *et al.*, 1996, Wittmann *et al.*, 1990).

Therefore, the second part of this investigation looked at the effect of RAS recirculation during aerobic treatment of ANPS on inorganic-N transformation concerning the oxidation of NH_4^+-N to NO_3^--N; a continuation study of the aeration protocols performed in Chapter 4.3.3.

Chapter 4.3.3 showed aeration to remove up to 20-76% of inlet (ANPS) NH_4^+-N, when applied to ANPS at a laboratory scale; controlled at various aeration regimes (aeration levels ≥ 10% saturation and THRT ≥ 5 d) (Figures 4.5-4.112). The highest (76%) NH_4^+ reduction yield occurred at a mean DO concentration of 5.1 ± 1.7 mg O_2 L^{-1} (7.5 mg O_2 L^{-1} set point) and a 10 d THRT in ST5 (Figure 4.11). Whilst, all trials were successful in the oxidation of inlet NH_4 to NO_2 (3-33%), only two carried out the oxidation process to NO_3^- (3and 17% in ST1, and ST5, respectively) (Figures 4.8-4.14). The highest NO_3^--N concentrations detected in ST5 (Figure 4.10).

Inclusion of a RAS clarification step post treatment was examined over six trials at various aeration regimes, to enhance this oxidative process. Quantitative changes in inorganic-N concentrations were measured in response to the incorporation of RAS, with all trials showing a significant increase in nitrification processes, with 75-86% of the inlet NH_4^+-N removed (Figures 5.6-5.13). In fact, mixed liquor (Outlet) NH_4^+-N concentrations were 1.18–3.75 times lower with RAS (Figures 5.6-5.14) than in the non-recycled trials of Chapter 4.3.3 when aerated at the same parameter (Figures 4.5-4.12).

Interestingly, that whilst 75-86% of the inlet NH_4^+-N was reduced across the six RAS trials, on average, it is the potential improvement in NO_3^--N accumulation that is of particular interest. In the non-recycled trials accumulation values of <BLOD–0.29 g NO_3^--N L^{-1} were obtained (Figures 4.5-4.12). These were considered low in comparison and likely the result of incomplete or partial nitrification through deficient nitrifying populations, denitrification, ammonium volatilisation (Buchanan *et al.*, 2013, Gerardi, 2002). One of the aims of the research is to conserve the N in the slurry for algal growth so that loss of ammonia by volatilisation is not desirable, hence the focus on conserving N as NO_3^--N. Returning 20% of the activated biomass (R1-R6) yielded NO_3^--N accumulations1.7–3.5 times higher than with just aeration (ST1-6): an improvement of 7-91%. This suggests a sufficient active nitrifying population was present in the mixed liquor (Gerardi, 2002,

Seviour and Nielsen, 2010). Findings from this research support the inclusion of a RAS feedback step to the AWTS.

The effect of RAS inclusion on ammonium oxidation at the different aeration levels

Since nitrifying populations are known to respond to environmental changes (i.e. DO, pH, temperature, THRT), establishing optimal operational parameters (air saturation and THRT) to maintain and run a successful AWTS is key to implementation (Buchanan *et al.*, 2013, Burton, 1992, Cumby, 1987a, Evans *et al.*, 1986, Hanaki *et al.*, 1990, Svoboda *et al.*, 2013, Svoboda, 1995).

The previous study (Chapter 4.3.3) examined NH_4 oxidation under four parameter sets comprised of three DO set points (0.8, 1.5, and up to 7.5 mg O_2 L^{-1}) and two THRTs (5 and 10 d). The findings denote a link between an increase in NH_4^+-N oxidation and increased DO concentrations when operated with a 5 d THRT (recommended minimum time required to oxidise NH_4^+ to NO_3^-) (ST1-ST4) (Evans *et al.*, 1986, Svoboda *et al.*, 2013, Svoboda, 1995). NH_4^+-N concentrations decreased post-treatment by 20-29%, 53% and 55% at the 3 set point values assessed, respectively with greater concentration decreases with increasing DO concentrations (Figures 4.5-10). The same however, could not be said concerning NO_3 accumulation, levels remained relatively low despite an increase in available DO. Insufficient nitrifying population due to the short THRT is a suggested cause (Evans *et al.*, 1986, Svoboda *et al.*, 2013, Svoboda, 1995). Extension of the mean THRT from 5 d to 10 d showed aeration at an uncontrolled DO set point 7.5 mg O_2 L^{-1} (~100% saturation; ST5) was considered the more suitable though, the improvement in NO_3 levels were still regarded as relatively low (Evans *et al.*, 1986, Svoboda *et al.*, 2013, Svoboda, 1995). This was based upon the findings of Chapter 4, with 76% of inlet NH_4^+-N removed, 17% oxidised to NO_3^-N (0.20 ± 0.11 g NO_3^-- N L^{-1}) in the mixed liquor (Figure 4.14).

Repetition to include a return of 20% clarified RAS in R1, demonstrated a 6% and 56% increase in NH_4^+-N removal (an 82% removal rate from 0.96 ± 0.26 g NH_4^+-N L^{-1} in the inlet to 0.17 ± 0.10 g NH_4^+-N L^{-1} in the outlet) and NO_3-N accumulation (0.45 g NO_3^--N L^{-1} in outlet mixed liquor) (Figure 5.6). Stipulating that at a high aeration level greater nitrification is shown (Gerardi, 2002).

However, one of the major concerns with the aeration of animal slurries is the high-energy cost associated with running this technology (Burton, 1992, Burton and Farrent, 1998, Evans *et al.*, 1986, Zhang and Zhu, 2005). Energy consumption and requirements although not addressed in this investigation, is an important aspect to keep in mind when designing and implementing an aerating system (Buchanan *et al.*, 2013, Evans *et al.*, 1986). Regardless, techniques designed at reducing running expenses at the same time accomplishing required treatment goals are essential for optimal use and greater employment of this technology according to Burton and Farrent (1998) and Evans *et al.* (1986), which need to be taken into consideration in order to make treatment more desirable and practical for farm use.

Based on this, aeration at 100% saturation, whilst effective in relation to NH_4 oxidation, may not be practical due to continued aerator use depending on the desired outcomes (Buchanan *et al.*, 2013, Burton and Farrent, 1998, Evans *et al.*, 1986, Gerardi, 2002). One avenue assessed, was to reduce saturation levels to below <100% saturation. Care had to be taken, to prevent potential population losses associated with a rapid decline in DO; therefore, concentrations were reduced by 20–30% at a time. High (70-100%), moderate (50%) and low (20%) saturation levels assessed when a 10 d THRT was maintained.

A drop in DO to ~70% saturation (5.3 mg O_2 L^{-1} set point) exhibited results similar to those at 100% saturation (7.5 mg O_2 L^{-1} set point; R1), yielding a reduction in inlet NH_4^+-N of 75% ($p \leq 0.05$); 7% lower than observed at 100% saturation ($p \leq 0.05$) (Figure 5.10). Of notable interest, was that 68% inlet NH_4^+-N was oxidised to NO_2^--N L^{-1} (2%) and NO_3^--N L^{-1} (66%; 0.70 ± 0.20 g NO_3^--N L^{-1}) in the mixed liquor (Figure 5.6). Clear evidence of complete nitrification, with majority of the oxidised NH_4^+ converted to NO_3^- (Gerardi, 2002), with nitrification found greatest during this period making it an ideal candidate for on-farm adoption. Not only did this exceed the NO_3^--N accumulated at 100% saturation by 1.6 times, but exceeded all trials run with and without RAS by 1.4 - 2.1 and 3.5 – 175.3 times, respectively regardless of saturation level ($p \leq 0.05$).

Lowering DO levels by a further 20% to maintain 2.8 ± 1.1 mg O_2 L^{-1} at approximately 50% saturation (3.8 mg O_2 L^{-1} set point) in R6, yielded mixed liquor NO_3^--N levels (0.51 ± 0.16 g L^{-1})

slightly lower than observed at 70% saturation by 1.4 times ($p \leq 0.05$). This was considered the next best nitration rate in terms of NO_3 accumulation. Although a slight drop in NO_3^--N levels was detected, almost 90% of inlet NH_4^+ was removed; the highest removal rate detected across all 12 aeration trials performed in this study and in Chapter 4.3.3 (Figures 4.14, 5.12 5.14 and 4.17). However, only 33% was oxidised to NO_2^--N (4%) and NO_3^--N (29%), the remaining 67% remained as NH_4^+-N (10%), organic-N or volatilised.

Finally the evaluation of inorganic-N transformations at low air saturation of 1.3 ± 0.5 mg O_2 L^{-1} (~20% saturation and 10 d THRT), showed an 80% NH_4^+-N (inlet) removal rate, 57% oxidised to NO_2^--N (24%) and NO_3^--N (33%), respectively (Figure 5.9). Outlet NO_3^--N (0.37 ± 0.09) were slightly lower than at either the moderate (50%; R6) or moderate-high (70% (R2), up to 100% (R1)) saturation levels by 1.4, 1.6 and 1.2 times (Figure 5.10). Suggesting that although some nitrification had taken place, DO levels may not be as sufficient as those with a higher percent of available DO present in the mixed liquor (Evans *et al.*, 1986). Nevertheless, RAS addition (R5) presented an increase in NO_3^--N accumulation 95-99% greater than either of the non-recycled trials of Chapter 4.3.3, when aerated at DO levels equivalent to 20% saturation and a 5 d THRT. Although, aerated at a slightly higher THRT to that of ST3 (Chapter 4.3.3), this finding provides further evidence to support the need to include a RAS clarification step to the treatment of ANPS.

Overall, the transformation of inorganic-N fractions was significantly affected by aeration rates. This coincided with the findings of Evans *et al.* (1986) using raw pig slurry. It is therefore feasible to assume that DO concentrations maintained at 20%, 50%, 70%, and 100% saturation is sufficient to satisfy the oxygen requirements for nitrification when combined with a 10 d THRT and 20% RAS recirculation.

The effect of RAS inclusion on ammonium oxidation at different THRTs

As demonstrated by Belser (1979), Evans *et al.* (1986), Svoboda *et al.* (2013), Svoboda (1995) THRT can have a significant effect on inorganic-N transformation during aerobic treatment. Therefore, it is imperative to have a balance between DO levels, THRT and nitrifier regeneration times (Belser, 1979, Gerardi, 2002).

This should be taken into consideration when optimising an aeration system, with the aim to get the largest amount of effluent treated in the shortest practical time possible to offset potential ongoing operational costs (Burton, 1992, Burton and Farrent, 1998, Evans *et al.*, 1986, Zhang and Zhu, 2005). For instance, when operated at a shorter THRT more slurry can be processed per unit time, which could potentially reduce ongoing operational and capital costs associated with operation over this time, as the required tanks size would be smaller (Burton, 1992, Burton and Farrent, 1998, Evans *et al.*, 1986, Zhang and Zhu, 2005). The opposite would be true when a longer THRT is used as the associated capital and ongoing costs would in theory be higher to accommodate for the larger tank size and storage requirements (Evans *et al.*, 1979, Evans *et al.*, 1986, Svoboda *et al.*, 2013). Although the current 10 d THRT lies within the recommended 2-14 d treatment range outlined by Evans *et al.* (1986), treatment towards the lower end of this range if possible would be preferable in terms of cost. As such, inorganic-N transformations were assessed at three THRT; 10 d, 5 d and 7 d.

At 10 d (THRT) 75-90% of NH_4^+-N was removed, 32-89% oxidised to NO_3^--N in the mixed liquor (R1, R2, R5 and R6) when operated at DO set point values of >7.5, 5.3, 1.5 and 3.8 mg O_2 L^{-1} (Figure 5.10). Shortening mixed liquor THRT from 10 d to 5 d at a DO set point of 7.5 mg O_2 L^{-1} (4.1 ± 1.9 mg O_2 L^{-1} maintained), saw a significant reduction (23%) in inorganic-N transformations, with 12% less NH_4^--N removed and 23% less NO_3^--N accumulated in the mixed liquor than at the longer THRT (Figure 5.13). This suggests that in this laboratory system, THRTs of 5 d were less effective in terms of nitrification than if retained over a longer period. This coincided with the findings of Chapter 4.3.3. Regardless, a significant improvement in nitrification was observed at this aeration regime (5 d THRT; ~100% air saturation) when RAS was used (R3) than when just aeration was used (ST4).Mixed liquor NO_3^--N concentrations were detected 0.33 g L^{-1} higher with RAS (0.33 g NO_3 L^{-1}; R3) than without (<BLOD g NO_3^--N L^{-1}; ST4).

At 7 d, NH_4^+-N removal was highest (75%) at a DO set point of 5.3 mg O_2 L^{-1} (~70% saturation) with 5% greater removal than achieved at 5 d (100% air saturation). However, no real improvement in nitrification rates were observed to those at 10 d when DO was set to maintain a 5.3 mg O_2 L^{-1} set point (~70% saturation). In fact, NO_3^--N concentrations were lower by almost one third.

Based on the findings from this research, shortening the slurry THRT to try to offset operational costs of the AWTS, offered no real improvement in nitrogen transformations, in fact nitrification activity was detected lower in each case. It could be that a slight population washout may have occurred, in spite of recirculation (Gerardi, 2002, 2011). Nevertheless, a RAS feedback step should be included into the AWTS design, with nitrification improved in each case with aeration found optimal when operated with a 10 d THRT and air saturation greater than 3.4 ± 1.5 mg O_2 L^{-1}.

5.4.3 Effect of RAS inclusion on carbon mass balance

Quantitative changes in carbon concentrations were also, monitored in the mixed liquor during the aeration of ANPS. A balanced TC mass account was, identified in each of the six RAS trials regardless of aeration regime used (Figures 5.15-5.17). This was evident in both inlet and mixed liquor carbon loads. IC losses of 82-97% were observed in all six of the RAS most likely consumed through nitrification processes (Figure 5.19).

During the activated sludge process, nitrifying bacteria consume IC as a carbon source required for growth and reproduction (Gerardi, 2002, 2011). A link between increased nitrification levels and IC consumption has been reported in the literature (Buchanan *et al.*, 2013, Burton, 1992, Gerardi, 2002, Guisasola *et al.*, 2007). This could explain the 82– 97% drop in IC observed in the current study across the different treatment setups (R1-R6) (Figure 5.19). The highest removal, 97% detected at 50% and 70% air saturation level when combined with at a THRT of 10 (R6 and R2, respectively). Interestingly, the fall in IC was shown to continue in association with the raise in NO_3^- accumulates, particularly at moderate aeration (50% and 100% saturation) and a 10 d THRT. This same pattern was observed in Chapter 4.3.4 at 100% aeration and a 10 d THRT. Nevertheless, outlet IC was detected 1.3 - 30.3 times lower overall when RAS was used with 1.1 - 2.3 more IC oxidised than in the trials with just aeration (Chapter 4). This was consistent with the higher nitrification rates observed.

5.4.4 Future research

Once a substantial reduction in both sunlight absorbing solids and NH_4^+-N concentrations were obtained and ideal operating conditions to drive nitrification in the AWTS identified, the next step in

the proposed integrated treatment system is to convert the slurry nutrients to biomass (microalgae) in a high rate algal pond (HRAP), for further disinfection and reuse (Buchanan *et al.*, 2013). However, prior to use in a HRAP, potential algal growth on the pre-conditioned mixed liquor needs to be determined. This will determine whether the slurry composition (i.e. SS and N concentrations) is favourable for growth or if further treatment is required. This will be examined in more detail in Chapter 6.

5.5 Conclusion

Aeration of ANPS was proven successful in the removal of toxic NH_4^+ and SS concentrations. Low nitrification rates, however highlighted the need for an additional treatment component, before the slurry is suitable for algal growth and reuse: recirculation of a clarified biomass, an apt solution. The current study clearly identified a significant improvement in nitrification upon inclusion, with greater NH_4^+-N to NO_3^--N conversions noted.

Like, in all treatment system designs operation parameters need to be managed for optimal use. The aeration regimes assessed in the current study altered the SS and NH_4^+ load of the treated ANPS significantly; both showed removal yields of >50% and 70%, respectively. If the removal of SS and NH_4^+-N are the main treatment objective of interest then aeration at any of the examined aeration regimes could be considered suitable in spite of statistical differences.

Whilst removal and NO_3^- accumulation was found at each combination, DO concentrations of greater than 3.4 mg O_2 L^{-1} and a saturation level of 45-70%, saturation is generally recommended in combination with 20% RAS and a THRT of 10 d based on the findings of this investigation.

The outcomes of these experiments can be applied to assist in the upscale from bench-top to pilot scale as a pre-conditioning step prior to use in a HRAP. However, before use in a HRAP, more research is required to establish whether the mixed liquor components are suitable for algal biomass production.

6. ALGAL GROWTH ON AERATED ANPS

6.1 Introduction

Since the over-arching aim of the research is to improve the sustainability of pig slurry as a renewable resource, the next phase of the integrated AWTS proposed by Buchanan *et al.* (2013) is the inclusion of a HRAP post aeration. HRAPs are shallow (usually 0.2–1 m depths) treatment ponds (Craggs *et al.*, 2011, Fallowfield, 2013, Park *et al.*, 2011). They are often in a raceway configuration, and are capable of achieving elevated nitrogen and phosphorous removal during the treatment of domestic, agricultural (including animal slurries) and industrial wastewaters (Fallowfield and Garrett, 1985b, Fallowfield *et al.*, 1999, Green *et al.*, 1996, Park *et al.*, 2011).

According to Green *et al.* (1996), Park *et al.* (2011) and Buchanan (2014) HRAP accomplish a higher degree of disinfection in a fraction of the time, due to the shorter retention times required and higher DO production. Nutrient removal in HRAPs occurs predominately, through assimilation during microalgae production in the mixed liquor, a by–product, that can be influenced and controlled by algal growth parameters (i.e. pH, light availability THRT, temperature, solar radiation) (Fallowfield *et al.*, 1999, Garcia *et al.*, 1992, Norsker *et al.*, 2011, Park *et al.*, 2011). Algal growth is not only important in the treatment of wastewaters, but successive harvesting of algal biomass can help to not only recover nutrients from wastewater, but offers a number of benefits through reuse (Gerchman *et al.*, 2017, Gutiérrez *et al.*, 2015, Park *et al.*, 2011). For instance, the end products would enable biomass energy production, improved water quality, reduced carbon output, and feed stocks, which could help lower the industry's greenhouse gas emissions and carbon footprint (Benemann, 2013, Borowitzka and Moheimani, 2013, Buchanan *et al.*, 2013, Fallowfield, 2013, Fallowfield *et al.*, 1999, Gerchman *et al.*, 2017, Gutiérrez *et al.*, 2015).

While the inclusion of a HRAP to treat piggery slurries is not new nor the possible use of the resulting biomass to produce biofuels, most of the work done in this area predominately used raw or diluted slurries (Borowitzka and Moheimani, 2013, Fallowfield and Garrett, 1985b, Fallowfield *et al.*, 1999, Kebede-Westhead *et al.*, 2006, Svoboda and Fallowfield, 1989). Dilution was often required due to the high opacity and nutrient load of the slurry, both of which are known to inhibit

growth (Fallowfield, 2013, Fallowfield and Garrett, 1985a). Hence, in order to accommodate the heavy dilution needed to reduce NH_4 inhibition and increase light penetration the HRAP would need to be of substantial size and require an excessive amount of fresh water (Fallowfield, 2013, Fallowfield and Garrett, 1985a). This would be both an impractical and costly exercise according to Fallowfield (2013), not to mention the strain on natural resources, particularly in arid regions like Australia; where fresh water supplies are scarce. Thereupon, dilution is not generally recommended, and should be avoided where possible (Fallowfield et al 2013).

Svoboda and Fallowfield (1989) indicated that an anaerobic or aerobic pre-treatment of piggery effluent prior to use in a HRAP could lower the high SS and NH_3 content, in doing so required light penetration needed for biomass production is improved. At present an anaerobic pre-treatment step in the form of anaerobic lagoons is already in place in many Australian piggeries (78%) (APL, 2015b, Buchanan *et al.*, 2013). Unfortunately, slurry characteristic (i.e. SS, NH_3) post-treatment are still considered unfavourable for HRAP application (Brown, 1984, Fallowfield, 2013). These include light limitations and NH_3 toxicity (Fallowfield, 2013, Park *et al.*, 2011, Sutherland *et al.*, 2015b).

For instance, any particulate matter in the slurry can cause both the depletion of DO concentrations and the attenuation of light particles in the mixed liquor, restricting the amount of light available for photosynthesis and DO generation (Bilotta and Brazier, 2008, Grobbelaar, 2010, Sutherland *et al.*, 2014, Sutherland *et al.*, 2015b). Whilst, NH_4 is the preferred nitrogen source for microalgae production, it is toxic at high concentrations (> 42 mg L^{-1}) exasperated by high pH, such that any algal growth with a high NH_4^+ concentration would shift the equilibrium towards NH_3 which is toxic (Chaiklahan *et al.*, 2010, Crofts, 1966, Matsudo *et al.*, 2009, Yuan *et al.*, 2011).

Therefore, the current investigation focussed on SS and nutrient removal from ANPS after undergoing consecutive aerobic treatment at different aeration regimes in a model system. Aeration was shown to reduce the SS and NH_4 content by a further (>) 51-79% and 24-76%, (Chapter 4), with 52-79% and 70-90% removed respectively when a RAS feed step was incorporated to the aeration regime (Chapters 5). Both displayed a significant change in slurry appearance and nutrient load, a vast improvement.

However, before HRAP inclusion, it is vital to first ascertain whether the slurry composition is indeed suitable for microalgae production. This will be examined throughout this chapter at a laboratory scale. Outcomes from this research will help to establish the suitability of aerated ANPS for biomass production and HRAP application that will assist in the generation of biofuels down the track. The particular aims of the current study were:

- To determine the growth potential of microalgae in undiluted piggery slurry undergone consecutive anaerobic-aerobic (with clarification) pre-treatment.

It was, hypothesised that the nutrient content of aerated ANPS would not be suitable for biomass production and thus algal growth would not occur in the aerated ANPS.

6.2 Methods

6.2.1 Algal growth experiments

6.2.1.1 Experimental set up - Slurry and inocula

To establish condition suitability for HRAP use, algal growth experiments were conducted using the pre-conditioned mixed liquor of trial R1 (aerated at 6.4 ± 2.6 mg O_2 L^{-1} and a 10 d THRT with 20% RAS) inoculated (10% algae: 90% slurry) with wastewater from a HRAP. Mixed liquor from trial R1 was selected based on nitrification performance and supply availability in order to obtain an understanding as to whether the nutrient load within the slurry is suffice for algal growth. Wastewater inocula were obtained from the HRAP located at Kingston on Murray (KoM), South Australia (E 140°20' S34°14).

Growth was assessed in 12, 1 L glass Erlenmeyer flasks containing 350 mL of mixed liquor for both treatment (mixed liquor inoculated with wastewater from KoM HRAP) and control (mixed liquor, only) samples. Each flask represented a replicate sample with six replicates per treatment group (Plate 6.1). Algal growth experiments were conducted in an incubated shaker (Innova® 44 Incubator Shaker series) set at 24℃ under continuous fluorescent light (Sylvania GRO-LUX® F15T8/GRO/AQ, 15W, 26.5 µmol m^{-2} s^{-1} irradiance) and a shaker speed of 100 rpm for 24 hours over a period of 28 days. In an attempt to reduce evaporative loss cling wrap was placed over the top of each flask with a small hole (s) pierced through to enable gas exchange.

