



**Advanced techniques for the upgrading of waste
stabilisation pond effluent: rock filtration;
duckweed; and attached-growth media**

**Michael Douglas Short
B.Tech(AQUA); B.Sc(Hons)**

**Department of Environmental Health
School of Medicine
Flinders University, Adelaide, South Australia**

PhD Thesis

December 2008

“It is striking that engineers have largely shaped the character of modern waste treatment, and even today one finds few aquatic biologists participating in it. Since most waste-treatment schemes are extensions of natural eutrophic ecosystems, ecologically oriented aquatic biologists could make a significant contribution to technologies heretofore dominated by sanitary engineers.”

W.S. Hillman and D.D. Culley Jr. (1978).

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.

Michael Douglas Short

Abstract

Waste Stabilisation Ponds (WSPs) are a relatively simplistic and non-intensive wastewater treatment technology; with various WSP configurations widely employed to treat a range of different wastewaters the world over. Whilst the advantages of WSP treatment are both numerous and well recognized, performance problems relating to the presence of occasionally large and unpredictable quantities of plankton (both algal and zooplankton) biomass in the final pond effluents have posed significant operational problems for WSP operators; with this suspended biomass representing the single biggest drawback associated with the technology. Research conducted during this project was concerned with assessing a selection of so-called ‘advanced’ in-pond treatment processes for the upgrading or polishing of a final WSP effluent. The particular research emphasis was on the removal of problematic algal and zooplankton biomass from WSP effluent prior to Dissolved Air Flotation/Filtration (DAF/F) treatment and wastewater reuse at the Bolivar Wastewater Treatment Plant (WWTP) north of Adelaide.

The *in situ* WSP upgrade systems assessed in this thesis were: the native floating plant ‘Duckweed’ (DW); ‘Rock Filters’ (RFs); and an artificial ‘Attached-Growth Media’ (AGM); all of which were assessed for their relative treatment efficacies parallel to a non-interventional ‘Open Pond’ (OP) system which served as an effective control. These performance comparisons were assessed on a pilot-scale using a custom made pilot treatment plant which was located at the Bolivar WWTP. Performance monitoring was periodically carried out over a 12 month period from July 2005–August 2006, with algal and zooplankton populations monitored in addition to the more conventional wastewater quality parameters.

Results from pilot plant investigations demonstrated that of the four pilot upgrade series, the RF and AGM systems displayed the greatest treatment potential in terms of both the magnitude and reliability of suspended solids, algal and zooplankton biomass removals. The DW system was also shown to be at least as effective and in some instances significantly more advanced than the uncovered OP system in terms of its ability to

significantly improve the final effluent quality of the Bolivar WSPs. Both the RF and AGM upgrades (and to a lesser degree also the DW system) were found to offer considerable potential for producing a higher quality WSP effluent for more efficient processing by the Bolivar DAF/F plant; although there were various operational advantages and disadvantages as well as varying capital establishment costs associated with each of the candidate technologies. This part of the research represented the first direct performance comparison between two popular pond upgrade technologies (i.e. RFs and DW) and also constituted the first assessment of a novel AGM for the upgrading of tertiary-level WSP effluent. In addition to this, results from ecological performance monitoring also provided the first detailed insights into algal and zooplankton population dynamics within these WSP upgrade environments.

In addition to these pilot-scale WSP upgrade performance investigations, another branch of the research project investigated additional research questions regarding the survival of algal cells within these pond upgrade environments. A series of laboratory experiments attempted to recreate the *in situ* conditions (in terms of light and oxygen availability) that might exist within the adopted upgrade environments. Using two common WSP algal species, long-term monitoring of the physiological status of phytoplankton cells during prolonged dark-exposure under conditions of reduced oxygen availability was performed in order to assess the likely effects of these particular environmental conditions on their survival potential *in situ*.

Results from these laboratory-based experiments showed that both algal species were capable of quickly adjusting their cellular metabolism in response to dark incubation. Results also showed that a reduced environmental oxygen concentration (25% of saturation) had no bearing on the ability of either *Chlorella* or *Chlamydomonas* species to withstand long-term dark-exposure; with both species retaining what was essentially full biological viability following up to two months of continuous dark-exposure. In an applied context, these results suggested that subjecting algal cells to conditions of simultaneous darkness and reduced oxygen availability would be expected to have no significant adverse effects on algal survivorship within an advanced in-pond upgrade system such as a duckweed-covered WSP, a rock filter or an AGM system.

Acknowledgements

I was once quoted saying “*there’s no aptitude without gratitude*”; so it’s now time to kneel down and give thanks!

I would firstly like to thank my principal supervisor Prof. Howard Fallowfield. Howard, thank you for being patient and supportive as I toiled away at the computer and for maintaining a sense of humour as the page number reached for the heavens... Your editorial feedback greatly improved the final thesis and your assistance during the early stages of the project was also invaluable in getting the beast off the ground and into the air, upon which it flew steadily—though not so rapidly at times—toward completion. Your *hands-off* supervisory approach was well suited to my desire to be largely left alone to get the job done, and has since helped me to become a highly independent and resourceful researcher; skills one requires if they are to be successful in this line of work. Thanks also to my co-supervisor Prof. Nancy Cromar for your involvement in my candidature, particularly the experimental design stage of the field work, and also for providing an open door throughout the project duration.