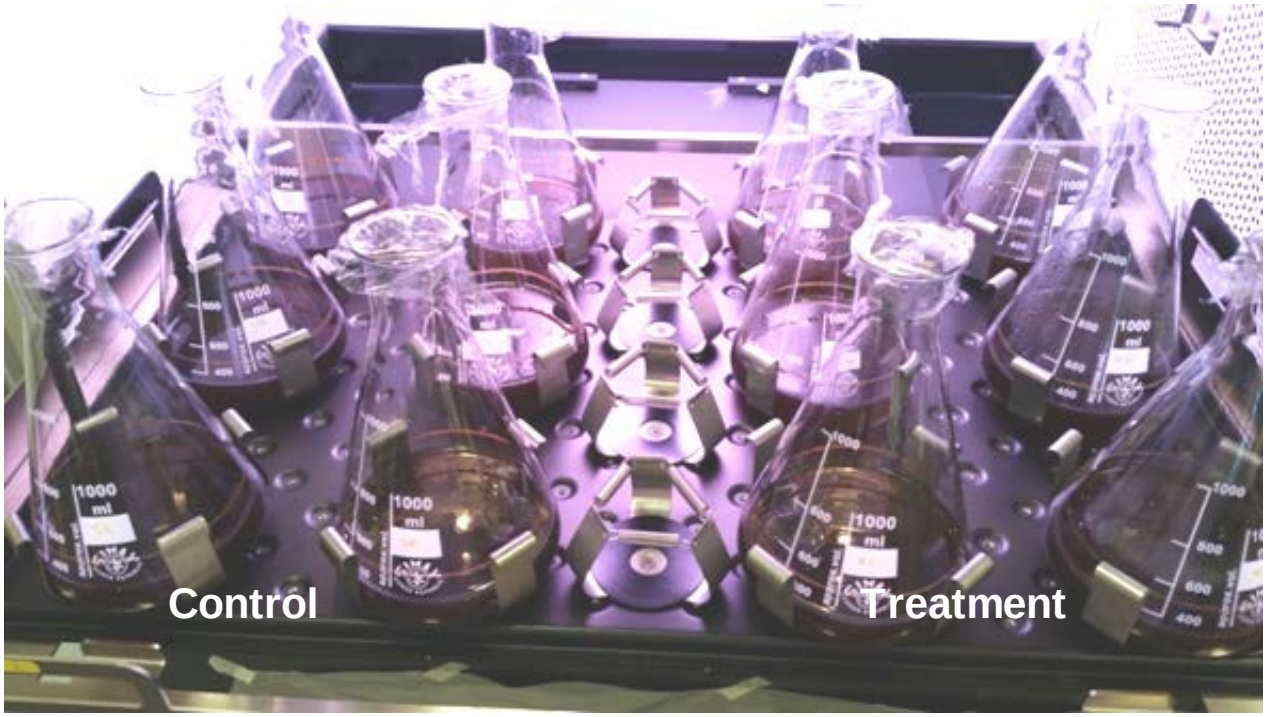

Plate 6.1: Algal growth in mixed liquor samples for the non-inoculated control (left) and inoculated (KOM HRAP water) treatment (Right) samples.

6.2.2 Sampling

50 mL random sample was collected every 3-4 d from both the treatment and control slurry flasks via glass pipette. Samples were either analysed within half an hour of collection or stored frozen (at -20°C, filtered) until required.

6.2.3 Water quality analyses

Analyses were performed on control and inoculated mixed liquor samples every 3-4 d for TSS (25 mL per filtrate), turbidity, chlorophyll *a* (Chl *a*), DO, pH, temperature, nitrogen (TIN, NH_4^+-N, NO_2^--N, and NO_3^--N), and carbon (TC, TOC, and IC) as described in Chapters 2.8, 6.2.3.1 and 6.2.3.2 with modifications (APHA, 1992). Values are presented as the mean ± SD in g L^{-1} of each analysis per collection day, tabulated and resultant graphs plotted using Microsoft Excel (2007).

6.2.3.1 Chlorophyll a

Chlorophyll *a* (Chl *a*) was used as an indicator for algal growth. 25 mL aliquots of the respective wastewater samples were vacuum filtered through 47 mm diameter GFC filter paper discs (GFC; LabServ, LBSOGF 090, Australia). The filters were then folded into eights and placed into 50 mL

capped (with rubber insert) glass McCartney bottles containing 10 mL of acetone (90%) ensuring the papers were fully submerged. To enable colour to develop samples were incubated in total darkness at 4°C for 24 h (APHA, 1992). 1 mL of the acetone extract was then transferred to a glass micro-cuvette and the absorbance read in triplicate at 664, 647 and 630 nm via a Shimadzu UV probe spectrophotometer (UV-1800, USA) against an acetone (90%) blank. Chl *a* concentrations were determined as µg L^{-1} using Equations 6.1 and 6.2 (APHA, 1992). Values were then converted to mg L^{-1}.

$$\boldsymbol{Abs_{Chl\ a} = 11.85\ (OD_{664}) - 1.54\ (OD_{647}) - 0.08\ (OD_{630})}$$ .....**Equation 6.1**

Where;

Abs $_{Chl\ a}$ = Chlorophyll *a* absorbance

OD_{664} = absorbance at 664 nm

OD_{647} = absorbance at 647 nm

OD_{630} = absorbance at 630 nm

$$\boldsymbol{C_{Chl\ a}(\mu g L^{-1}) = Abs_{Chl\ a} \times (\frac{acetone volume(mL)}{sample volume(L)})}$$ .....**Equation 6.2**

6.2.3.2 Turbidity

Turbidity (NTU) was measured regularly in the respective wastewater samples (25 mL) at 450 nm using a HACH DR2000 Spectrophotometer. Samples were diluted 1:10 (2.5 mL sample: 22.5 mL RO water) to fit within the testable range.

6.2.4 Statistical Analysis

Data were analysed for statistical significance according to the protocols described in Chapter 2.6.

6.3 Results

The ANPS was pre-treated by combined aeration-clarification (Chapter 5; trial R1) with the aim of reducing the high SS and NH_4^+ loads for use in a HRAP. Establishment of an algal biomass on the treated mixed liquor was examined in 12 flasks over a 28 d period. Each flask contained identical volumes and incubation conditions; six were inoculated with algal rich wastewater from a HRAP while the other six served as a control (no inoculation).The KOM inocula had a mean Chl *a* concentration of 1.31 ± 0.06 mg L^{-1}. Values (mean ± SD) of the collated biological and physical parameters are summarised in Table 6.1 and the relationship correlations for each parameter are outlined in Tables 6.2 and 6.3 for both control and inoculated samples. During this period, temperature of the mixed liquor at the time of sampling varied from 18–21.8°C in both the treatment groups. Mean DO and pH were observed slightly lower in the inoculated sample than in the control by 1.0-1.3 times (Table 6.1). However, the differences were not statistically significant, between the treatment and control for either DO (t (56) = 1.00, p = 0.32 ≥ 0.05) or pH (t (56) = 1.63 p = 0.11 ≥ 0.05).

Table 6:1: Average environmental parameters measured in the inoculated (treatment) and non-inoculated (control) mixed liquor from trial R1 (Chapter 5) incubated under continuous light over a 28 d period to assess biomass production.

Mean (± SD)	Control (n = 28)	Treatment (n = 30)
Temperature (°C)	20.2 ± 1.0	20.8 ± 0.9
pH	5.1 ± 0.7	4.8 ± 0.7
DO (mg O_2 L^{-1})	6.3 ± 0.4	6.1 ± 0.5
Turbidity (NTU)	271.14 ± 104.56	274.6 ± 100.36

6.3.1 Algal growth

6.3.1.1 Chlorophyll a

Algal content (Chl *a*) at the start of the experiment measured 0.10 ± 0.05 mg L^{-1} (n = 6) in the control and 0.26 ± 0.06 mg L^{-1} (n = 6) in the inoculated mixed liquor. Initial colouration of the mixed liquor was a brownish-orange in all 12 flasks, irrespective of inoculation.

Figure 6.1 shows that over time an increase in biomass production within the inoculated mixed liquor had occurred as shown by the gradual rise in Chl *a* concentrations during the 28 d incubation period from 0.05 to 4.96 mg Chl *a* L^{-1} (n = 30). A mean maximum of 2.74 ± 1.75 mg L^{-1} (n = 6) was detected after 28 d. There was a statistical significance detected in the inoculated mixed liquor between the initial (0d) and final (28d) Chl *a* content (t (10) = 4.36, $p \leq 0.001$). Irrespective of incubation time (days) algal content (Chl *a*) was consistently greater in the inoculated mixed liquor than the non-inoculated control, the difference was statistically significant (t (11) = 2.89, p= 0.02 ≤ 0.05) from 17 d onwards (Figure 6.1).

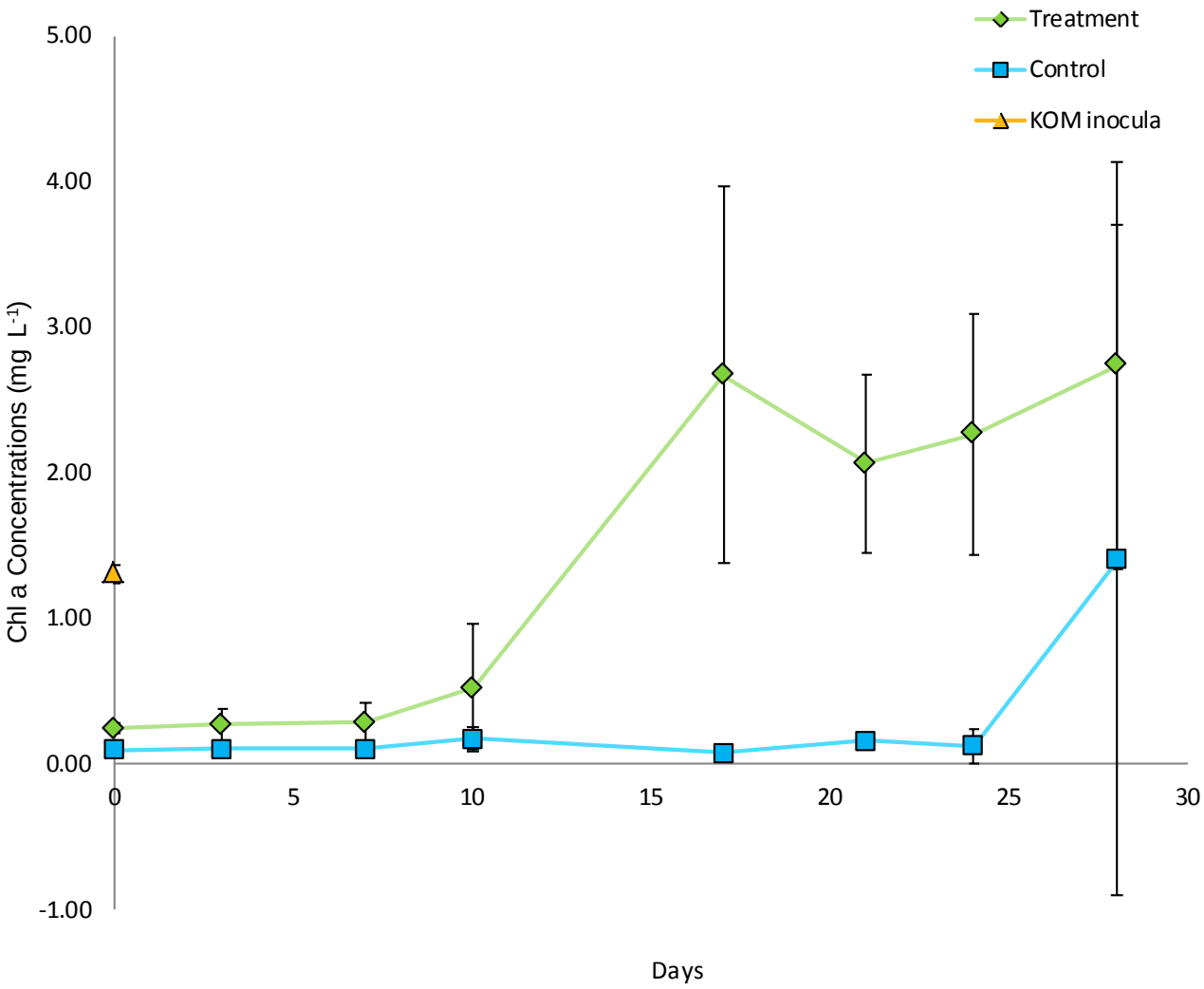

Figure 6.1: Chl *a* concentrations measured in the inoculated (treatment) and non-inoculated (control) mixed liquor over a 28 d growth period under continuous light (PAR400-700 nm 26.5 µmol m^{-2} s^{-1}) at 24°C on an orbital shaker (100 rpm).

A correlation between slurry appearance and increased Chl *a* was observed, particularly in relation to the colour of the mixed liquor, which transitioned from brownish orange to a brownish-olive green during this time.

The mean Chl *a* concentration in the control was 0.12 ± 0.03 mg L^{-1} (*n* = 22) over the first 24 days which increased to 1.41 ± 2.30 mg L^{-1} (*n* =5) after 28 days (Figure 6.1). Interestingly, some algal growth was observed to a small degree in five of the six control flasks after 28 d (mean 1.27 ± 0.95 mg Chl*a*L^{-1}; n = 6; Figure 6.1), which suggests that a natural algal population had developed within the non-inoculated control. Further investigation is required to confirm this finding and identify the strain.

6.3.1.2 Suspended solids and Light penetration

Initial (0d) SS concentrations were 0.44 ± 0.08 g L^{-1} (*n* = 6) and 0.40 ± 0.02 g L^{-1} (*n* =6) in the control and the inoculated treatment slurry, respectively (Figure 6.2). Over, time a gradual increase in SS was noted for both slurry types as shown in Figure 6.2, reaching concentrations of 0.70 ± 0.18 g L^{-1} (*n* = 5) in the control and 1.35 ± 0.84 g L^{-1} (*n* =6) in the inoculated samples after 28 d (final). This was more noticeable in the inoculated samples, an increase of up to 70% detected (Figure 6.2). Data analysis showed SS to be significantly higher at the end of the experiment (28d) than at the start of the experiment (0d) by up to 3.38 times in the inoculated slurry (t (5) = 2.77,p = 0.04 ≤ 0.05) and 1.59 times in the control (W = 0, p ≤ 0.001). This could be attributed to an increase in algal content (Chl *a*) and a loss of volume through sampling and evaporation, samples would have been more concentrated as a result. A positive correlation between SS levels and Chl *a* supports this finding for both inoculated (r = 0.59, p ≤ 0.05) and non-inoculated (r = 0.56, p ≤ 0.05) samples, (Tables 6.2 and 6.3). Although concentrations measured up to 1.93 times higher in the inoculated slurry than in the control, the differences between the samples were not statistically significant (W = 416, p = 0.96 ≥ 0.05) (Figure 6.2).

In Figure 6.2 turbidity levels demonstrated a similar trend rise to that of SS, in that after 28d of continuous light exposure, an increase in turbidity levels of up to 83% was detected in both control (from 461.00 to 2504.00 NTU) and inoculated (461.00 to 2651.67 NTU) samples. The difference

between initial (0d) and final (28d) turbidity was statistically significant for both the treatment (inoculated) (t (5) = 13.19, $p \leq 0.001$) and control (t (4) = 9.13, $p \leq 0.001$). However, like SS, no statistically significant differences existed between the control and inoculated samples (t (18) = 0.14, p= 0.89 ≥ 0.05). A positive correlation was shown to have occurred between Chl *a* and turbidity (r = 0.64, $p \leq 0.05$; inoculated and r = 0.38, $p \leq 0.05$; control) and between SS and turbidity (r = 0.67, $p \leq 0.05$; inoculated and r = 0.73, $p \leq 0.05$; control), which could explain the increase in turbidity concentrations over time (Table 6.2 and 6.3). An increase in algal particles the most plausible cause. Nevertheless, the presence of biomass in the treated mixed liquor is a clear indication that suffice light penetration essential for photosynthesis had occurred and that both SS load and turbidity of the mixed liquor had not in fact impeded growth.

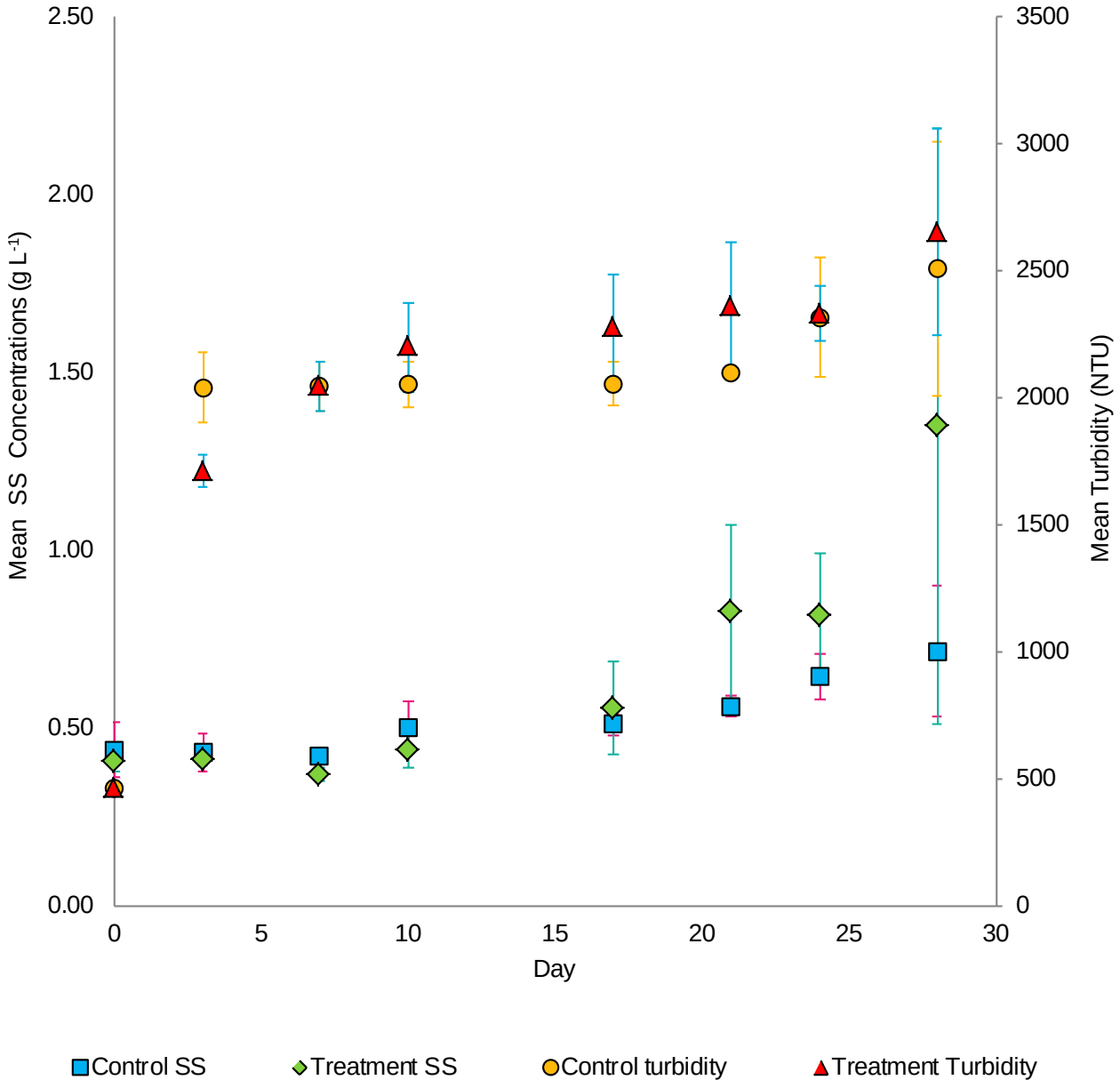

Figure 6.2: Average (mean ± SD) SS (g L^{-1}) and turbidity (NTU) measured in the inoculated (treatment) and non-inoculated (control) mixed liquor over a 28 d growth period under continuous illumination (26.5 µmol m^{-2} s^{-1}) at 20.5 ± 1.1℃ and stirred constantly at 100 rpm

6.3.2 Removal of nutrients

A potential benefit of HRAP is the removal of ammonia through nitrification, carbon and other dissolved nutrients (Fallowfield *et al.*, 1999). The N (Figure 6.3) and C (Figure 6.4) loads of the mixed liquor were monitored in triplicate every 3-4 days (of the 28 d incubation period) for both inoculated and non-inoculated samples and presented as the average measurable fraction of TIN (NH_4^+-N, NO_2^--N, and NO_3^--N) and TC (TOC and IC). The various inorganic-N and C species measured showed similar responses to the changes in incubation day and algal concentrations. For both mixed liquor types, similar initial TIN and TC concentrations were recorded; 0.85 ± 0.26 g TIN L^{-1} (n = 6) and 0.45 ± 0.02 g TC L^{-1} (n =6) the control and 0.77 ± 0.03 g TIN L^{-1} (n = 6) and 0.43 ± 0.04 g TC L^{-1} (n = 6) in the inoculated samples.

NH_4^+-N levels at the start of the experiment were 0.26 ± 0.06 g L^{-1} (Control; n = 6) and 0.25 ± 0.02 g L^{-1} (Inoculated treatment; n = 6) in the two liquor types. Interestingly, over time, little to no additional nitrogen or carbon removal was exhibited in the inoculated samples compared to those of the control; in fact, inorganic-N and carbon concentrations had increased slightly for both sample groups (NO_2^--N and IC the exception) (Figure 6.3 and 6.4). NO_3^--N in particular, an indication that continued nitrification had occurred. Over the 28 d, an estimated evaporative loss of 21% was detected in the flasks over the 28 d. This and the fact the removed volume was not replaced post collection could account for the increase in the inorganic- N, TOC and IC concentrations. No statistically significant differences existed between the treatment and control for any of the measured N species despite mean concentrations on average measured 1.1 times higher in the treatment slurry for all SS, N, and C with the exception of NO_2^--N and IC where concentrations read the same.

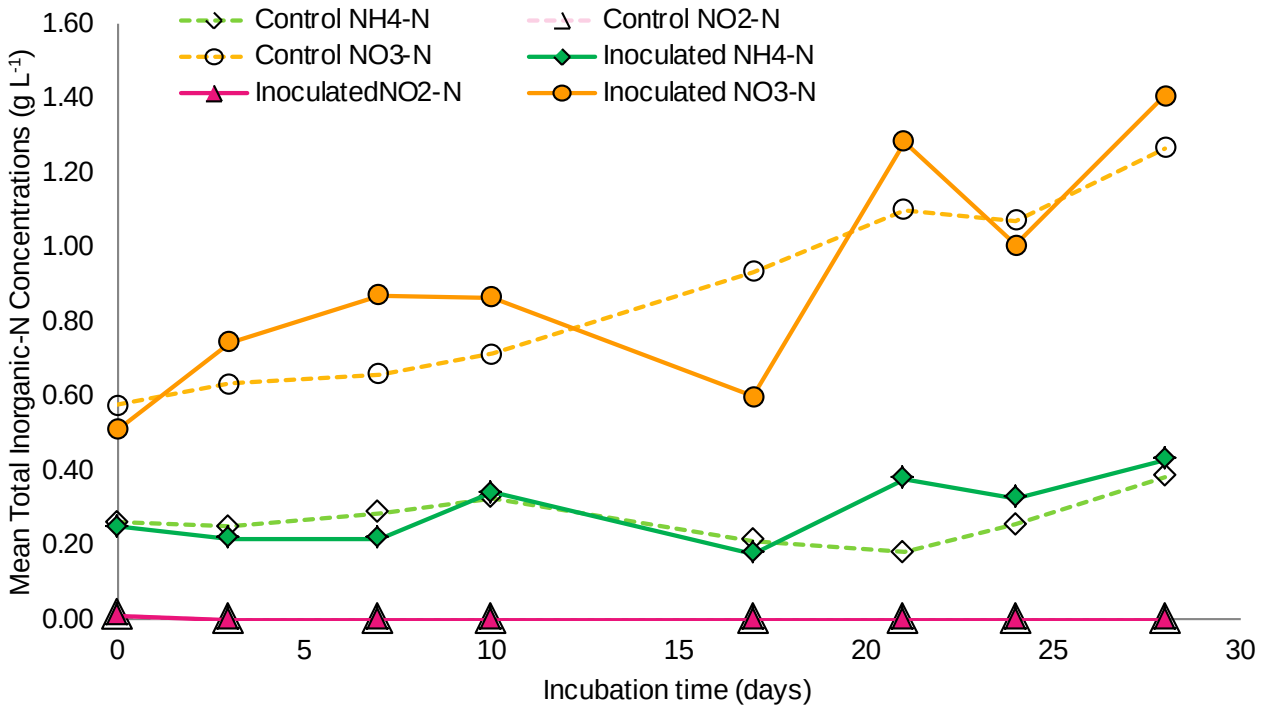

Figure 6.3: Average concentrations of inorganic-N fractions measured in the filtered inoculated (treatment) and non-inoculated (control) mixed liquor, stirred constantly at 100 rpm under continuous illumination (26.5 µmol m^{-2} s^{-1}) exposure at 20.5 ± 1.1℃ and 100 rpm over a 28 d incubation period.

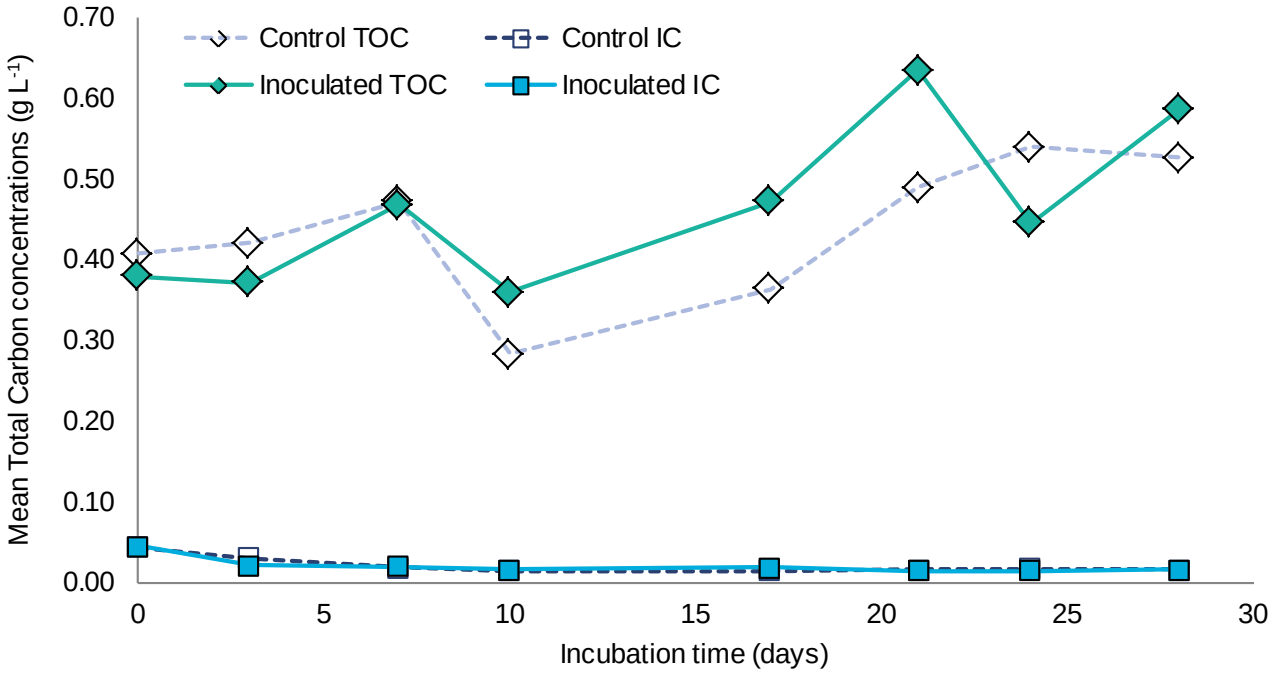

Figure 6.4: Mean carbon concentrations measured in the filtered inoculated and non-inoculated (control) mixed liquor (filtered), stirred constantly at 100 rpm under continuous light exposure (26.5 µmolm^{-2} s^{-1}) at 20.5 ± 1.1℃ and 100 rpm over a 28 d incubation period.

Tables 6.2 and 6.3 presents a correlation matrix between the different inorganic-N and carbon fractions examined in relation to the inoculated and non-inoculated mixed liquor. The results showed Chl *a* concentrations to also be correlated positively with TN, TIN, NH_4^+-N, NO_3^--N, TC, and TOC and negatively with NO_2^--N and IC.

There is enough evidence to reject the null hypothesis that the nutrient composition and SS load of the aerated ANPS (with RAS feedback) would be unsuitable for algal biomass development, with clear algal growth in aerobically pre-treated ANPS.

Table 6:2: Correlation data matrix between all parameters examined within the non-inoculated mixed liquor samples (Control).

	SS ($g\ L^{-1}$)	TN ($g\ L^{-1}$)	TIN-N ($g\ L^{-1}$)	NH_4^+-N ($g\ L^{-1}$)	NO_2^--N ($g\ L^{-1}$)	NO_3^--N ($g\ L^{-1}$)	TC ($g\ L^{-1}$)	TOC ($g\ L^{-1}$)	IC ($g\ L^{-1}$)	Turbidity (NTU)	Chl *a* ($mg\ L^{-1}$)	pH	DO ($mg\ L^{-1}$)	Temperature (°C)
SS ($g\ L^{-1}$)	1													
TN ($g\ L^{-1}$)	0.75	1												
TIN-N ($g\ L^{-1}$)	0.97	0.74	1											
NH_4^+-N ($g\ L^{-1}$)	0.36	-0.01	0.34	1										
NO_2^--N ($g\ L^{-1}$)	-0.39	-0.21	-0.48	-0.06	1									
NO_3^--N ($g\ L^{-1}$)	0.93	0.78	0.97	0.11	-0.51	1								
TC ($g\ L^{-1}$)	0.50	0.92	0.50	0.01	-0.04	0.52	1							
TOC ($g\ L^{-1}$)	0.55	0.94	0.56	0.00	-0.15	0.58	0.99	1						
IC ($g\ L^{-1}$)	-0.50	-0.19	-0.60	-0.10	0.92	-0.62	0.05	-0.07	1					
Turbidity (NTU)	0.56	0.38	0.64	0.20	-0.97	0.63	0.21	0.30	-0.86	1				
Chl *a* ($mg\ L^{-1}$)	0.73	0.47	0.78	0.74	-0.18	0.63	0.38	0.39	-0.23	0.38	1			
pH	-0.22	-0.03	-0.29	-0.37	-0.14	-0.21	-0.07	-0.04	-0.14	0.01	-0.61	1		
DO ($mg\ L^{-1}$)	-0.70	-0.89	-0.67	0.29	0.22	-0.78	-0.74	-0.76	0.19	-0.36	-0.27	0.10	1	
Temperature (°C)	0.26	0.16	0.31	0.65	0.23	0.16	0.30	0.27	0.14	-0.09	0.67	-0.76	0.04	1

Where; (-) 0.85 to (-) 1 represents a strong correlation (green), (-) 0.5 – (-) 0.85; moderate correlation (blue); (-) 0.1 to (-) 0.5; weak correlation (yellow) and 0 to (-) 0.1; weak correlation (yellow).

Table 6:3: Correlation data matrix between all parameters examined within the algae inoculated mixed liquor samples.

	SS (g L^{-1})	TN (g L^{-1})	TIN-N (g L^{-1})	NH_4^+-N (g L^{-1})	NO_2^--N (g L^{-1})	NO_3^--N (g L^{-1})	TC (g L^{-1})	TOC (g L^{-1})	IC (g L^{-1})	Turbidity (NTU)	Chl *a* (mg L^{-1})	pH	DO (mg L^{-1})	Temperature (°C)
SS (g L^{-1})	1													
TN (g L^{-1})	0.85	1												
TIN-N (g L^{-1})	0.85	0.83	1											
NH_4^+-N (g L^{-1})	0.79	0.74	0.92	1										
NO_2^--N (g L^{-1})	-0.36	-0.37	-0.51	-0.27	1									
NO_3^--N (g L^{-1})	0.84	0.83	0.99	0.87	-0.57	1								
TC (g L^{-1})	0.72	0.93	0.72	0.54	-0.34	0.76	1							
TOC (g L^{-1})	0.74	0.94	0.75	0.56	-0.43	0.79	1.00	1						
IC (g L^{-1})	-0.41	-0.43	-0.58	-0.37	0.98	-0.63	-0.34	-0.43	1					
Turbidity (NTU)	0.59	0.56	0.66	0.45	-0.96	0.71	0.49	0.57	-0.96	1				
Chl *a* (mg L^{-1})	0.67	0.71	0.41	0.31	-0.50	0.43	0.61	0.64	-0.52	0.64	1			
pH	-0.64	-0.56	-0.28	-0.13	0.06	-0.31	-0.55	-0.54	0.10	-0.22	-0.68	1		
DO (mg L^{-1})	-0.66	-0.64	-0.45	-0.37	0.11	-0.46	-0.52	-0.52	0.23	-0.27	-0.67	0.89	1	
Temperature (°C)	0.41	0.31	0.07	-0.02	0.10	0.10	0.46	0.43	0.19	0.02	0.47	-0.58	-0.28	1

Where; (-) 0.85 to (-) 1 represents a strong correlation (green), (-) 0.5 – (-) 0.85; moderate correlation (blue); (-) 0.1 to (-) 0.5; weak correlation (yellow) and 0 to (-) 0.1; weak correlation (yellow).

6.4 Discussion

The notion of using wastewaters as a nutrient source for microalgae growth has been reported to be one of the most efficient, low cost and environmentally friendly waste management strategies currently available (Hii *et al.*, 2011). Identified in the literature, the use of biomass derived from HRAPs for biofuels and bio-feedstock production has the potential to lower greenhouse gas emissions and provide alternative energy resources (Borowitzka and Moheimani, 2013, Fallowfield, 2013).

Several studies documented the success of HRAPs for the production of algal biomass on piggery effluent when diluted slurries were used (Fallowfield and Garrett, 1985b, Fallowfield *et al.*, 1999, Garcia *et al.*, 1992, Groeneweg *et al.*, 1980, Martin and Fallowfield, 1989). Dilution was performed to increase light penetration as much as possible at the same time lower the likelihood of NH_4^+ inhibition (Fallowfield, 2013). A strategy not generally advisable as indicated in Section 6.1 (Fallowfield, 2013, Fallowfield and Garrett, 1985a). Alternative pre-conditioning techniques are required (Borowitzka and Moheimani, 2013, Fallowfield, 2013, Fallowfield *et al.*, 1999)

One of the main research challenges with treating pig slurry in a HRAP is to manage the issues related to NH_4^+ toxicity and light attenuation associated with the high opacity, nutrient, and SS loads in pig slurry that can negatively influence algal growth (Borowitzka and Moheimani, 2013, Fallowfield, 2013, Fallowfield *et al.*, 1999, Gerardi, 2002, Groeneweg *et al.*, 1980). This supports the need for an integrated pre-treatment regime prior to application in a HRAP(Fallowfield, 2013, Fallowfield and Garrett, 1985b)

Consecutive anaerobic, aerobic and clarification treatments proved effective in NH_4^+-N (24-89%) and SS (51-79%) removal as shown in Chapters 4 and 5; reducing concentrations to 0.17–1.04 g NH_4^+-N L^{-1} and 0.28–1.12 g SS L^{-1} across the various operating conditions examined. This chapter presents the results of an assessment of the ability of pre-conditioned ANPS from trial R1 of Chapter 5 to support algal biomass production and enable use in a HRAP.

6.4.1 Algal growth

Over the course of the experiment algal growth, determined using the surrogate indicator chlorophyll *a* became clearly apparent within the inoculated mixed liquor samples, demonstrating a capability to withstand NH_4^+-N concentrations up to 0.87 g L^{-1}. Positive algal growth was documented in all six of the inoculated samples, including one out of the six controls (Flask C2) after a 28 d incubation period: a mean maximum of 2.74 ± 1.75 mg Chl *a* L^{-1} reached (Figure 6.1). These coincided with the 2.89 ± 0.29 mg L^{-1} (range 0.22 – 16.35 mg L^{-1}) Chl *a* concentrations acquired in the HRAP water of Fallowfield *et al.* (1999) batch fed with raw diluted (1:10) piggery slurry. García *et al.* (2006) also noted an average Chl *a* concentration of 2.44 – 1.06 and 2.44 – 1.30 mg L^{-1} in the mixed liquor of two HRAPs fed with raw urban wastewater. This provides evidence to suggest that an integrated AWTS is as effective pre-treatment for the growth of microalgae on piggery effluent. It is interesting that the highest Chl *a* concentration recorded during the experiment was detected within one of the control samples (C2) 5.50 mg L^{-1} after 28 d (Figure 6.1). This implies that a native algal strain may have already been present within the mixed liquor. Further research is required to identify the exact algal strain found within the two mixed liquors (inoculated and control).Time constraints would not allow this to be carried out in the current study.

6.4.2 Suspended solids and Light

Light availability is one of the main factors that influence microalgae performance in a HRAP. The quantity of light (sunlight) available for photosynthesis and disinfection will be affected by the attenuating properties of the slurry (Borowitzka and Moheimani, 2013, Fallowfield, 2013). In this case, the strong colouration and SS load of the ANPS. Issues surrounding light limitations and a high SS content during the treatment of animal wastes has been documented throughout the literature (Boersma *et al.*, 1975, Brown, 1984, Fallowfield, 2013, Groeneweg *et al.*, 1980). An integrated AWTS as demonstrated in Chapters 4 and 5 proved effective in lowering the SS content of ANPS by up to 79%. The highest detected during trial R1 (Chapter 5) when aerated at a DO saturation level of up to 100% combined with a 10 d THRT and 20% RAS feedback. The same mixed liquor was used in the current experiment to assess whether the SS content (initial; 0.29 - 0.49 g SS L^{-1}) interfered with algal growth. On average, SS concentrations ranged between 0.37 ±

0.02 – 1.35 ± 0.84 g L^{-1} (*n*=30) and 0.42 ± 0.00 – 0.70 ± 0.18 g L^{-1} (*n* = 28) in both the inoculated and non-inoculated mixed liquor. However, it is important to understand that as light penetrates HRAP water, a large proportion (>80%) is absorbed via the microalgae leading to an increase in biomass (Mehrabadi *et al.*, 2015, Sutherland *et al.*, 2015a, Sutherland *et al.*, 2015b). Sutherland *et al.* (2014) and Sutherland *et al.* (2015b) noted that at high biomass concentrations, increased attenuation often results and can lead to self-shading. Consequently, reducing light penetration, leaving at least one third of the pond light deficient (Sutherland *et al.*, 2015b). While this was not observed to a great extent during this investigation, it is something that should be taken into account in the field.

6.4.3 Nutrient removal

While NH_4^+ is the favoured source of N by microalgae, high concentrations can be inhibitory if not toxic to most microalgae species (Borowitzka and Moheimani, 2013, Britto and Kronzucker, 2002). Growth performance in the mixed liquor suggests there to be some tolerance to NH_4^+ concentrations up to 0.87 g L^{-1}. This is a promising sign for the intended treatment of undiluted APS in a HRAP.

One of the prospective benefits of wastewater treatment using HRAP is the removal of nitrogen, carbon and other dissolved nutrients (Fallowfield *et al.*, 1999). Water of a higher quality is produced as a result. Previous studies noted nitrogen removal rates in both full and pilot scale HRAPs to be as high as 60-75% (Craggs *et al.*, 2012, Cromar and Fallowfield, 1997, García *et al.*, 2006, Garcia *et al.*, 2000, Park *et al.*, 2011, Sutherland *et al.*, 2015a)

However, in the current experiment this was not the case. A decrease in nitrogen removal was detected in the two mixed liquors over the 28 d (Figure 6.3). Like the SS loads, a gradual increase in both the inorganic-N and carbon (TOC and IC) content was observed in all 12 samples (Figure 6.6 and 6.7). This was reflected in the respective fractions (exception of NO_2^--N and IC) (Figure 6.6 and 6.7) and could be attributed to the effect of water loss, both as a result of evaporation (estimated to be around 21% on average) and sample collection (as the volume removed was not replaced post treatment). In fact, NO_3^--N content increased by up to 55% (control) and 64%

(inoculated) over time. A strong positive correlation between the inorganic-N (exception of NO_2^--N) and algal biomass content was identified irrespective of inoculation. This coupled with the very low NO_2^--N levels indicate that nitrification had taken place, which could explain why there was a decrease in nitrogen removal during this period, with NH_4^+-N found to have still been nitrified during this period using DO produced from the algal biomass to carry out this process.

This investigation demonstrated successful algal growth in undiluted APS. This is a huge advantage in the integration of AWTS and HRAPs for the treatment of undiluted pig slurry.

6.4.4 Future research

While this was considered a necessary initial experiment designed solely to determine whether growth was indeed possible within the aerated ANPS, further work is needed to establish the growth yields both in the field and for the other aeration parameters examined (as only one aeration set was used). The algae strain (s) tolerant to these conditions also requires identification. This will help to model and implement the integrated system on a larger scale.

Nevertheless, now that the potential for algal biomass production has been established in the aerated ANPS, the next step would be to look at a) establishing optimal operating conditions for treatment in a HRAP, and b) upscale to a pilot in-field scale for farm application. The findings of which can assist in the generation and manufacture of biofuels-and bio-feedstock as a means to lower greenhouse gas emissions and enhance the environmentally friendliness of the industry.

6.5 Conclusion

Growth of microalgae on undiluted pig slurry has been demonstrated in an aerated ANPS. Aerobic treatment including RAS has been demonstrated as a suitable pre-conditioning step prior to application of a HRAP for integrated wastewater treatment and algal biomass production.

7. GENERAL CONCLUSIONS

This chapter summarises the main discussion points and conclusions observed from the work described in this thesis for each of the relevant experimental chapters.

Effluent management in Australian piggeries occurs primarily through anaerobic lagoons; the pond water recycled on farm. However, as indicated throughout this study the retained SS and NH_4^+ loads are concerning for reuse. Proven successful in the management of various wastewaters, aerobic treatment was identified to be an ideal intermediate treatment step for pig slurry between the pre-existing anaerobic lagoons and the proposed HRAPs (APL, 2015b, Buchanan *et al.*, 2013). This thesis therefore demonstrated at a laboratory scale the integrated AWT of ANPS; system characterisation the objective.

As highlighted throughout, very little work has been done on either the aerobic treatment or algal biomass production of ANPS, post lagoon treatment. A review of the current literature identified that majority of the characterization work regarding integrated treatments of pig slurry was done using whole or diluted raw slurries (Buchanan *et al.*, 2013, Evans *et al.*, 1979, Evans *et al.*, 1983, Evans *et al.*, 1986, Gerardi, 2002, Svoboda, 1995, Svoboda and Fallowfield, 1989). The research conducted in this thesis is therefore novel, in that it looked to bridge the knowledge gap surrounding the aeration and characterisation of an integrated treatment (AD + AT + HRAPs) of ANPS in which a large proportion of the organic-C was removed during pre-treatment

A rational approach was therefore used to identify and configure a suitable apparatus setup of the aeration vessel and optimised operation regime (aeration conditions), to achieve the desired outcomes of this research (SS removal, nitrification and algal biomass production). The information obtained, will allow for a better understanding of how the AWTS works at a laboratory scale, that can be used to assist in the design and scale-up to an in-field pilot scale (Buchanan *et al.*, 2013).

The main findings of this thesis are now reiterated in accordance to the research objectives outlined in Chapter 1 and again in each of the relevant chapters.

Aim 1: Establish a suitable equipment set up for the aeration of ANPS in a small scale AWTS by evaluating the influence of equipment configuration on the re-aeration of tap water that will assist in characterising the system.

During aerobic treatment, nitrifying microorganisms utilise DO to carry out the transformation of inorganic-N during nitrification (Gerardi, 2002, Svoboda *et al.*, 2013, Svoboda and Evans, 1987, Svoboda and Fallowfield, 1989). It is of upmost importance then that sufficient DO and recirculation of the aerated mixed liquor occurs within the aeration vessel (Evans *et al.*, 1986, Gerardi, 2002, Svoboda *et al.*, 2013). Such that placement of aeration equipment (air stones and DO probe) within the vessel was considered fundamental.

Chapter 3 reported on the oxygen transfer rates of tap water in the system under four different configurations to assess the influence of air-stone orientation and DO probe depth on re-aeration rates and DO measurements, respectively (Table 3.2 and 3.3; Chapter 3). Stones were configured to either face towards (in) or away from (out) the vessels centre, with a DO probe depth 4-6 or 19-22 cm below water surface. The reported oxygen transfer rates of tap water were found to be up to 61.5 times higher when stones faced towards the centre than facing away from (Table 3.2; Chapter 3). This was more noticeable when DO measurements were taken at the shallower probe depth (4-6 cm below water) (Configuration 1). Whilst both probe, heights could justifiably be used upon deliberation it was decided that for this study, the system was to be arranged according to configuration 1.

Once arranged, the air saturation (100%) value of ANPS was assessed, reported to be 7.5 mg O_2 L^{-1} in Chapter 3. This value was then used as an aid to help characterise the AWTS for optimal performance, by providing the saturation value to which the DO control set point examined in the subsequent chapters (Chapters 4 and 5) were based upon. This leads to the next research aim.

Aim 2: Identify optimal aeration regimes to operate an integrated AWTS by assessing the influence of aeration conditions (i.e. DO saturation and THRT) on nitrification and SS removal in ANPS, with or without the inclusion of a RAS feedback step.

Once configured for a suitable apparatus arrangement, characterisation of the system for optimal performance (SS removal and nitrification) was performed at different combinations of DO and THRTs. These two operating parameters have demonstrated the ability to manipulate the speciation of inorganic-N during nitrification, and were thus, chosen accordingly (Béline and Martinez, 2002, Béline *et al.*, 1999, Buchanan *et al.*, 2013, Evans *et al.*, 1979, Evans *et al.*, 1983, Evans *et al.*, 1986).

Effective management of these parameters would enable the system to be operated in a more practical and economical manner using controlled DO set points (Åmand *et al.*, 2013, Guo *et al.*, 2009). Success would reduce treatment and associated running costs. The lowest practical set points desired.

Chapters 4 and 5 reported on the removal of SS and NH_4^+-N concentrations from ANPS under various conditions of DO and THRTs in the absence or presence of a RAS (20%) feedback step. In general, Chapters 4 and 5 reported SS removal rates of over 50% were achieved using this technique for each of the DO set points assessed. These rates coincided with the gradual decline in TSS observed in raw pig slurry reported by Evans *et al.* (1983) after an increase in THRT from 1 to 8 days. Based on the results of Chapters 4 and 5, that whilst SS removal was detected highest (79%) at both 20% saturation (1.5 mg O_2 L^{-1} DO setpoint, and 5 d THRT; ST3, Chapter 4) and up to 100% saturation (7.5 mg O_2 L^{-1}, set point, a 10 d THRT and 20% RAS; R1, Chapter 5), it would be also justifiable to operate the system at a DO concentrations as low as 10% saturation if the main objective of operation is simply to remove the SS content.

However, the running of an aeration system is generally more complicated than simple solid removal and other factors such as nitrification performance have to be taken into account when characterising an aeration system.

Chapter 4 presented the nitrification outcomes of the AWTS at four parameter sets comprising of three DO set points (0.8, 1.5, >7.5) and two THRT (5 and 10 d). The main objective of the chapter was to optimise nitrification in the system, in the simplest manner possible without expending too much energy. The influence of DO saturation and THRT on inter-conversions of inorganic-N was

assessed per condition. NH_4^+-N removal rates of 20 -76% were achieved across the different conditions increasing with increased DO concentrations and THRT. The remaining NH_4^+-N levels a vast improvement. However, in order to stimulate algal growth there needs to be adequate levels of NO_3^--N conserved within the mixed liquor (Fallowfield, 2013). Unfortunately, not all of the removed NH_4^+-N was oxidised to NO_3^--N during the aeration experiments described in Chapter 4 resulting in incomplete or partial nitrification. Insufficient DO or a deficient nitrifying population within the mixed liquor was identified to be a likely cause. One solution to address this issue was to incorporate a RAS feedback step into the matrix as a means of boosting the microbial population (Burton and Turner, 2003, Gerardi, 2002). Incorporation of a RAS feedback was examined in Chapter 5.

As described in Chapter 5, RAS feedback is a process in which a percent of the active biomass is recycled back through the aeration tank and diversified within the mixed liquor (Gerardi, 2011, Seviour and Nielsen, 2010, Wittmann *et al.*, 1990). Chapter 5 reported on the nitrification rates of the integrated AWTS following the incorporation of a 20% RAS feedback step. Like Chapter 4, the RAS trials yielded NH_4^+-N removal rates of 70-90%; an improvement of 14-66% (Chapter 5). However, of more significance was the vast improvement in NO_3^--N production (0.33-0.70 g NO_3^--N L^{-1}; Chapter 5) of 7-91% detected in the trials featuring this inclusion (R1-R6; Chapter 5) than those without (<BLOD-0.20 g NO_3^--N L^{-1}; ST1-ST5; Chapter 4). The findings of this study clearly support the inclusion of a RAS feedback step as part of its treatment regime. Whilst NH_4^+-N removal and NO_3^--N accumulation was clearly shown for each of the operation regimes assessed, nitrification was detected greatest at 3.4 mg O_2 L^{-1} (45-70% saturation level), 20% RAS feedback and a THRT of 10 d (R2; Chapter 5) and thus the recommended operating regime for this laboratory system based on the findings of this research.

However, further investigation is required to assess the economic viability and cost of short and long-term aeration at these levels and the implications this may entail. These factors were not measured for any of the research conducted during the investigation as the information obtained at a laboratory scale would offer no real significant value due to the size and scale of the reactor used and was thus, outside the scope of research for this current project. However, these would need to

be examined at a pilot scale where there would be more value in the results.

During the investigation, nitrification was shown to be influenced by changes to the operational characteristics (aeration level and THRT) applied. Unfortunately, confounding factors such as the natural change in environmental conditions and influent characteristics may have also influenced this oxidative process, with ANPS characteristics shown to have varied from one trial to the next despite being sampled from the same lagoon (attached to a working piggery). This is due to the variable nature of the slurry being used affected by changes in environmental conditions, herd sizes, diet, handling and storage practices (flushing, filling and settling) and from the anaerobic processes taking place (APL, 2015b, Buchanan *et al.*, 2013). Consequently, it is difficult to obtain and maintain an effluent of constant consistency on a regular basis. The corresponding results from the study are to be treated with care accordingly. Whilst it is not ideal, it is something that will need to be taken into consideration when put into practice in the field.

From the results reported in Chapter 4 and 5, Chapter 6 focused on investigating the suitability of the mixed liquor for HRAP application and microalgae production. HRAP achieve a higher degree of disinfection with the added benefit of biomass recovery for various reuse applications (Gerchman *et al.*, 2017, Gutiérrez *et al.*, 2015, Park *et al.*, 2011). Therefore, the ability to promote and maintain an active algal culture in wastewater is not only the next logical step and vital aspect of HRAP treatments (Fallowfield *et al.*, 1999, Sutherland *et al.*, 2015b) but also the outcomes would help to tie the research together nicely, which leads to the final aim of this thesis.

Aim 3: Evaluation of the microalgae growth potential of aerated ANPS prior to application in a HRAP

This was assessed in a one off laboratory-based experiment during Chapter 6, which reported algal concentrations (Chl *a*) of 2.74 ± 1.75 mg Chl *a* L^{-1} in the inoculated mixed liquor and 1.41 ± 2.30 mg Chl *a* L^{-1} in the control after 28 d of continuous illumination (26.5 µmol m^{-2} S^{-1}) at 20.5 ± 1.1°C and stirred constantly at 100 rpm. This finding was significant in that not only was the slurry conditions found suitable for algal biomass production, but the biomass present exhibited a tolerance to NH_4^+-N concentrations of up to 0.87 g NH_4^+-N L^{-1}. A good sign for HRAP treatment.

What was also interesting was the 5.50 mg Chl *a* L^{-1} detected in one of the control flasks (C2) after 28 d, believed to be a native strain already present within the mixed liquor. Since this, was purely a proof of concept experiment further research is required to identify and establish the exact algal strain, growth yields, and ideal operating conditions for HRAP treatment and bio-energy production both in the laboratory and in the field.

Taken as a whole, the research carried out in this thesis demonstrated at a laboratory scale the successful characterisation and implementation of an integrated AWTS into the sequential treatment of ANPS, with a high degree of SS and NH_4^+-N removal exhibited. Furthermore, the results demonstrated aerated ANPS (undiluted) with RAS feedback to be suitable for microalgae biomass production. Future research is vital to fully evaluate the integrated system at a farm level and the mechanisms to harvest and exploit the desired by-products. What is more, it would contribute to the knowledge surrounding the treatment of pig slurry for both better reuse and as a sustainable resource. By ascertaining, both the optimal apparatus set up and operating conditions for the treatment of ANPS, and show casing algal growth potential, valuable information has been attained to assist in the design and construction of an on-site pilot system at a local South Australian piggery. Anticipated future research and directions can be summarised as follow;

- Conduct a detailed assessment of how the system performs (aeration, nitrification, and SS removal) following an up-scale to pilot system. This will provide a more rounded and realistic overview, of the implications of this technology operated under field conditions.
- Evaluate the efficiency of operating the AWTS at the recommended levels (outlined from this research), by conducting energy efficiency and associated costs analysis, which would comment on the economical viability of the system in the field.
- Evaluate the influence the AWTS might have on other aspects of the slurry, pathogen removal for instance.
- Identify and further evaluate the growth and reuse potential (i.e. bio-fuels, alternative feed) of microalgae on the treated pig slurry, by identifying the NH_4^+-N tolerant algae strains and growth yields under the different aeration regimes assessed (as only one was examined in this study).

8. APPENDICES

8.1 Appendix A – Tap water aeration plots

8.1.1 Re-aeration of tap water using equipment configuration 1 (DO probe top + air stones facing inwards)

8.1.1.1 Re-aeration of Tap water using equipment configuration 1– Experiment B

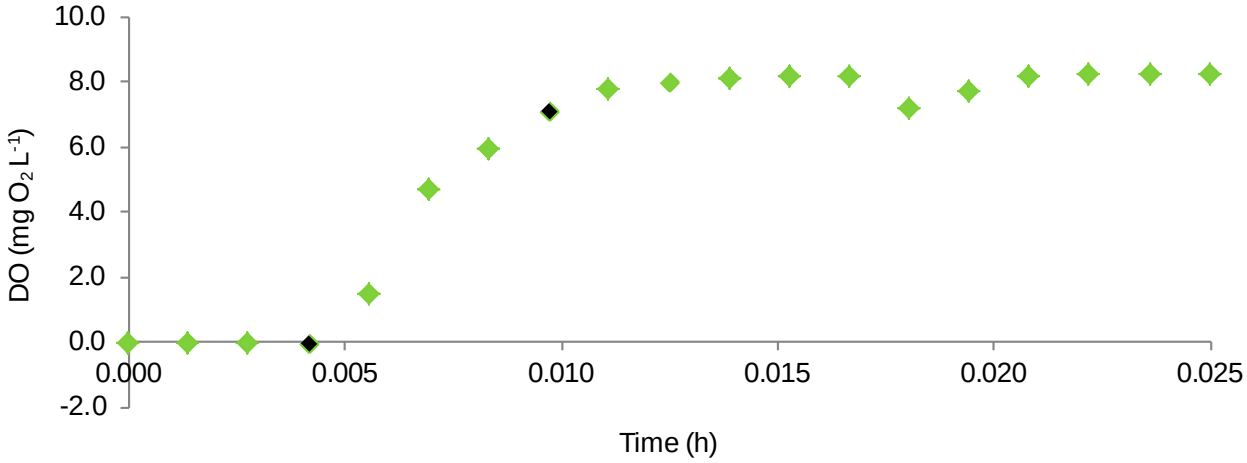

Figure 8.1: Re-aeration A2 of deoxygenated tap water (3 L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. Data collection occurs every 5 s over 0.03h.Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

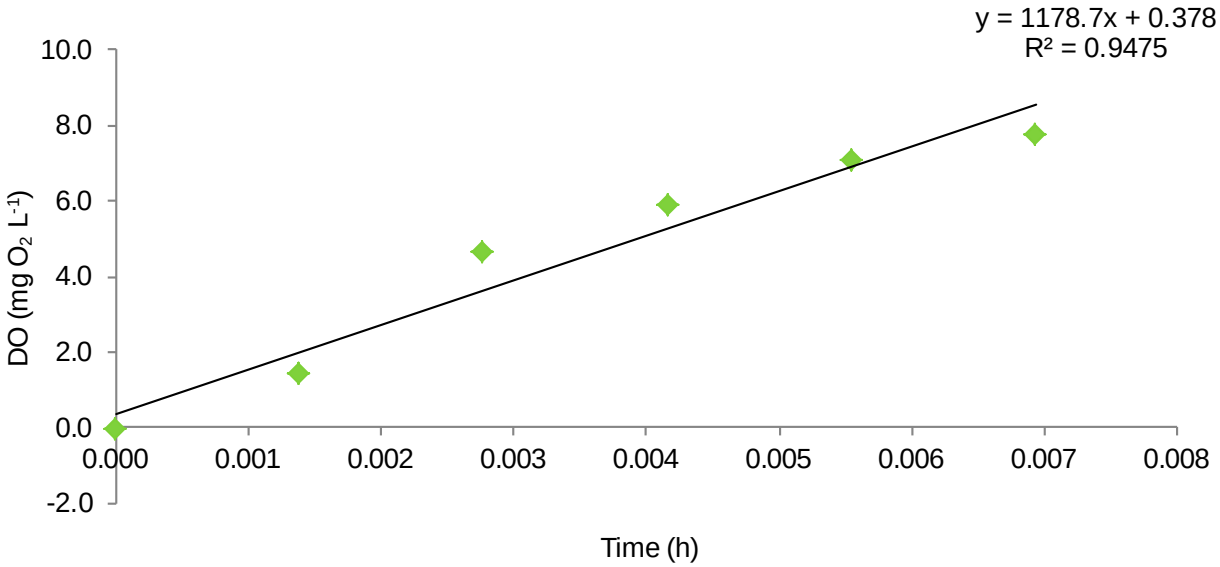

Figure 8.2: Slope of re-aeration A2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. The represented data refers to the linear regression (p = 6.86e^{-04} ≤0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.1.

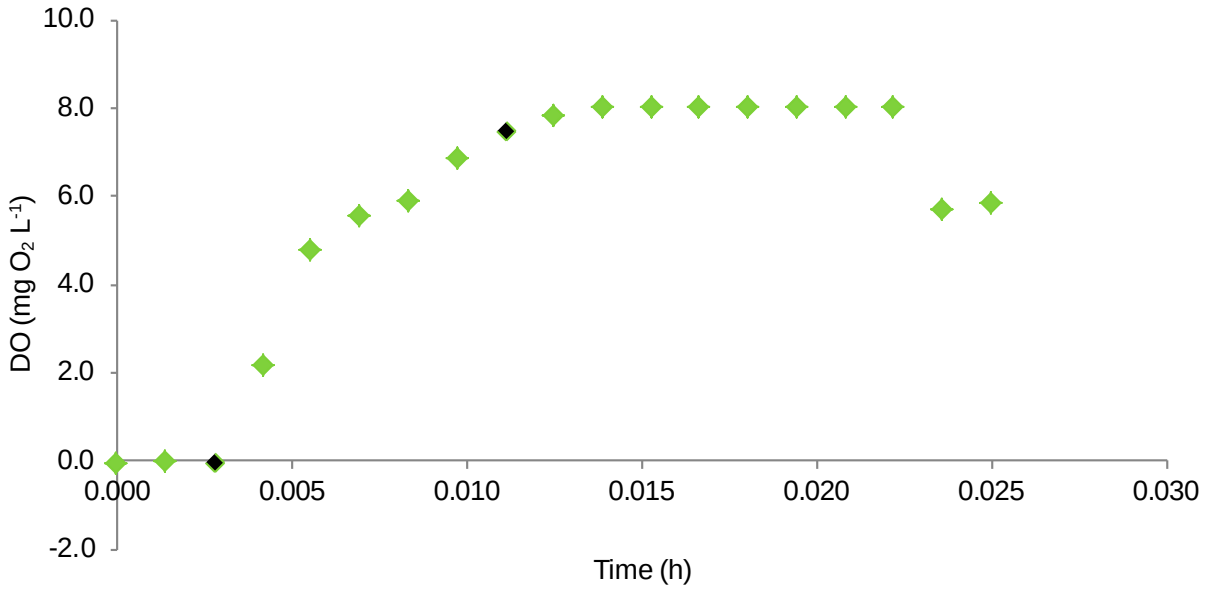

Figure 8.3: Re-aeration A3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7°C. Data collection occurred every 5 s over 0.03h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

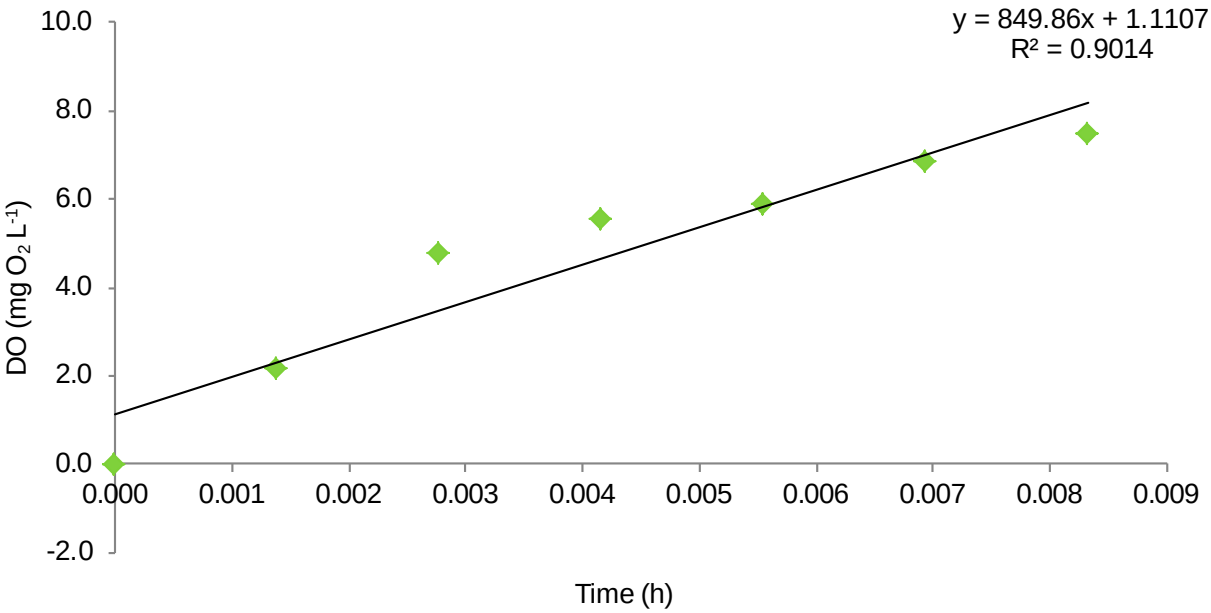

Figure 8.4: Slope of re-aeration A3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.7^{0}C. The represented data refers to the linear regression (p = 0.001 ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.3.

8.1.1.2 Re-aeration of Tap water using equipment configuration 1– Experiment B

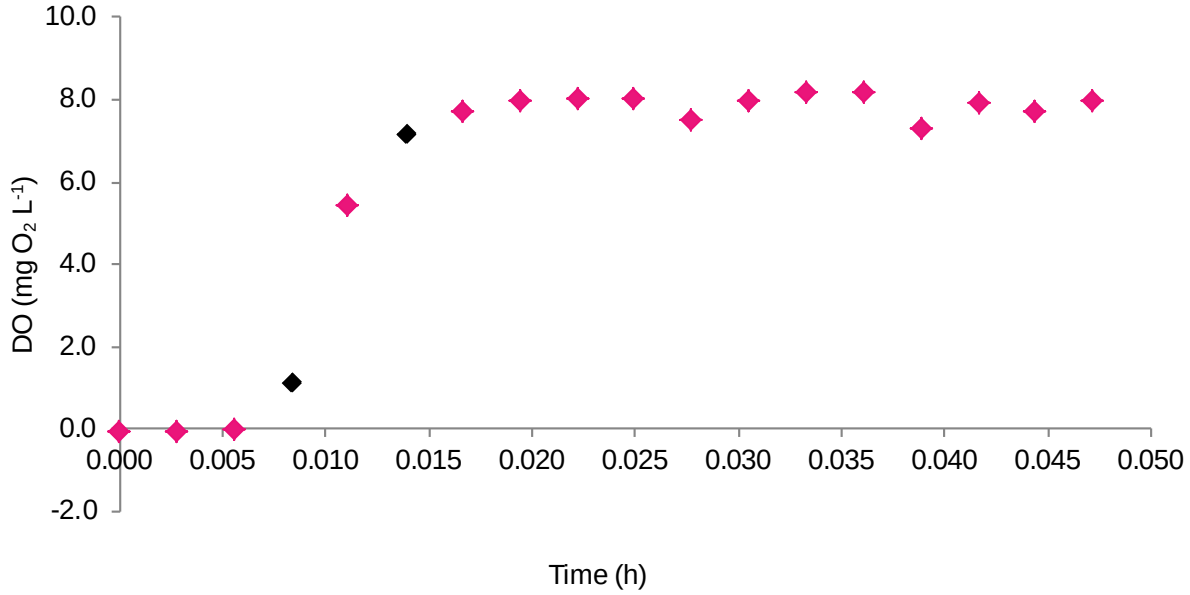

Figure 8.5: Re-aeration B1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. Data collection occurred every 10 s over 0.05h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

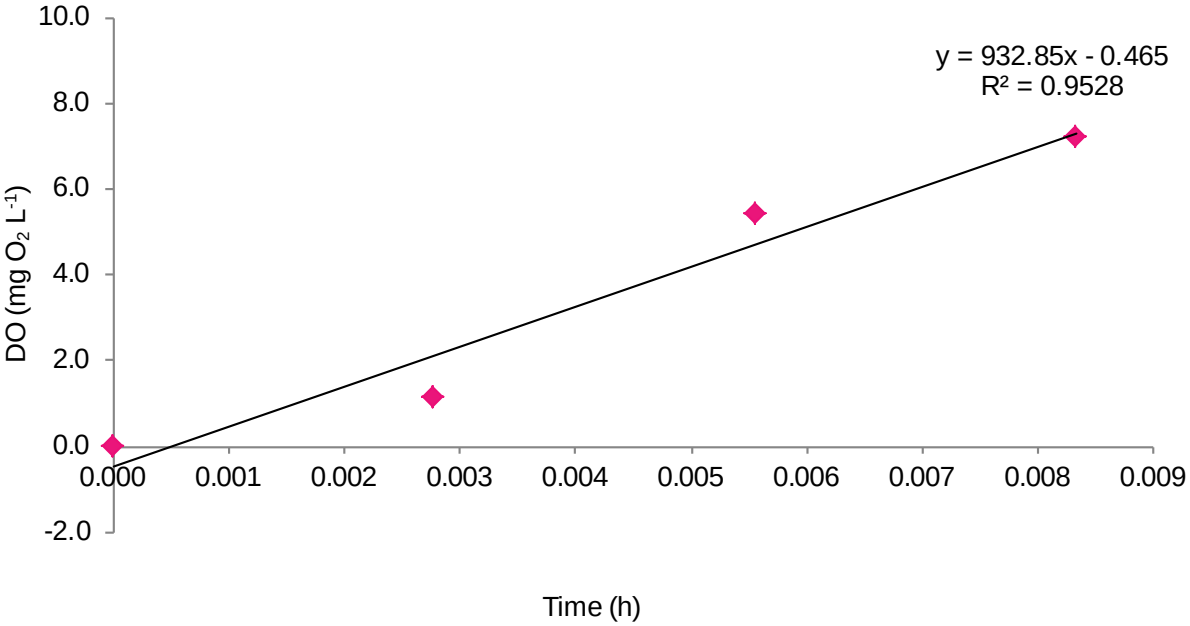

Figure 8.6: Slope of re-aeration B1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23^{0}C (above). The represented data refers to the linear regression (p = 0.008 ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.5.

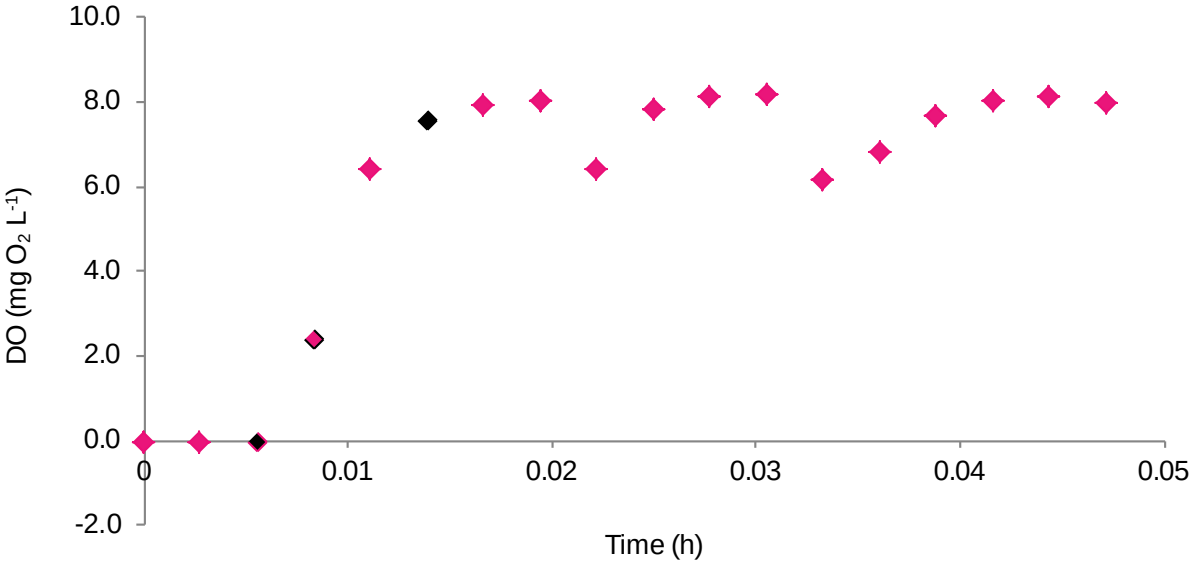

Figure 8.7: Re-aeration B2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. Data collection occurred every 10 s over 0.05h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

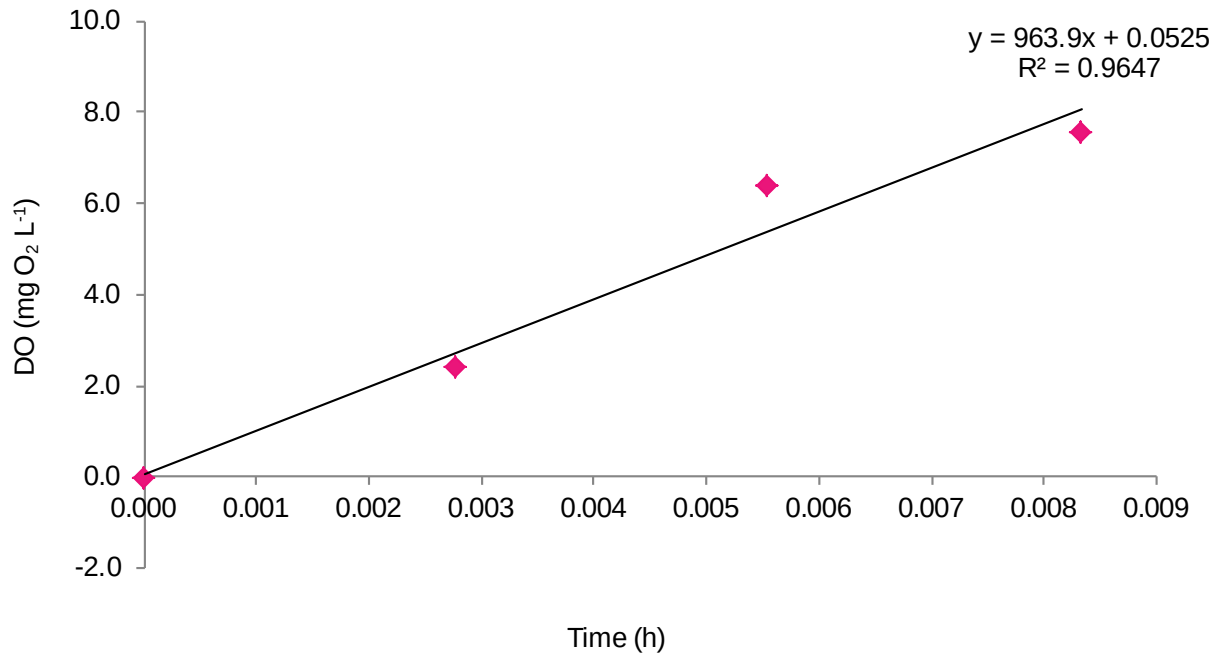

Figure 8.8: Slope of re-aeration A3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. The represented data refers to the linear regression ($p = 0.01 \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.7.

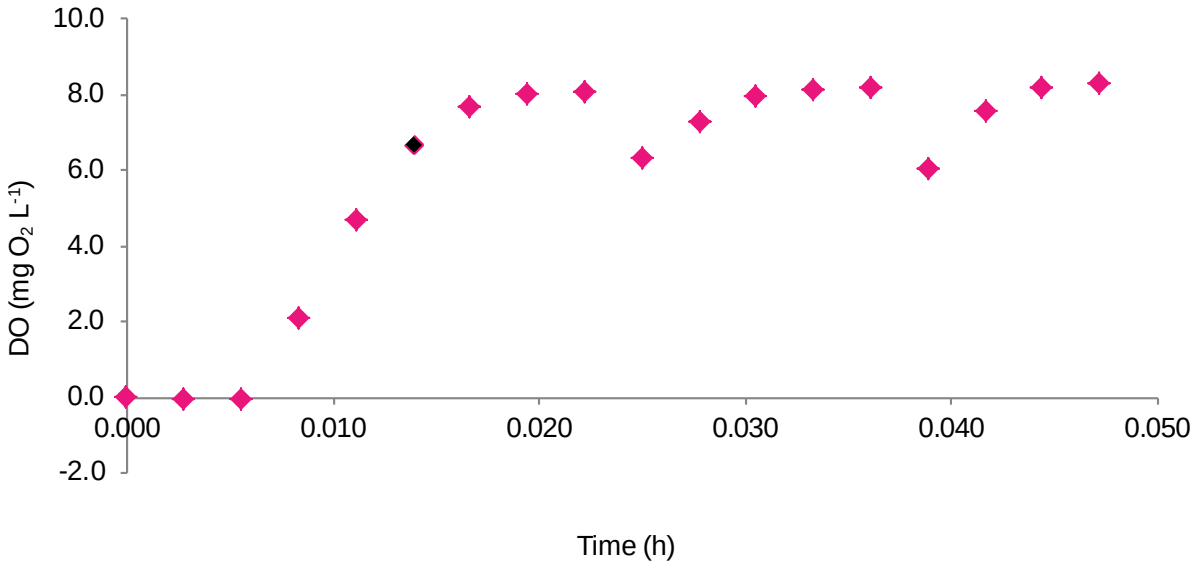

Figure 8.9: Re-aeration B3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.0°C. Data collection occurred every 10 s over 0.05h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

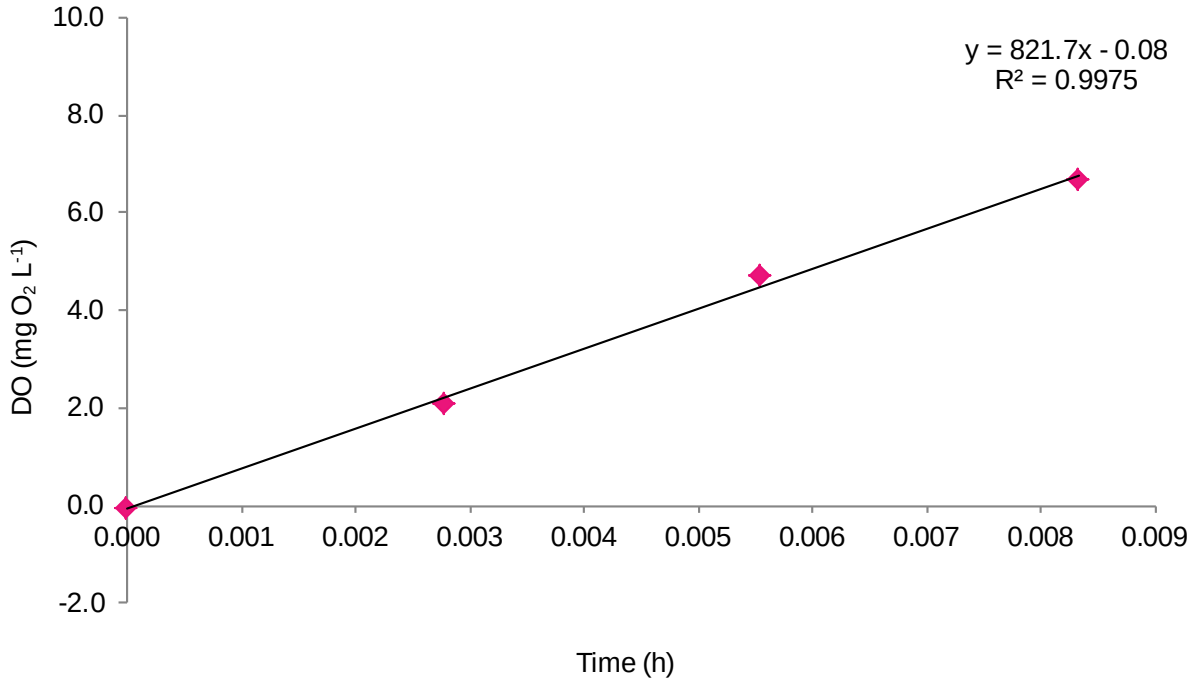

Figure 8.10: Slope of re-aeration B3 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23^{0}C. The represented data refers to the linear regression ($p = 0.001 \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.9.

8.1.1.3 Re-aeration of Tap water using equipment configuration 1– Experiment E

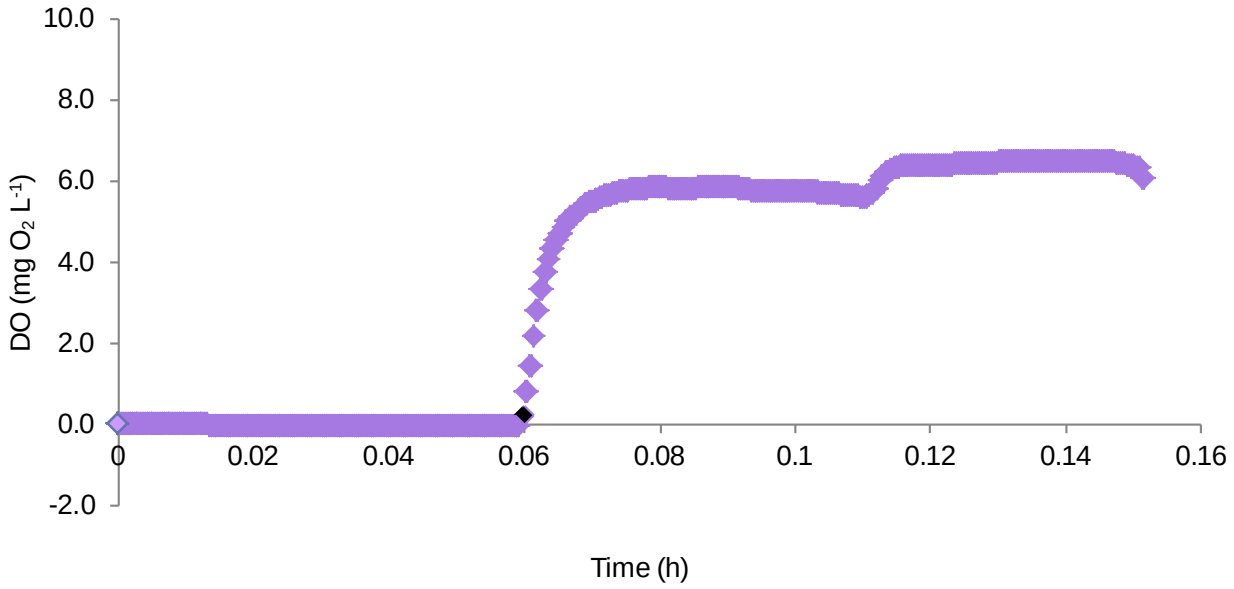

Figure 8.11: Re-aeration E1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9°C. Data collection occurred every second over 0.15h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

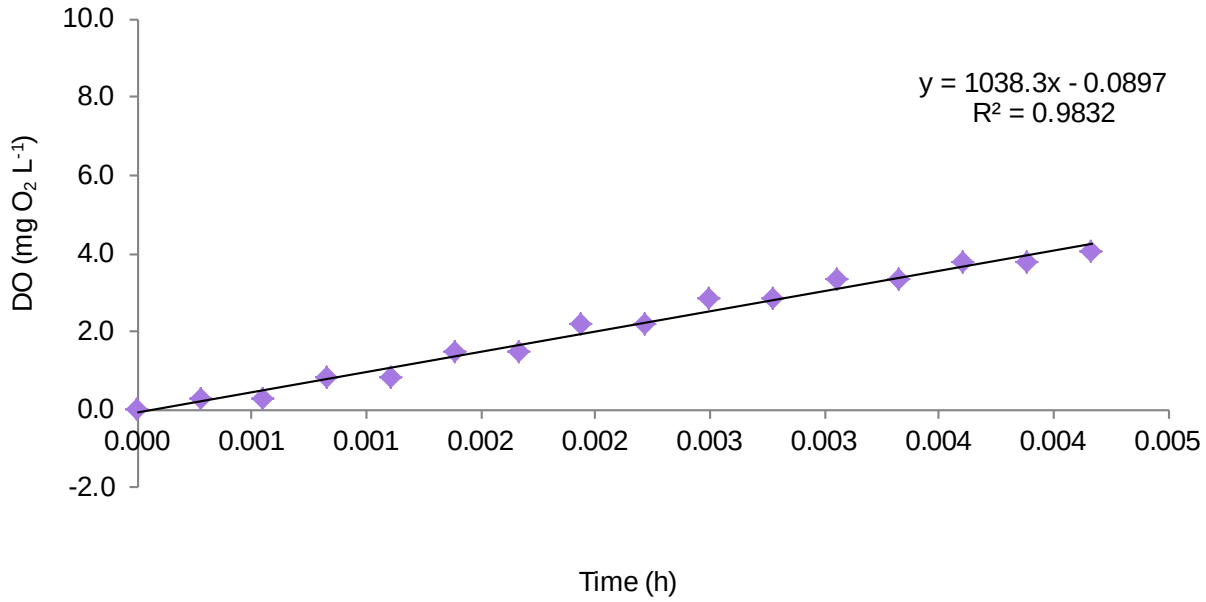

Figure 8.12: Slope of re-aeration E1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9^{0}C (above). The represented data refers to the linear regression (p = 8.10e^{-14} ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.11.

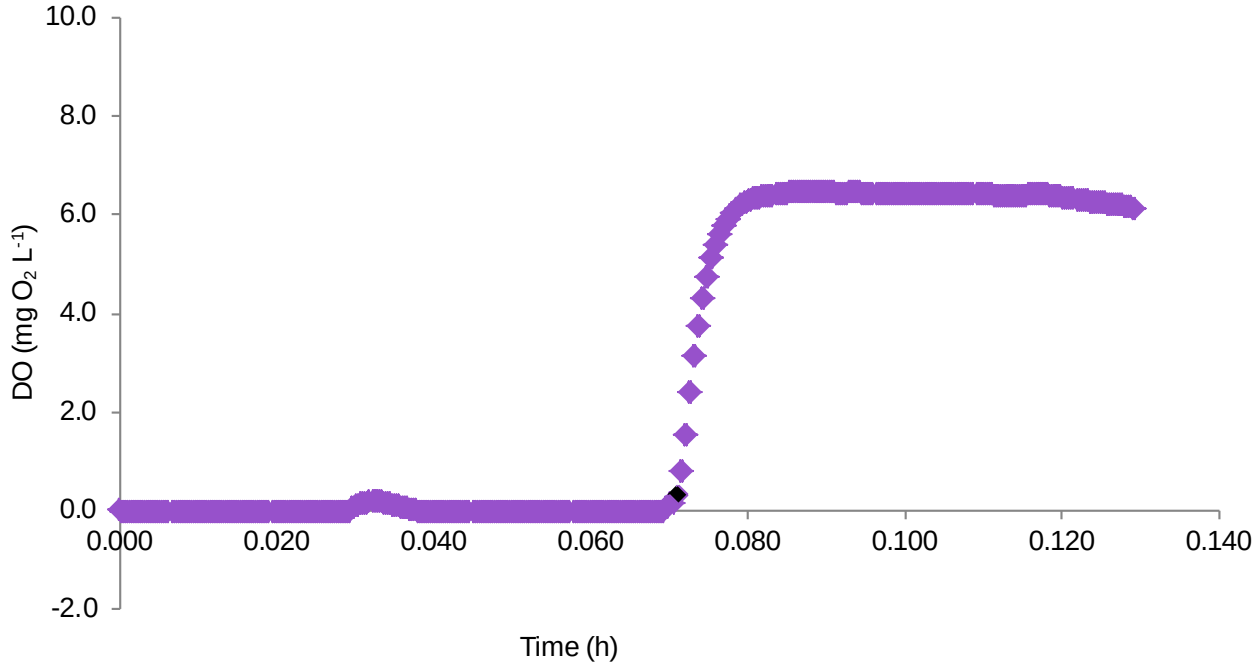

Figure 8.13: Re-aeration E2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9°C. Data collection occurred every second over 0.13h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

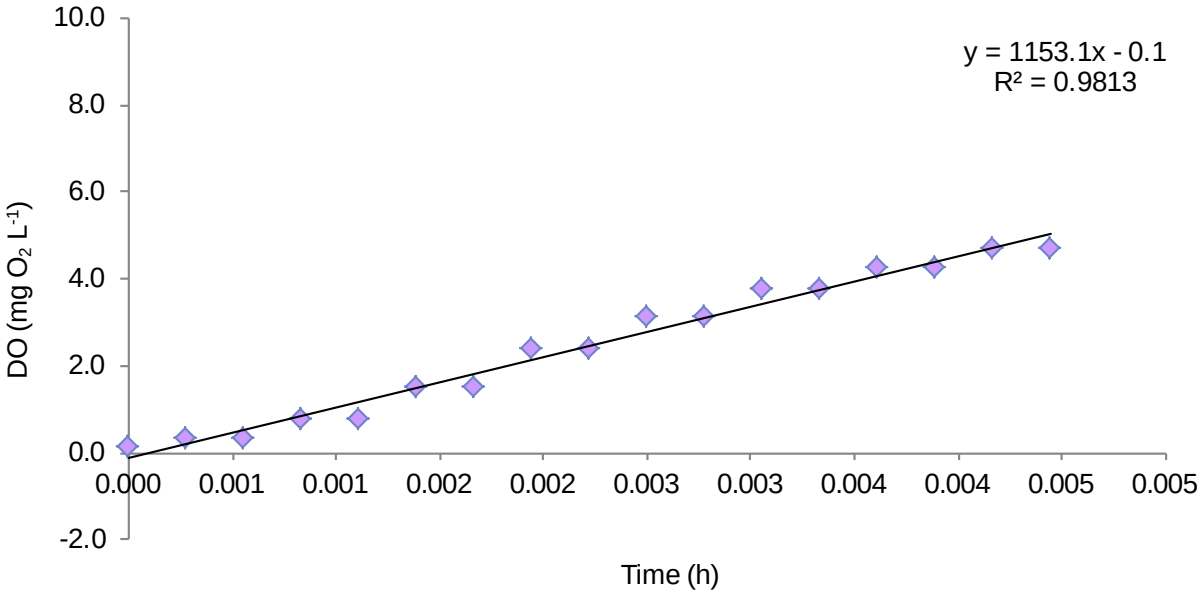

Figure 8.14: Slope of re-aeration E2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 21.9^{0}C (above). The represented data refers to the linear regression (p = 2.22e^{-14} ≤0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.13.

8.1.2 Re-aeration of tap water using equipment configuration 2 (DO probe top + air stones facing out)

8.1.2.1 Re-aeration of Tap water using equipment configuration 2– Experiment D

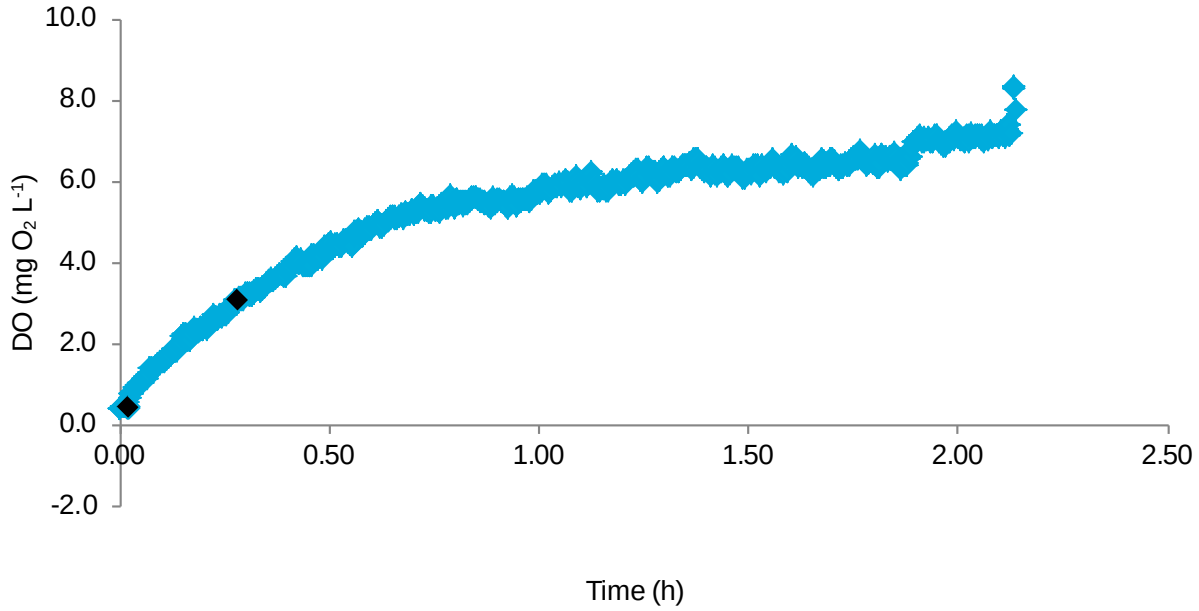

Figure 8.15: Re-aeration D1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 22.0°C. Data collection occurred every 10 s over 2.14 h. Aeration equipment arranged according to configuration 2; DO probe ~4-6 cm below water, with air stones facing outwards (away from centre). The black dots represent the respective data points between which the linear regression was determined.

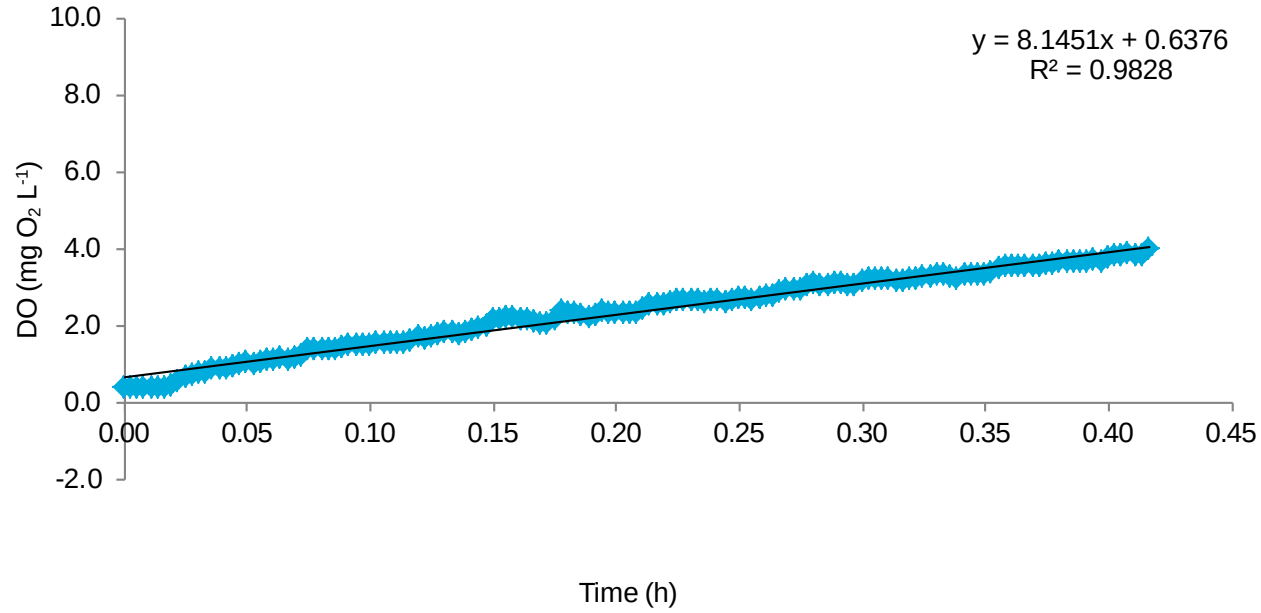

Figure 8.16: Slope of re-aeration D1 of deoxygenated tap water (3L) at a flow rate of 26.31 ccmin^{-1} at 22^{0}C.The represented data refers to the linear regression (p =4.44e^{-121} ≤ 0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.15.

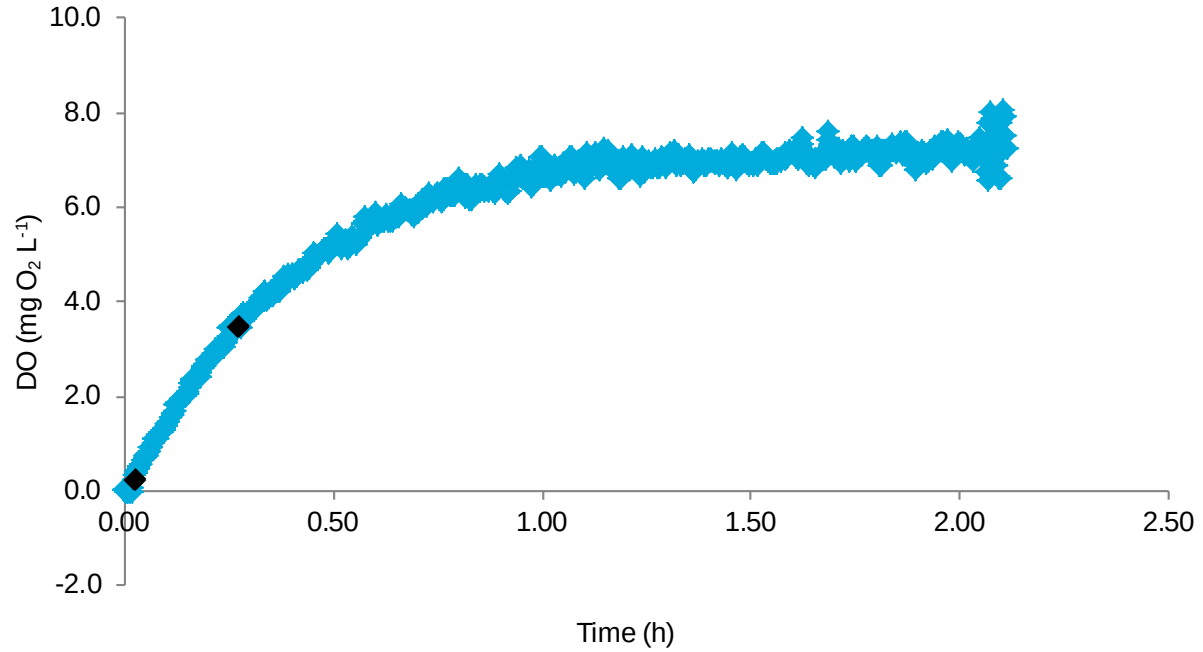

Figure 8.17: Re-aeration D2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 22.0°C. Data collection occurred every 10 s over 2.14 h. Aeration equipment arranged according to configuration 2; DO probe ~4-6 cm below water, with air stones facing outwards (away from centre).The black dots represent the respective data points between which the linear regression was determined.

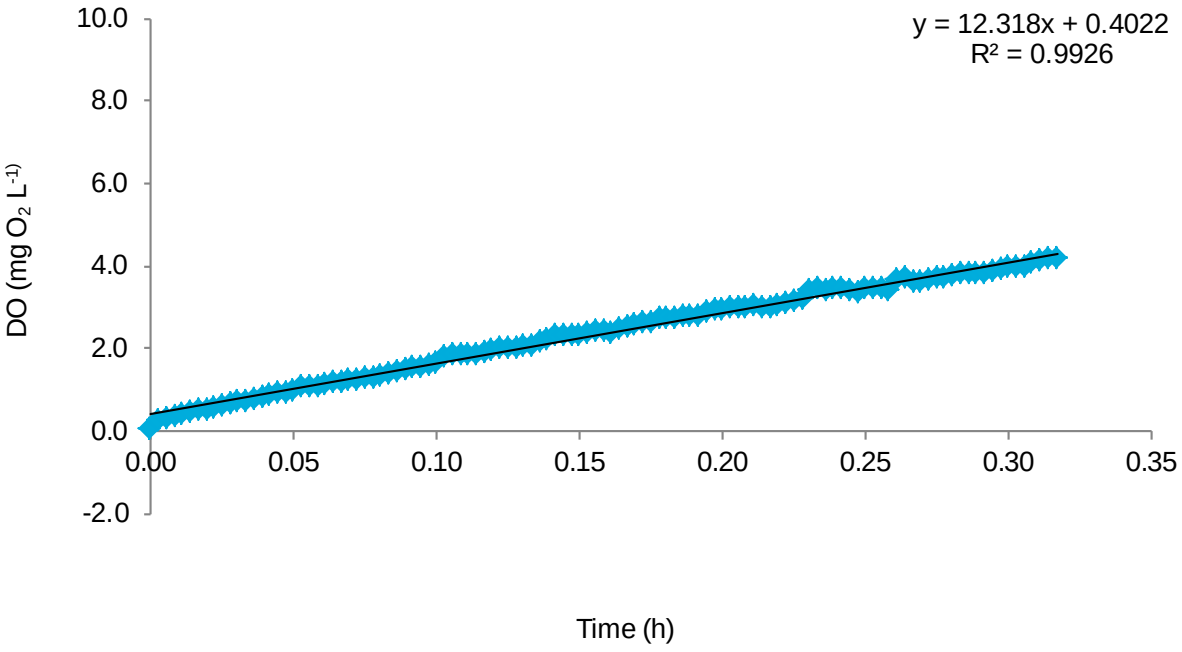

Figure 8.18: Slope of re-aeration D2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 22^0C. The represented data refers to the linear regression ($p = 4.44e^{-121} \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.17.

8.1.3 Re-aeration of tap water using equipment configuration 3 (DO probe bottom + air stones facing in)

8.1.3.1 Re-aeration of Tap water using equipment configuration 3– Experiment F

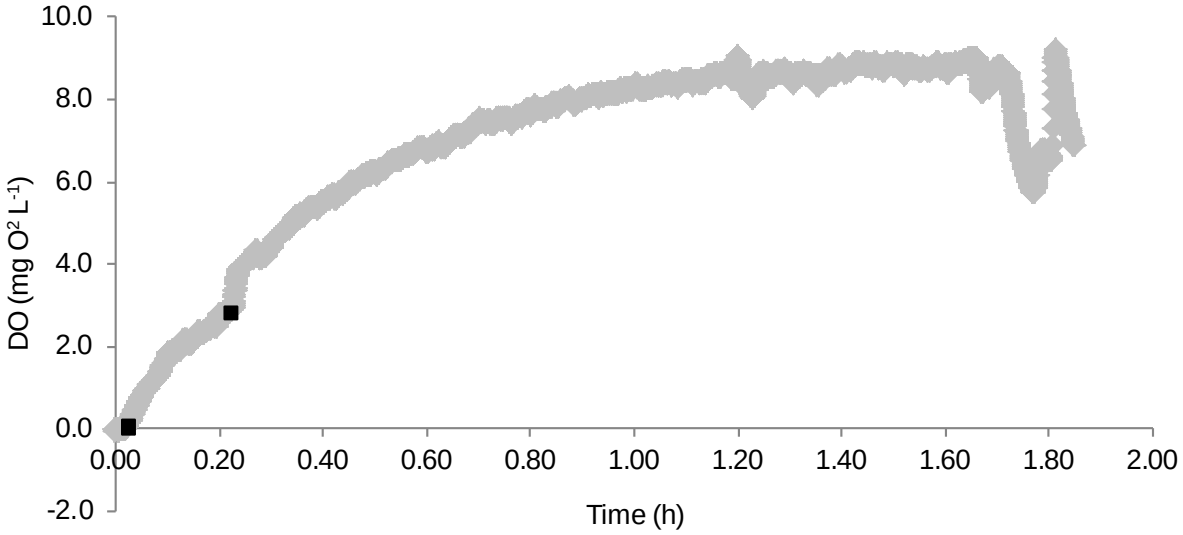

Figure 8.19: Re-aeration F1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.4°C. Data collection occurred every second over 1.85h. Aeration equipment arranged according to configuration 3; DO probe ~19-22 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

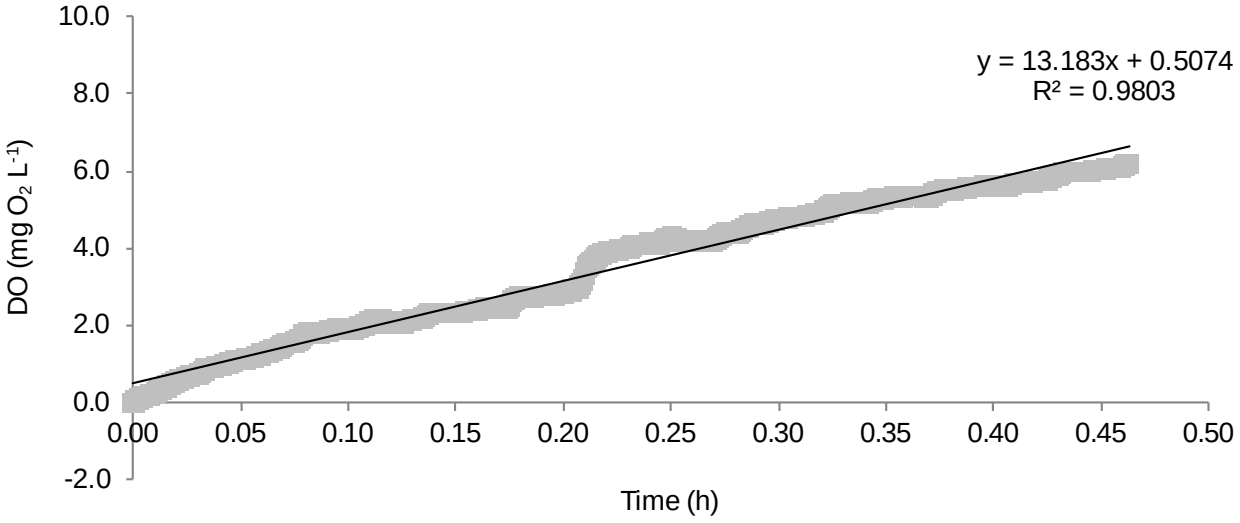

Figure 8.20: Slope of re-aeration F1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.5^{0}C. The represented data refers to the linear regression ($p \geq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.19. Position of Do probe ~4-6 cm above the bottom of the vessel, with air stones (bottom) facing inwards (towards the centre).

8.1.3.2 Re-aeration of Tap water using equipment configuration 3– Experiment G

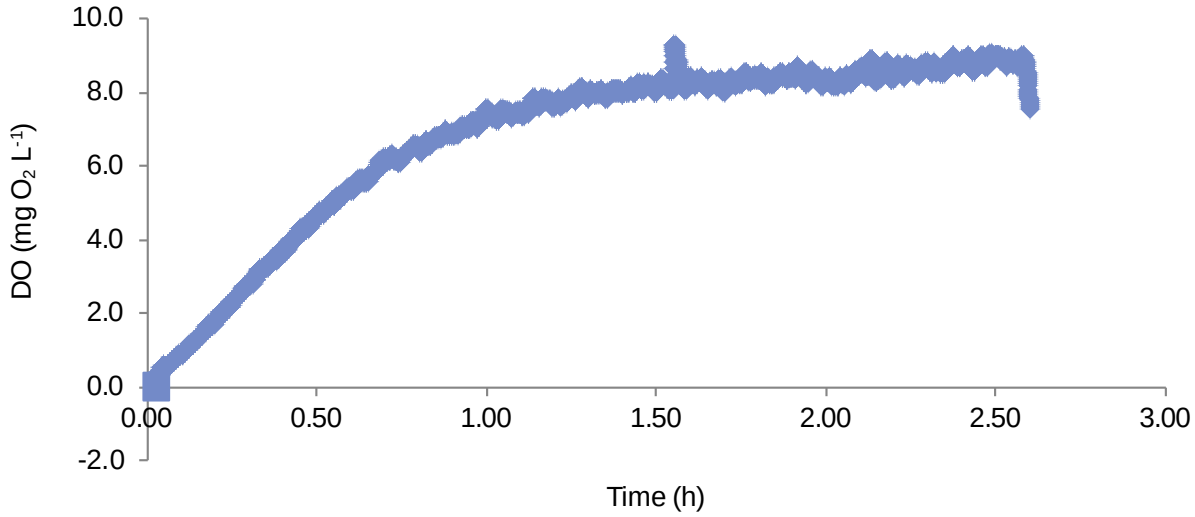

Figure 8.21: Re-aeration G1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.5°C. Data collection occurred every second over 2.6 h. Aeration equipment arranged according to configuration 3; DO probe ~19-22 cm below water, with air stones facing inwards (towards centre). The black dots represent the respective data points between which the linear regression was determined.

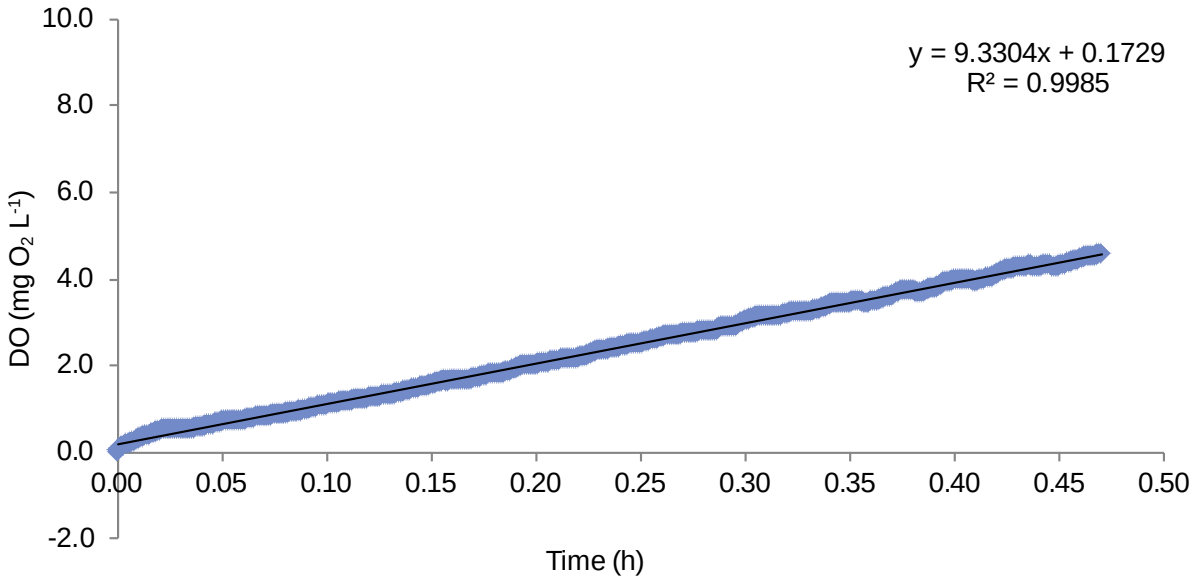

Figure 8.22: Slope of re-aeration G1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 20.5^{0}C. The represented data refers to the linear regression ($p \geq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the lighter coloured dots shown in Figure 8.21. Position of Position of DO probe ~4-6 cm above the bottom of the vessel, with air stones (bottom) facing inwards (towards the centre).

8.1.4 Re-aeration of tap water using equipment configuration 4 (DO probe bottom + air stones facing out)

8.1.4.1 Re-aeration of Tap water using equipment configuration 3– Experiment C

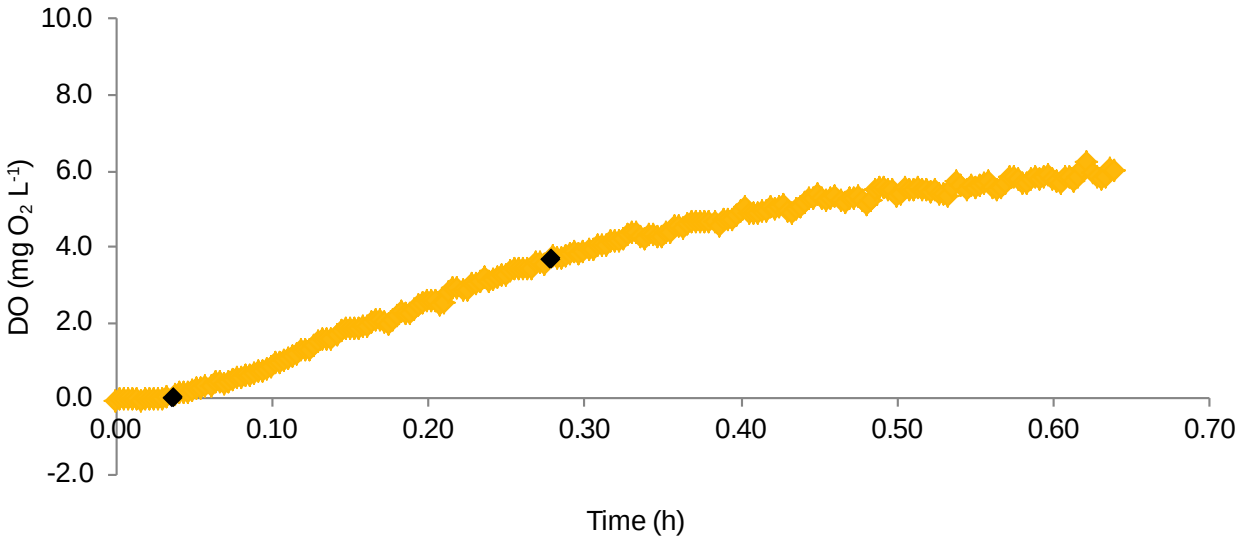

Figure 8.23: Re-aeration C1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.8°C. Data collection occurred every 10 s over 0.63h. Aeration equipment arranged according to configuration 4; DO probe ~19-22 cm below water, with air stones facing outwards (away from centre). The black dots represent the respective data points between which the linear regression was determined.

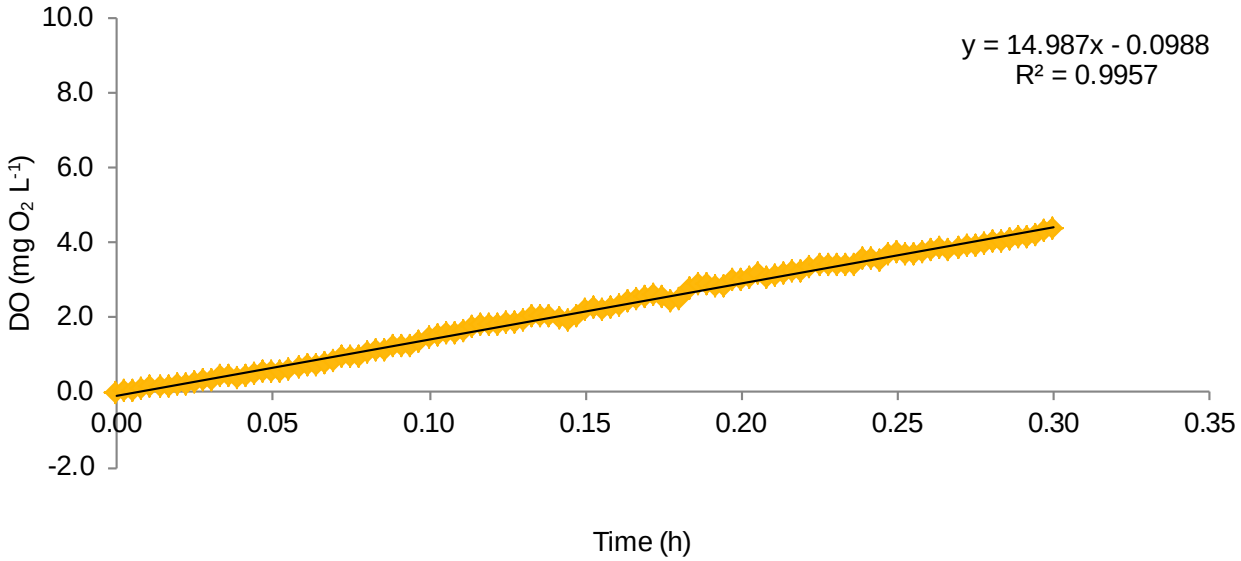

Figure 8.24: Slope of re-aeration C1 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.8^{0}C. The represented data refers to the linear regression (p = 1.09e^{-127} ≤0.05) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.23.

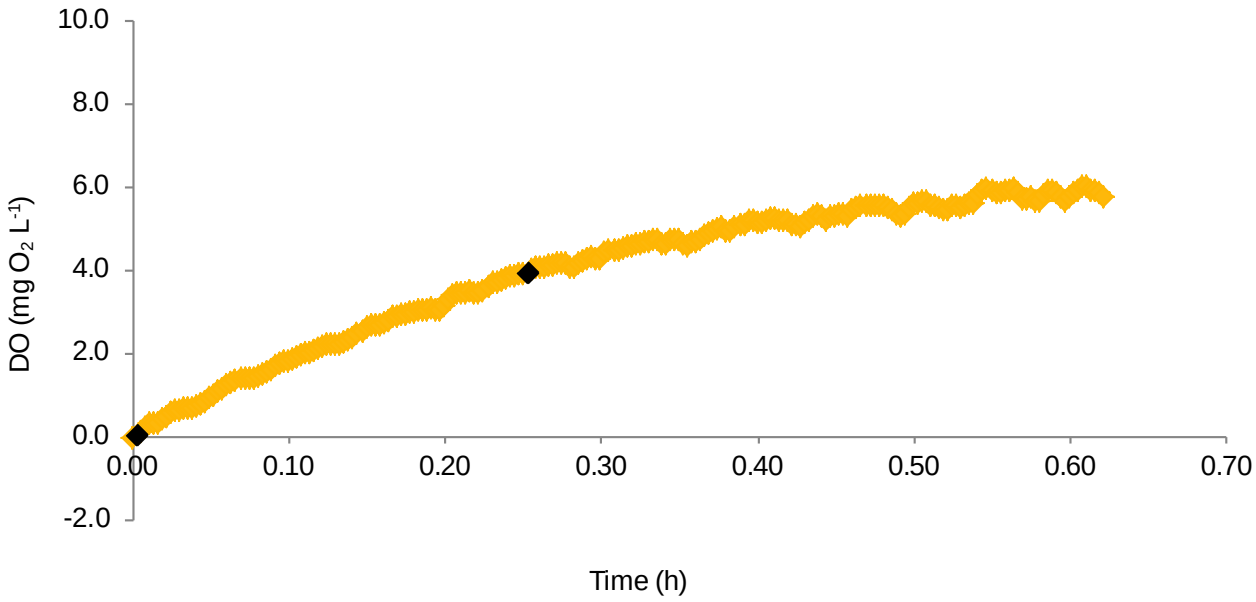

Figure 8.25: Re-aeration C2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.8°C. Data collection occurred every 10 s over 0.62 h. Aeration equipment arranged according to configuration 4; DO probe ~19-22 cm below water, with air stones facing outwards (away from centre). The black dots represent the respective data points between which the linear regression was determined.

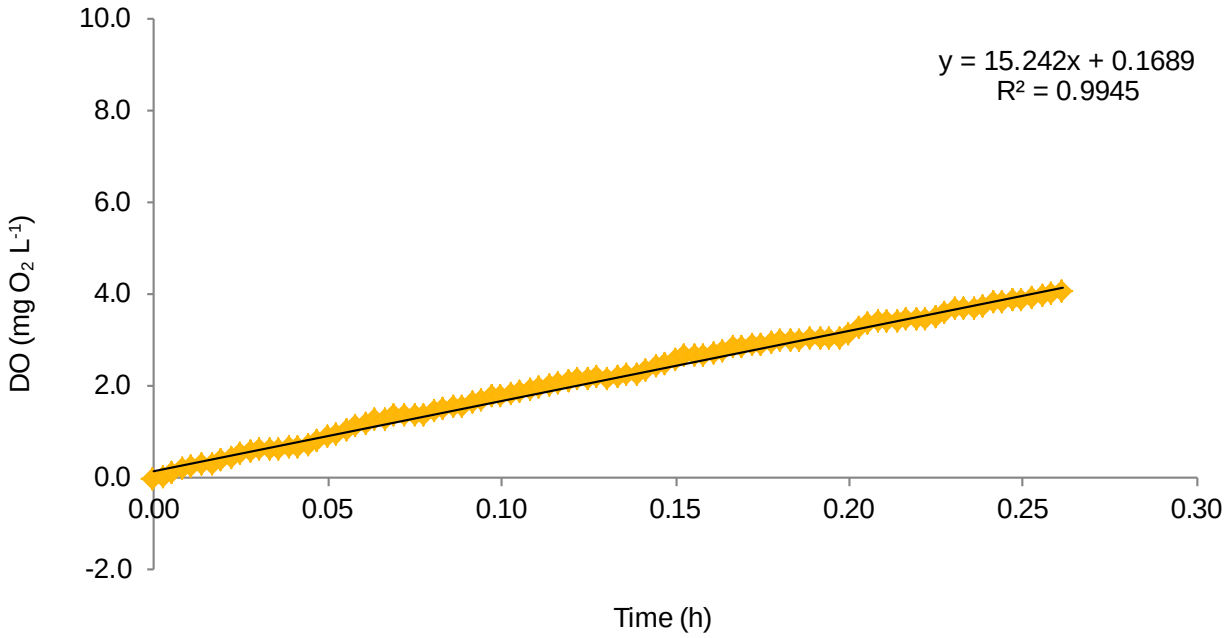

Figure 8.26: Slope of re-aeration C2 of deoxygenated tap water (3L) at a flow rate of 26.31 cc min^{-1} at 23.8^{0}C. The represented data refers to the linear regression ($p = 7.08e^{-107} \leq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.25.

8.2 Appendix B – ANPS aeration plots

8.2.1 Re-aeration of ANPS using equipment configuration 1 (DO probe top + air stones facing inwards)

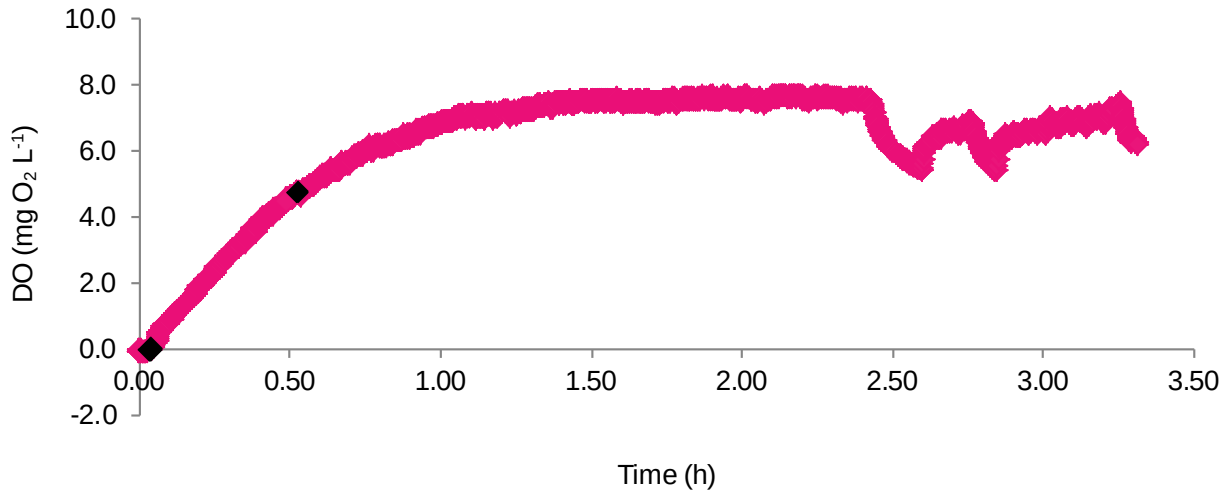

Figure 8.27: Re-aeration 1A of ANPS (2 L) at a flow rate of 26.31 cc min^{-1} at 20.6°C. Data collection occurred every 5 s over 3.50 h. Aeration equipment arranged according to configuration 1; DO probe ~4-6 cm below water, with air stones facing inwards (away from centre). The black dots represent the respective data points between which the linear regression was determined.

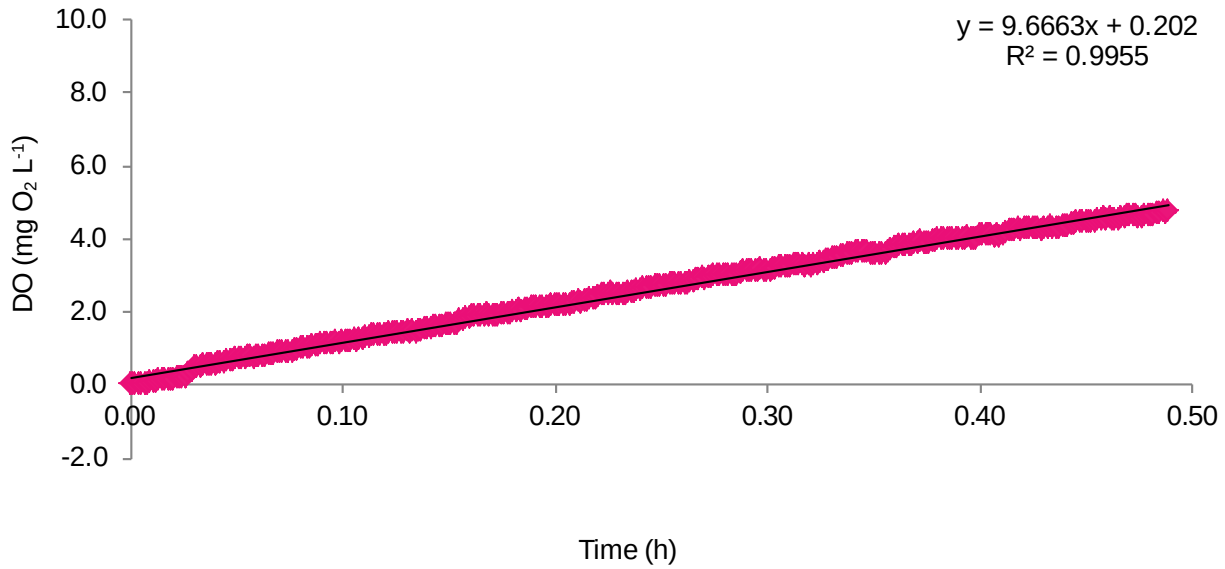

Figure 8.28: Slope of re-aeration 1A of ANPS (3L) at a flow rate of 26.31 cc min^{-1} at 20.6^{0}C. The represented data refers to the linear regression ($p \geq 0.05$) of aeration data between the points of first oxygenation to the point of inflection as stipulated via the darker coloured dots shown in Figure 8.27 (Dark squares). Position of DO probe ~4-6 cm below slurry surface (BW), with air stones (bottom) facing inwards (towards the centre).

9. REFERENCES

ABARES, AUSTRALIAN BUREAU OF AGRICULTURAL AND RESOURCE ECONOMICS AND SCIENCES 2013. Agricultural commodities: September quarter 2013. *In:* FORESTRY, D. O. A. F. A. (ed.). Canberra: ABARES.

ABDEL-RAOUF, N., AL-HOMAIDAN, A. A. & IBRAHEEM, I. B. M. 2012. Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences,* 19**,** 257-275.

ABOU-SHANAB, R. A. I., JI, M.-K., KIM, H.-C., PAENG, K.-J. & JEON, B.-H. 2013. Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. *Journal of Environmental Management,* 115**,** 257-264.

ABS 2012. 2012 Year Book Australia. Canberra: Commonwealth of Australia.

ABS 2013a. LIVESTOCK SLAUGHTERINGS AND OTHER DISPOSALS, 7503.0 - Value of Agricultural Commodities Produced, Australia, 2011-12

Canberra: Commonwealth of Australia.

AGUIRRE, P., ÁLVAREZ, E., FERRER, I. & GARCÍA, J. 2011. Treatment of piggery wastewater in experimental high rate algal ponds. *Revista Latinoamericana de Biotecnología Ambiental y Algal,* 2.

ALLEMAN, J. 1985. Elevated nitrite occurrence in biological wastewater treatment systems. *Water Science and Technology,* 17**,** 409-419.

ÅMAND, L., OLSSON, G. & CARLSSON, B. 2013. Aeration control – a review. *Water Science and Technology,* 67**,** 2374-2398.

AMOO, A. E. & BABALOLA, O. O. 2017. Ammonia-oxidizing microorganisms: key players in the promotion of plant growth. *Journal of soil science and plant nutrition,* 17**,** 935-947.

APHA, (AMERICAN PUBLIC HEALTH ASSOCIATION) 1992. *Standard Methods for the Examination of Water and Wastewater,* Washington D.C., APHA, WEF & AWWA.

APL, AUSTRALIAN PORK LIMITED 2004. National Environmental Guidelines for Piggeries. Deakin, A.C.T.: APL.

APL, AUSTRALIAN PORK LIMITED 2010. Australian Pork Limited Industrial Survey Report. Canberra.

APL, AUSTRALIAN PORK LIMITED, 2015a. project 2012/1028 Piggery Manure and Effluent Management and Reuse Guidelines.

APL, AUSTRALIAN PORK LIMITED, 2015b. Project 2012/1028, Piggery Manure and Effluent Management and Reuse Guidelines.

ASHLEY, K. I., MAVINIC, D. S. & HALL, K. J. 1992. Bench-scale study of oxygen transfer in coarse bubble diffused aeration. *Water Research,* 26**,** 1289-1295.

AYRE, J. 2013. Microalgae culture to treat piggery anaerobic digestion effluent. *Murdoch University,* 88.

BANHAZI, T. & CARGILL, C. 1996. Measuring Air Quality Parameters. *PIN Pig Industry News,* 64**,** 23-25.

BARATI ROSHVANLO, R., REZAEE, A., HOSSINI, H. & SHIRI, M. 2014. Ammonium Removal by Nitrification and Denitrification in an Integrated Fixed Film Activated Sludge Process. *Health Scope,* 3**,** e18347.

BARLOW, E. W. R., BOERSMA, L., PHINNEY, H. K. & MINER, J. R. 1975. Algal growth in diluted pig waste. *Agriculture and Environment,* 2**,** 339-355.

BECKER, E. W. 1994. *Microalgae: biotechnology and microbiology*, Cambridge University Press.

BÉLINE, F., DAUMER, M. L., LOYON, L., POURCHER, A. M., DABERT, P., GUIZIOU, F. & PEU, P. 2008. The efficiency of biological aerobic treatment of piggery wastewater to control nitrogen, phosphorus, pathogen and gas emissions. *Water Science and Technology,* 57**,** 1909-1914.

BÉLINE, F. & MARTINEZ, J. 2002. Nitrogen transformations during biological aerobic treatment of pig slurry: effect of intermittent aeration on nitrous oxide emissions. *Bioresource Technology,* 83**,** 225-228.

BÉLINE, F., MARTINEZ, J., CHADWICK, D., GUIZIOU, F. & COSTE, C.-M. 1999. Factors affecting nitrogen transformations and related nitrous oxide emissions from aerobically treated piggery slurry. *Journal of Agricultural Engineering Research,* 73**,** 235-243.

BELSER, L. W. 1979. Population ecology of nitrifying bacteria. *Annual reviews in microbiology,* 33**,** 309-333.

BENEMANN, J. 2013. Microalgae for Biofuels and Animal Feeds. *Energies,* 6**,** 5869-5886.

BERNET, N. & BÉLINE, F. 2009. Challenges and innovations on biological treatment of livestock effluents. *Bioresource Technology,* 100**,** 5431-5436.

BERNET, N., DELGENES, N., AKUNNA, J. C., DELGENES, J. P. & MOLETTA, R. 2000. Combined anaerobic–aerobic SBR for the treatment of piggery wastewater. *Water Research,* 34**,** 611-619.

BICUDO, J. & SVOBODA, I. F. 1995. Effects of intermittent-cycle extended-aeration treatment on the fate of nutrients, metals and bacterial indicators in pig slurry. *Bioresource Technology,* 54**,** 63-72.

BILOTTA, G. S. & BRAZIER, R. E. 2008. Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research,* 42**,** 2849-2861.

BLOK, J. 1976. Measurements of the viable biomass concentration in activated sludge by respirometric techniques. *Water Research,* 10**,** 919-925.

BOERSMA, L., BARLOW, E., MINER, J., PHINNEY, H. & OLDFIELD, J. 1975. Protein production rates by algae using swine manure as a substrate.

BOLTON, N. 2013. Pathogens in piggery waste and their removal during wastewater treatment. *ALGAE FOR ENERGY AND FEED: A***,** 153.

BONMATÍ, A. & FLOTATS, X. Year. Pig slurry treatment strategy in a high livestock concentration area: anaerobic digestion as the key process. *In:* Latin American Workshop and Symposium on Anaerobic Digestion, 7, 2002. UNAM, 8.

BONMATí, A. & FLOTATS, X. 2003. Air stripping of ammonia from pig slurry: characterisation and feasibility as a pre- or post-treatment to mesophilic anaerobic digestion. *Waste Management,* 23**,** 261-272.

BOROWITZKA, M. A. & MOHEIMANI, N. R. 2013. ALGAE–CULTURE AND APPLICATIONS 7. *ALGAE FOR ENERGY AND FEED: A***,** 79.

BORTONE, G. 2009. Integrated anaerobic/aerobic biological treatment for intensive swine production. *Bioresource technology,* 100**,** 5424-5430.

BRITTO, D. T. & KRONZUCKER, H. J. 2002. NH4+ toxicity in higher plants: a critical review. *Journal of Plant Physiology,* 159**,** 567-584.

BROCKMEIER, S. L., HALBUR, P. G. & THACKER, E. L. 2002. Porcine Respiratory Disease Complex. *In:* BROGDEN, K. A. & M., G. J. (eds.) *Polymicrobial Diseases.* Washington (DC): ASM Press.

BROWN, R. 1984. RELATIONSHIPS BETWEEN SUSPENDED SOLIDS, TURBIDITY, LIGHT ATTENUATION, AND ALGAL PRODUCTIVITY. *Lake and Reservoir Management,* 1**,** 198-205.

BUCHANAN, A. N. 2014. Comparing the performance of a high rate algal pond with a waste stabilisation pond in rural South Australia. *Flinders University*.

BUCHANAN, A. N., BOLTON, N., MOHEIMANI, N., SVOBODA, I. F., GRANT, T., BATTEN, D., CHENG, N. N., BOROWITZKA, M. & FALLOWFIELD, H. J. 2013. Algae For Energy and Feed: A Wasterwater Solution. A Review (Project 4A-101 112). 210.

BURTON, C. H. 1992. A review of the strategies in the aerobic treatment of pig slurry: Purpose, theory and method. *Journal of Agricultural Engineering Research,* 53**,** 249-272.

BURTON, C. H. & FARRENT, J. W. 1998. Continuous Aerobic Treatment of Pig Slurry: Evaluation of Options based on Long-treatment Time and Two-stage Processing. *Journal of Agricultural Engineering Research,* 69**,** 159-167.

BURTON, C. H. & SNEATH, R. W. 1995. Continuous Farm Scale Aeration Plant for Reducing Offensive Odours from Piggery Slurry: Control and Optimization of the Process. *Journal of Agricultural Engineering Research,* 60**,** 271-279.

BURTON, C. H. & TURNER, C. 2003. *Manure Management: Treatment Strategies for Sustainable Agriculture*, Silsoe Research Institute.

CALVET, S., HUNT, J. & MISSELBROOK, T. H. 2017. Low frequency aeration of pig slurry affects slurry characteristics and emissions of greenhouse gases and ammonia. *Biosystems Engineering,* 159**,** 121-132.

CARGILL, C., MURPHY, T. & BANHAZI, T. 2002. Hygiene and air quality in intensive housing facilities in Australia.

CARGILL, C. & SKIRROW, S. C. 1997. Air quality in pig housing facilities. *Pig Prod. Proc. Post Graduate Foundation Veterinary Science.,* The University of Sydney**,** 85-103.

CASEY, K., MCGAHAN, E., ATZENI, M., GARDNER, E. & FRIZZO, R. 1999. PIGBAL: A Nutrient Balance Model for Intensive Piggeries. Version.

CHAIKLAHAN, R., CHIRASUWAN, N., SIANGDUNG, W., PAITHOONRANGSARID, K. & BUNNAG, B. 2010. Cultivation of Spirulina platensis using pig wastewater in a semi-continuous process. *J Microbiol Biotechnol,* 20**,** 609-14.

CHEN, S., TIMMONS, M. B., ANESHANSLEY, D. J. & BISOGNI, J. J. 1992. Bubble size distribution in a bubble column applied to aquaculture systems. *Aquacultural Engineering,* 11**,** 267-280.

CHEN, Y. 1983. Kinetic analysis of anaerobic digestion of pig manure and its design implications. *Agricultural Wastes,* 8**,** 65-81.

CHENG, J. & LIU, B. 2002. Swine wastewater treatment in anaerobic digesters with floating medium. *Transactions of the ASAE,* 45**,** 799-805.

CHOI, E. 2007. *Piggery waste management*, IWA Publishing.

CHYNOWETH, D., WILKIE, A. & OWENS, J. 1999. Anaerobic treatment of piggery slurry. *Asian–Australian Journal of Animal Science,* 12**,** 607-628.

CHYNOWETH, D. P., WILKIE, A. C. & OWENS, J. M. Year. Anaerobic treatment of piggery slurry.

In: Managing Animal Wastes and their Utilisation, 8th World Conference in Animal Production, Seoul, South Korea June, 1998. 391-427.

COLINA, J., LEWIS, A. & MILLER, P. S. 2000. A Review of the Ammonia Issue and Pork Production.

CÔTÉ, C., MASSÉ, D. I. & QUESSY, S. 2006. Reduction of indicator and pathogenic microorganisms by psychrophilic anaerobic digestion in swine slurries. *Bioresource Technology,* 97**,** 686-691.

CRAGGS, R. 2005. Advanced integrated wastewater ponds. *Pond treatment technology. IWA scientific and technical report series. IWA, London***,** 282-310.

CRAGGS, R., PARK, J., HEUBECK, S. & SUTHERLAND, D. 2014. High rate algal pond systems for low-energy wastewater treatment, nutrient recovery and energy production. *New Zealand Journal of Botany,* 52**,** 60-73.

CRAGGS, R., SUTHERLAND, D. & CAMPBELL, H. 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology,* 24**,** 329-337.

CRAGGS, R. J., HEUBECK, S., LUNDQUIST, T. J. & BENEMANN, J. R. 2011. Algal biofuels from wastewater treatment high rate algal ponds. *Water Science and Technology,* 63**,** 660-665.

CROFTS, A. R. 1966. Uptake of ammonium ion by chloroplasts, and the mechanism of amine uncoupling. *Biochemical and Biophysical Research Communications,* 24**,** 127-134.

CROMAR, N. J. & FALLOWFIELD, H. J. 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. *Journal of Applied Phycology,* 9**,** 301-309.

CUMBY, T. R. 1987a. A review of slurry aeration 1. Factors affecting oxygen transfer. *Journal of Agricultural Engineering Research,* 36**,** 141-156.

CUMBY, T. R. 1987b. A review of slurry aeration 2. Mixing and foam control. *Journal of Agricultural Engineering Research,* 36**,** 157-174.

CUMBY, T. R. 1987c. A review of slurry aeration 3. Performance of aerators. *Journal of Agricultural Engineering Research,* 36**,** 175-206.

CUMBY, T. R. 1990. Slurry mixing with impellers: Part 1, theory and previous research. *Journal of Agricultural Engineering Research,* 45**,** 157-173.

DEPARTMENT OF AGRICULTURE FISHERIES AND FORESTRY. 2012. *Establishing a piggery* [Online]. Queensland. Available: http://www.daff.qld.gov.au/animal-industries/pigs/getting-started/establishing-a-piggery [Accessed].

DOSMAN, J. A., SENTHILSELVAN, A., KIRYCHUK, S. P., LEMAY, S., BARBER, E. M., WILLSON, P., CORMIER, Y. & HURST, T. S. 2000. Positive Human Health Effects of Wearing a Respirator in a Swine Barn. *Chest,* 118**,** 852-860.

DOWNING, L. S. & NERE, R. Year. Nitrification in the activated sludge process. *In:* J. Proc. Inst. Sewage Purification, 1964. Citeseer.

DROSTE, R. L. 1997. *Theory and practice of water and wastewater treatment*, John Wiley & Sons Incorporated.

DUNCAN, D., WALKER, M. & HARVEY, F. 2007. *Regulatory Monitoring and Testing: Water and Wastewater Sampling*, Environment Protection Authority.

EK, A., HALLIN, S., VALLIN, L., SCHNURER, A. & KARLSSON, M. Year. Slaughterhouse waste co-digestion-Experiences from 15 years of full-scale operation. *In:* World Renewable Energy Congress-Sweden 8-13 May, 2011: Volume 1 (Bioenergy Technology), 2011.

Linköping University Electronic Press, Linköpings universitet, 64-71.

ENVIRONMENT PROTECTION AGENCY, AUSTRALIA., M. B. C. S. & LTD, F. S. A. P. 2000. Alternative Systems for Piggery Effluent Treatment: Report Prepared for : Environment Protection Agency (South Australia) and the Rural City of Murray Bridge. Environment Protection Agency and the City of Murray Bridge.

EVANS, M., HISSETT, R., SMITH, M., ELLAM, D., BAINES, S. & WOODS, J. 1979. Effect of microorganism residence time on aerobic treatment of piggery waste. *Agricultural Wastes,* 1**,** 67-85.

EVANS, M., HISSETT, R., SMITH, M., THACKER, F. & WILLIAMS, A. 1980. Aerobic treatment of beef cattle and poultry waste compared with the treatment of piggery waste. *Agricultural Wastes,* 2**,** 93-101.

EVANS, M. R., DEANS, E. A., HISSETT, R., SMITH, M. P. W., SVOBODA, I. F. & THACKER, F. E. 1983. The effect of temperature and residence time on aerobic treatment of piggery slurry—Degradation of carbonaceous compounds. *Agricultural Wastes,* 5**,** 25-36.

EVANS, M. R., SMITH, M. P. W., DEANS, E. A., SVOBODA, I. F. & THACKER, F. E. 1986. Nitrogen and aerobic treatment of slurry. *Agricultural Wastes,* 15**,** 205-213.

EVANS, M. R., SVOBODA, I. F. & BAINES, S. 1982. Heat from aerobic treatment of piggery slurry. *Journal of Agricultural Engineering Research,* 27**,** 45-50.

FALLOWFIELD, H. 2013. INTEGRATED PIGGERY WASTEWATER TREATMENT AND 8 MICROALGAL BIOMASS PRODUCTION. *ALGAE FOR ENERGY AND FEED: A***,** 128.

FALLOWFIELD, H. J. & GARRETT, M. K. 1985a. The photosynthetic treatment of pig slurry in temperate climatic conditions: A pilot-plant study. *Agricultural Wastes,* 12**,** 111-136.

FALLOWFIELD, H. J. & GARRETT, M. K. 1985b. The treatment of wastes by algal culture. *Journal of Applied Bacteriology,* 59**,** 187S-205S.

FALLOWFIELD, H. J., MARTIN, N. J. & CROMAR, N. J. 1999. Performance of a batch-fed High Rate Algal Pond for animal waste treatment. *European Journal of Phycology,* 34**,** 231-237.

FÄNDRIKS, I. 2011. Alternative Methods for Evaluation of Oxygen Transfer Performance in Clean Water.

FAO 2009. *The state of food and agriculture, 2009*, Economic and Social Development Department.

FAO, FOOD AND AGRICULTURE ORGANIZATION. 2013. *FAOSTAT* [Online]. Available: http://faostat3.fao.org/home/index.html#VISUALIZE). [Accessed 2013].

FAOSTAT, FOOD AND AGRICULTURE ORGANIZATION 2017. FAOSTAT Live Animals.

FLOTATS RIPOLL, X., FOGED, H., BONMATÍ BLASI, A., PALATSI CIVIT, J., MAGRÍ ALOY, A. & SCHELDE, K. M. 2012. Manure processing technologies.

FUJIE, K., URANO, K., KUBOTA, H. & KASAKURA, T. 1992. Hydrodynamics and Oxygen Transfer Characteristics in Activated Sludge Aeration Tanks. *Water Science and Technology,* 26**,** 791-800.

GARCIA, C., HERNANDEZ, T., COSTA, F., CECCANTI, B. & CIARDI, C. 1992. Changes in ATP content, enzyme activity and inorganic nitrogen species during composting of organic wastes. *Canadian Journal of Soil Science,* 72**,** 243-253.

GARCÍA, J., GREEN, B. F., LUNDQUIST, T., MUJERIEGO, R., HERNÁNDEZ-MARINÉ, M. & OSWALD, W. J. 2006. Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. *Bioresource Technology,* 97**,** 1709-1715.

GARCIA, J., MUJERIEGO, R. & HERNANDEZ-MARINE, M. 2000. High rate algal pond operating strategies for urban wastewater nitrogen removal. *Journal of Applied Phycology,* 12**,** 331-339.

GERARDI, M. H. 2002. *Nitrification in the Activated Sludge Process*, Wiley Online Library.

GERARDI, M. H. 2011. *Troubleshooting the sequencing batch reactor*, John Wiley & Sons.

GERCHMAN, Y., VASKER, B., TAVASI, M., MISHAEL, Y., KINEL-TAHAN, Y. & YEHOSHUA, Y. 2017. Effective harvesting of microalgae: Comparison of different polymeric flocculants. *Bioresource Technology,* 228**,** 141-146.

GINNIVAN, M. J. 1983. The effect of aeration rates on odour and solids of pig slurry. *Agricultural Wastes,* 7**,** 197-207.

GIRARD, M., PALACIOS, J. H., BELZILE, M., GODBOUT, S. & PELLETIER, F. 2013. Biodegradation in Animal Manure Management. *In:* CHAMY, R. & ROSENKRANZ, F. (eds.) *Biodegradation - Engineering and Technology,*. https://www.intechopen.com/books/biodegradation-engineering-and-technology/biodegradation-in-animal-manure-management: IntechOpen.

GREEN, F. B., BERNSTONE, L. S., LUNDQUIST, T. J. & OSWALD, W. J. 1996. Advanced integrated wastewater pond systems for nitrogen removal. *Water Science and Technology,* 33**,** 207-217.

GROBBELAAR, J. U. 2010. Microalgal biomass production: challenges and realities. *Photosynthesis Research,* 106**,** 135-144.

GROENEWEG, J., KLEIN, B., MOHN, F., RUNKEL, K. & STENGEL, E. 1980. First results of outdoor treatment of pig manure with algal-bacterial systems. *Algae biomass: production and use/[sponsored by the National Council for Research and Development, Israel and the Gesellschaft fur Strahlen-und Umweltforschung (GSF), Munich, Germany]; editors, Gedaliah Shelef, Carl J. Soeder*.

GUISASOLA, A., PETZET, S., BAEZA, J. A., CARRERA, J. & LAFUENTE, J. 2007. Inorganic carbon limitations on nitrification: Experimental assessment and modelling. *Water Research,* 41**,** 277-286.

GUO, J., PENG, Y., WANG, S., ZHENG, Y., HUANG, H. & WANG, Z. 2009. Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. *Bioresource Technology,* 100**,** 2796-2802.

GUTIÉRREZ, R., PASSOS, F., FERRER, I., UGGETTI, E. & GARCÍA, J. 2015. Harvesting microalgae from wastewater treatment systems with natural flocculants: Effect on biomass settling and biogas production. *Algal Research,* 9**,** 204-211.

HANAKI, K., WANTAWIN, C. & OHGAKI, S. 1990. Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor. *Water Research,* 24**,** 297-302.

HII, Y., SOO, C., CHUAH, T., MOHD-AZMI, A. & ABOL-MUNAFI, A. 2011. Interative Effect of Ammonia and Nitrate on the Nitrogen Uptake by Nannochloropsis sp. *Journal of Sustainability Science and Management,* 6**,** 60-68.

HOLM-NIELSEN, J. B., AL SEADI, T. & OLESKOWICZ-POPIEL, P. 2009. The future of anaerobic digestion and biogas utilization. *Bioresource Technology,* 100**,** 5478-5484.

HUDSON, N., BELL, K., MCGAHAN, E., LOWE, S., GALVIN, G. & CASEY, K. 2007. Odour emissions from anaerobic piggery ponds. 2: Improving estimates of emission rate through recognition of spatial variability. *Bioresource technology,* 98**,** 1888-1897.

IMBEAH, M. 1998. Composting piggery waste: A review. *Bioresource Technology,* 63**,** 197-203.

ISLAM, M. N., PARK, K. J. & ALAM, M. J. 2011. Treatment of swine wastewater using sequencing batch reactor. *Engineering in Agriculture, Environment and Food,* 4**,** 47-53.

JIMÉNEZ-PÉREZ, M. V., SÁNCHEZ-CASTILLO, P., ROMERA, O., FERNÁNDEZ-MORENO, D. & PÉREZ-MARTíNEZ, C. 2004. Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. *Enzyme and Microbial Technology,* 34**,** 392-398.

KAMPSCHREUR, M. J., TEMMINK, H., KLEEREBEZEM, R., JETTEN, M. S. M. & VAN LOOSDRECHT, M. C. M. 2009. Nitrous oxide emission during wastewater treatment. *Water Research,* 43**,** 4093-4103.

KAPARAJU, P. & RINTALA, J. 2011. Mitigation of greenhouse gas emissions by adopting anaerobic digestion technology on dairy, sow and pig farms in Finland. *Renewable Energy,* 36**,** 31-41.

KEBEDE-WESTHEAD, E., PIZARRO, C. & MULBRY, W. W. 2006. Treatment of swine manure effluent using freshwater algae: Production, nutrient recovery, and elemental composition of algal biomass at four effluent loading rates. *Journal of Applied Phycology,* 18**,** 41-46.

KIM, K. Y., KO, H. J., KIM, H. T., KIM, Y. S., ROH, Y. M. & KIM, C. N. 2007. Effect of ventilation rate on gradient of aerial contaminants in the confinement pig building. *Environmental Research,* 103**,** 352-357.

KLEANTHOUS, A. 2009. Pigs and the Environment. *In:* BPEX (ed.). Warwickshire: BPEX.

KRUGER, I. R., TAYLOR, G. & FERRIER, M. 1995. *Effluent at Work*, NSW Agricultre.

KUTTY, S., ISA, M. & LEONG, L. 2011a. The Effect of Ammonia Loading on the NitrificationKinetic of Aerobic Baffled Continuous Biological Reactor. *International Journal of Environmental Science and Development,* 2**,** 259.

KUTTY, S., ISA, M. & LEONG, L. 2011b. Removal of ammonia-nitrogen (NH3-N) and nitrate (NO3-) by modified conventional activated-sludge system to meet new DOE regulations.

LIN, Q., HE, G., RUI, J., FANG, X., TAO, Y., LI, J. & LI, X. 2016. Microorganism-regulated mechanisms of temperature effects on the performance of anaerobic digestion. *Microbial Cell Factories,* 15**,** 96.

LUSK, P. & WISELOGEL, A. 1998. *Methane recovery from animal manures: the current opportunities casebook*, National Renewable Energy Laboratory Golden, CO

LYERLY, C. N. 2005. Swine Wastewater Treatment in an Integrated System of Anaerobig Digestion and Duckweed Nutrient Removal: Pilot Study.

MARTIN, N. & FALLOWFIELD, H. 1989. Computer modelling of algal waste treatment systems. *Water science and technology,* 21**,** 1657-1660.

MATSUDO, M. C., BEZERRA, R. P., SATO, S., PEREGO, P., CONVERTI, A. & CARVALHO, J. C. M. 2009. Repeated fed-batch cultivation of Arthrospira (Spirulina) platensis using urea as nitrogen source. *Biochemical Engineering Journal,* 43**,** 52-57.

MCCARTY, P. L. 1964. Anaerobic waste treatment fundamentals. *Public works,* 95**,** 107-112.

MCGAHAN, E. J., OUELLET-PLAMONDON, C. & WATTS, P. J. 2010. Estimates of Manure Production from Animals for Methane Generation *In:* CORPORATION, R. I. R. A. D. (ed.). Kingston: RIRDC.

MCGLONE, J. J. 2013. The Future of Pork Production in the World: Towards Sustainable, Welfare-Positive Systems. *Animals,* 3**,** 401-415.

MEHRABADI, A., CRAGGS, R. & FARID, M. M. 2015. Wastewater treatment high rate algal ponds (WWT HRAP) for low-cost biofuel production. *Bioresource Technology,* 184**,** 202-214.

MOBIN, S. & ALAM, F. Year. Biofuel production from algae utilizing wastewater. *In*, 2014.

MOHAIBES, M. & HEINONEN-TANSKI, H. 2004. Aerobic thermophilic treatment of farm slurry and food wastes. *Bioresource Technology,* 95**,** 245-254.

MOHEDANO, R. A., COSTA, R. H. R., TAVARES, F. A. & BELLI FILHO, P. 2012. High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. *Bioresource Technology,* 112**,** 98-104.

MÖLLER, K. & MÜLLER, T. 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences,* 12**,** 242-257.

MOREAUX, B., NEMMAR, A., BEERENS, D. & GUSTIN, P. 2000. Inhibiting effect of ammonia on citric acid-induced cough in pigs: a possible involvement of substance P. *Pharmacology & toxicology,* 87**,** 279-285.

MOUTAFCHIEVA, D., POPOVA, D., DIMITROVA, M. & TCHAOUSHEV, S. 2013. Experimental determination of the volumetric mass transfer coefficient. *Journal of Chemical Technology and Metallurgy,* 48**,** 351-356.

MULLER, E. B., STOUTHAMER, A. H., VAN VERSEVELD, H. W. & EIKELBOOM, D. H. 1995. Aerobic domestic waste water treatment in a pilot plant with complete sludge retention by cross-flow filtration. *Water Research,* 29**,** 1179-1189.

MURPHY, T. 2011. *The effects of individual and combinations of airbourne pollutants on feed intake, immune function and physiology of the pig.* pHD, University of Adelaide.

MURPHY, T., CARGILL, C., RUTLEY, D. & STOTT, P. 2012. Pig-shed air polluted by α-haemolytic cocci and ammonia causes subclinical disease and production losses. *Veterinary Record,* 171**,** 123-123.

NEUFELD, R., GREENFIELD, J. & RIEDER, B. 1986. Temperature, cyanide and phenolic nitrification inhibition. *Water Research,* 20**,** 633-642.

NORSKER, N.-H., BARBOSA, M. J., VERMUË, M. H. & WIJFFELS, R. H. 2011. Microalgal production — A close look at the economics. *Biotechnology Advances,* 29**,** 24-27.

ÖZBEK, B. & GAYIK, S. 2001. The studies on the oxygen mass transfer coefficient in a bioreactor. *Process Biochemistry,* 36**,** 729-741.

PARK, J. B. K., CRAGGS, R. J. & SHILTON, A. N. 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology,* 102**,** 35-42.

PETERSEN, S. O., SOMMER, S. G., BÉLINE, F., BURTON, C., DACH, J., DOURMAD, J. Y., LEIP, A., MISSELBROOK, T., NICHOLSON, F., POULSEN, H. D., PROVOLO, G., SØRENSEN, P., VINNERÅS, B., WEISKE, A., BERNAL, M. P., BÖHM, R., JUHÁSZ, C. & MIHELIC, R. 2007. Recycling of livestock manure in a whole-farm perspective. *Livestock Science,* 112**,** 180-191.

PITTOORS, E., GUO, Y. & VAN HULLE, S. W. H. 2014. Oxygen transfer model development based on activated sludge and clean water in diffused aerated cylindrical tanks. *Chemical Engineering Journal,* 243**,** 51-59.

PORTEJOIE, S., MARTINEZ, J., GUIZIOU, F. & COSTE, C. M. 2003. Effect of covering pig slurry stores on the ammonia emission processes. *Bioresource Technology,* 87**,** 199-207.

POURCHER, A. M., MARTI, R., THORIGNÉ, A., JÉGOU, B., DABERT, P. & ALAND, A. Year. Effect of anaerobic storage and aerobic digestion on micro-organisms in pig manure: cultural and molecular approaches. *In:* Proceedings XIII International Congress in Animal

Hygiene ISAH Vol. 1, Estonian University of Life SciencesTartu, Estonia, June 17-21, 2007, Tartu, 2007.

PRAKASAM, T. B. S. & LOEHR, R. C. 1972. Microbial nitrification and denitrification in concentrated wastes. *Water Research,* 6**,** 859-869.

PRINČIČ, A., MAHNE, I., MEGUŠAR, F., PAUL, E. A. & TIEDJE, J. M. 1998. Effects of pH and Oxygen and Ammonium Concentrations on the Community Structure of Nitrifying Bacteria from Wastewater. *Applied and Environmental Microbiology,* 64**,** 3584-3590.

R DEVELOPMENT CORE TEAM. 2016. *A language and environment for statistical computing. R Foundation for Statistical Computing* [Online]. Vienna, Austria. Available: https://www.R-project.org/. [Accessed].

RIEGER, L., GILLOT, S., LANGERGRABER, G., OHTSUKI, T., SHAW, A., TAKACS, I. & WINKLER, S. 2012. *Guidelines for Using Activated Sludge Models*, IWA Publishing.

RIGOLOT, C., ESPAGNOL, S., POMAR, C. & DOURMAD, J. 2010. Modelling of manure production by pigs and NH3, N2O and CH4 emissions. Part I: animal excretion and enteric CH4, effect of feeding and performance. *Animal: an international journal of animal bioscience,* 4**,** 1401.

ROBERTSON, G. & GROFFMAN, P. 2007. Nitrogen transformations. *Soil microbiology, ecology, and biochemistry,* 3**,** 341-364.

ROMAN, M.-D. & MUREŞAN, M.-V. 2014. Analysis of oxygen Requirement and Transfer Efficiency in a Wastewater Treatment Plant. *International Journal of Latest Research in Science and Technology,* 3**,** 30-33.

SAFLEY JR, L. M. & WESTERMAN, P. W. 1990. Psychrophilic anaerobic digestion of animal manure: Proposed design methodology. *Biological Wastes,* 34**,** 133-148.

SARMIENTO, F. B., LEIGH, J. A. & WHITMAN, W. B. 2011. Chapter three - Genetic Systems for Hydrogenotrophic Methanogens. *In:* ROSENZWEIG, A. C. & RAGSDALE, S. W. (eds.) *Methods in Enzymology.* Academic Press.

SEVIOUR, R. & NIELSEN, P. H. 2010. *Microbial Ecology of Activated Sludge*, IWA Publishing.

SHAMMAS, N. K. 1986. Interactions of temperature, pH, and biomass on the nitrification process. *Journal (Water Pollution Control Federation)***,** 52-59.

SHERRARD, J. H. 1976. Destruction of Alkalinity in Aerobic Biological Wastewater Treatment. *Journal (Water Pollution Control Federation),* 48**,** 1834-1839.

SIALVE, B., BERNET, N. & BERNARD, O. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances,* 27**,** 409-416.

SNEATH, R., BURTON, C. & WILLIAMS, A. Year. The performance of a farm scale aerobic treatment plant for reducing the odours from piggery slurry. *In:* Agricultural and food processing waste: proceedings fo the 6th International Symposium on agricultural and food processing wastes, December 17-18, 1990, Chicago, USA., 1990. American Society of Agricultural Engineers, 460-469.

SNEATH, R. W., SHAW, M. & WILLIAMS, A. G. 1988. Centrifugation for separating piggery slurry 1. The performance of a decanting centrifuge. *Journal of Agricultural Engineering Research,* 39**,** 181-190.

SPELLMAN, F. R. 2013. *Handbook of water and wastewater treatment plant operations*, CRC Press.

STAFFORD, D. A., HAWKES, D. L. & HORTON, R. 1980. *Methane production from waste organic*

matter, CRC Press.

STRAIN, J. J., FALLOWFIELD, H. J., FRASER, T. W. & GARRETT, M. K. 1986. A nutritional evaluation of farm waste grown and axenically cultured algal biomass. *Agricultural Wastes,* 15**,** 235-252.

STRAUSS, E. A. & LAMBERTI, G. A. 2000. Regulation of nitrification in aquatic sediments by organic carbon. *Limnology and Oceanography,* 45**,** 1854-1859.

SURMACZ-GORSKA, J., GERNAEY, K., DEMUYNCK, C., VANROLLEGHEM, P. & VERSTRAETE, W. 1996. Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements. *Water Research,* 30**,** 1228-1236.

SUTHERLAND, D. L., HOWARD-WILLIAMS, C., TURNBULL, M. H., BROADY, P. A. & CRAGGS, R. J. 2014. Seasonal variation in light utilisation, biomass production and nutrient removal by wastewater microalgae in a full-scale high-rate algal pond. *Journal of Applied Phycology,* 26**,** 1317-1329.

SUTHERLAND, D. L., HOWARD-WILLIAMS, C., TURNBULL, M. H., BROADY, P. A. & CRAGGS, R. J. 2015a. Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology,* 184**,** 222-229.

SUTHERLAND, D. L., MONTEMEZZANI, V., HOWARD-WILLIAMS, C., TURNBULL, M. H., BROADY, P. A. & CRAGGS, R. J. 2015b. Modifying the high rate algal pond light environment and its effects on light absorption and photosynthesis. *Water Research,* 70**,** 86-96.

SVOBODA, I. 2003. Anaerobic Digestion, Storage, Oligolysis, Lime, Heat and Aerobic Treatment of Livestock Manures. *F. Services (Ed.)***,** 110.

SVOBODA, I., BUCHANAN, N. & FALLOWFIELD, H. 2013. AEROBIC TREATMENT OF PIGGERY WASTEWATERS 6. *ALGAE FOR ENERGY AND FEED: A***,** 62.

SVOBODA, I. F. 1989. A computer program for farm waste management. *Water Science & Technology,* 21**,** 1865-1868.

SVOBODA, I. F. Year. Nitrogen removal from pig slurry by nitrification and denitrification. *In:* Proceedings of the Seventh International Symposium on Agricultural and Food Processing Wastes, 1995. 18-20.

SVOBODA, I. F. & EVANS, M. R. 1987. Heat from aeration of piggery slurry. *Journal of Agricultural Engineering Research,* 38**,** 183-192.

SVOBODA, I. F. & FALLOWFIELD, H. J. 1989. An Aerobic Piggery Slurry Treatment System with Integrated Heat Recovery and High-Rate Algal Ponds. *Water Science & Technology,* 21**,** 277-287.

TAUSEEF, S., PREMALATHA, M., ABBASI, T. & ABBASI, S. 2013. Methane capture from livestock manure. *Journal of environmental management,* 117**,** 187-207.

TCHOBANOGLOUS, G., BURTON, F. L., STENSEL, H. D., METCALF & EDDY, I. 2003. *Wastewater Engineering: Treatment and Reuse*, McGraw-Hill Education.

THAKRE, S., BHUYAR, L. & DESHMUKH, S. 2008. Effect of different configurations of mechanical aerators on oxygen transfer and aeration efficiency with respect to power consumption. *International Journal of Aerospace and Mechanical Engineering,* 2**,** 100-108.

TRIBE, L., BRIENS, C. & MARGARITIS, A. 1995. Determination of the volumetric mass transfer coefficient (kLa) using the dynamic “gas out–gas in” method: analysis of errors caused by dissolved oxygen probes. *Biotechnology and bioengineering,* 46**,** 388-392.

TUCKER, R. W. 2015. Piggery Manure and Effluent Management and Reuse Guidelines 2015.

TUCKER, R. W. 2018. *National Environmental Guidelines for Indoor Piggeries - Third Edition, APL Project 2015-2221,* Kingston, ACT, Australia, Australian Pork Ltd.

TUCKER, R. W., MCGAHAN, E. J., GALLOWAY, J. L. & O'KEEFE, M. F. 2010. *National Environmental Guidelines for Piggeries - Second Edition,* Deakin, ACT, Australia

TUCKER, R. W. & O'KEEFE, M. F. 2013. National Environmental Guidelines for Rotational Outdoor Piggeries, Project 2011/1039 (2013) Barton, ACT, 2600 (revised): Australian Pork Ltd;.

VAN DE GRAAF, A. A., MULDER, A., DE BRUIJN, P., JETTEN, M. S., ROBERTSON, L. A. & KUENEN, J. G. 1995. Anaerobic oxidation of ammonium is a biologically mediated process. *Applied and Environmental Microbiology,* 61**,** 1246-51.

VAN HAANDEL, A. & VAN DER LUBBE, J. 2012. *Handbook of Biological Wastewater Treatment*, IWA Publishing.

VAN LIER, J. B., MAHMOUD, N. & ZEEMAN, G. 2008. Anaerobic wastewater treatment. *biological wastewater treatment, principles, modelling and design***,** 415-456.

VAN LIER, J. B., MARTIN, J. L. S. & LETTINGA, G. 1996. Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. *Water Research,* 30**,** 199-207.

VANOTTI, M. & HUNT, P. 2000. Nitrification treatment of swine wastewater with acclimated nitrifying sludge immobilized in polymer pellets.

VANOTTI, M. B., SZÖGI, A. A. & VIVES, C. A. Year. Greenhouse gas emission reductions and carbon credits from implementation of aerobic manure treatment systems in swine farms. *In:* Proceedings of the workshop on agricultural air quality: State of the science, 2006.

VEEKEN, A., KALYUZHNYI, S., SCHARFF, H. & HAMELERS, B. 2000. Effect of pH and VFA on hydrolysis of organic solid waste. *Journal of environmental engineering,* 126**,** 1076-1081.

VELHO, V., MOHEDANO, R., FILHO, P. & COSTA, R. 2012. The viability of treated piggery wastewater for reuse in agricultural irrigation. *International Journal of Recycling of Organic Waste in Agriculture,* 1**,** 1-9.

WADLEIGH, C. H. 1968. *Wastes in relation to agriculture and forestry*, US Govt. Print. Off.

.

WANAPAT, M., CHERDTHONG, A., PHESATCHA, K. & KANG, S. 2015. Dietary sources and their effects on animal production and environmental sustainability. *Animal Nutrition,* 1**,** 96-103.

WESNER, G. M., CULP, G. L. & LINECK, T. 1978. Energy conservation in municipal waste water treatment. *EPA Publication.* EPA.

WETT, B. & RAUCH, W. 2003. The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. *Water Research,* 37**,** 1100-1110.

WIEDEMANN, S. G., MCGAHAN, E. J. & MURPHY, C. M. 2012. Energy, Water and Greenhouse Gas Emissions in Australian Pork Supply Chains: A life Cycle Assessment. *Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry***,** 1-103.

WILLERS, H., DERIKX, P., TEN HAVE, P. & VIJN, T. 1998. Nitrification limitation in animal slurries at high temperatures. *Bioresource technology,* 64**,** 47-54.

WITTMANN, J. W., THIEL, D. J. & SMITH, G. W. 1990. Activated sludge wastewater treatment process. Google Patents.

YUAN, X., KUMAR, A., SAHU, A. K. & ERGAS, S. J. 2011. Impact of ammonia concentration on

Spirulina platensis growth in an airlift photobioreactor. *Bioresource Technology,* 102**,** 3234-3239.

ZABRANSKA, J. & POKORNA, D. 2018. Bioconversion of carbon dioxide to methane using hydrogen and hydrogenotrophic methanogens. *Biotechnology Advances,* 36**,** 707-720.

ZHANG, M., LAWLOR, P. G., WU, G., LYNCH, B. & ZHAN, X. 2011. Partial nitrification and nutrient removal in intermittently aerated sequencing batch reactors treating separated digestate liquid after anaerobic digestion of pig manure. *Bioprocess and biosystems engineering,* 34**,** 1049-1056.

ZHANG, Z. & ZHU, J. 2005. Effectiveness of short-term aeration in treating swine finishing manure to reduce odour generation potential. *Agriculture, Ecosystems & Environment,* 105**,** 115-125.

ZHEN, H., PETIRAKSAKUL, A. & MEESAPYA, W. 2003. Oxygen-transfer measurement in clean water. *J. KMITNB,* 13**,** 14-19.

ZHOU, X., WU, Y., SHI, H. & SONG, Y. 2013. Evaluation of oxygen transfer parameters of fine-bubble aeration system in plug flow aeration tank of wastewater treatment plant. *Journal of Environmental Sciences,* 25**,** 295-301.

ZHU, K., GAMAL EL-DIN, M., MOAWAD, A. & BROMLEY, D. 2004. Physical and chemical processes for removing suspended solids and phosphorus from liquid swine manure. *Environmental technology,* 25**,** 1177-1187.

ZOPPAS, F. M., MENEGUZZI, A., URRUTIA, H., BERNARDES, A. M. & ANTILEO, C. 2017. Comparison of different strategies for nitrogen removal by simultaneous nitrification and denitrification process in a batch rotating disk reactor. *American Journal of Engineering Research,* 6**,** 76-82.