Thanks to United Water for providing financial sponsorship for the project and also for making room for me down at Bolivar WWTP. Incidentally, “*This research project was proudly supported by United Water International as part of its commitment to innovation and responsible water management.*” Thank you Dr. John Nixon and Dr. David Sweeney for your insightful advice with all things Bolivar-esque and wastewatery. Dave, your assistance with the fluid hydraulics side of the work was both first class and greatly appreciated (which reminds me, I still have your copy of *Levenspiel!*). A special thanks to Danny Tintor for your freely-offered and invaluable assistance on the ground at Bolivar. No job was ever too big or small and you sure kept everyone down there honest; you’re undoubtedly a shiny pearl within the unrefined oyster that is the Bolivar treatment works. A big thank you goes also to Sheree Bailey for your invaluable assistance and technical advice with the flow cytometry side of the project. I know I learnt a lot about flow cytometry and I hope that you learned something about algae too...

Thanks to Darcy and Laurie down in Biomedical Engineering for all your help designing and building parts of the pilot plant, and also for your willingness to offer technical guidance (often without notice). Thank you Alana, Trish and Cheryl for taking care of all the nitty-gritty stuff over the past 5+ years. Each of you always made it your business to do whatever you could to help and I thank you all sincerely for it! Thank you also Dr. Richard Bentham, Dr. John Edwards and Dr. Kirstin Ross for your advice and scholarly assistance at various points along the way. Thanks Shazza and Emily for many a stimulating and at times fiery lunch break conversation; I wish you both the best for your future careers. A retrospective thanks goes to the gone but not forgotten legends of EH: Louigi; Ben Pol; Adele; Michelle; Duncan; and Iry; for many a good time over a cup of morning and/or afternoon tea, even if milky thumbs are your style Louis.

Thanks to Ben and Akio and for providing both practical and technical advice and project support. Thanks Ben for schooling me on some of the lab analyses and much respect for helping out with the pilot plant construction; I don’t think either of us will

forget those rocks as long as we live. Thanks Akio for being a good mate over the past few years and for coming surfing with me when Ben was sleeping. Thank you also for teaching me about good coffee and for the Japanese bean grinder which I now use on a daily basis ☺. Thanks to Richard Evans for yielding significant amounts of technical know-how and also for providing many an *in vivo* insight into how one might go about thinking ‘outside the square’ and then slightly to the left of the neighbouring triangle. What do you mean you can’t see it, its right there?! No not that, that’s a prism... oh boy, here we go again... Thanks to the G-man for many a glorious (but sometimes downright brutally frigid) morning spent punishing a frequently deserted and wanting Goolwa lineup, and less so for providing the occasional pearl of scientific wisdom. A big thank you also goes to the resident south coast aquatic megafauna for routinely bypassing an easy—albeit somewhat lean—meal.

Thanks to the classical masters J.C.W.T. Mozart and J.S. Bach for providing both a profoundly relaxing and also intellectually-stimulating soundtrack to the thesis writing process – one wonders whether you have forever tainted my prior appreciation for heavy metal. A big thanks should also go to the ‘Doctor of Philosophy’ degree itself, through which I have been afforded many an intellectual and lifestyle freedom. Through your flexible and relaxed academic nature, you have also enabled—if not thoroughly encouraged—me to hone my surfing skills over the past several years; a luxury for which I will be forever indebted. To this end, thanks also to the Australian Government, and in turn Flinders University, for providing me with the necessary financial assistance so that I was able to undertake such a process in the first instance.

Thanks to Mum and Dad for working around me on occasion and for allowing me to sleep in following many a late night spent in the lab. Although the PhD machine left me somewhat detached from family life at times, you have always supported me in my endeavours and for that I am eternally grateful! Finally, thanks to my now darling wife (previously darling fiancée and darling girlfriend) Miška for being patient with me all of the times when I didn’t have enough spare time to catch up, and also for saying “yes” to that age-old question. You have provided me with both personal support and also inspiration for too many things to list, and it’s a testament to your character that you still went ahead and enrolled in a PhD of your own even after seeing what a beast mine had become; although I do take great comfort in knowing that your University has a strictly enforced page limit for Doctoral theses ☺.

Table of contents

1	ADVANCED WASTEWATER TREATMENT FOR ALGAL REMOVAL: LITERATURE	
	REVIEW AND GENERAL THESIS INTRODUCTION	1
1.1	Background.....	1
1.2	Waste Stabilisation Ponds	3
1.2.1	Facultative WSPs.....	4
1.2.2	Aerobic ‘maturation’ WSPs.....	7
1.2.2.1	WSP technology and treatment performance	8
1.2.3	Heterotrophic microbes and algae—the backbone of effective WSP treatment	9
1.2.4	Waste stabilisation: a state change from liquid to solids	12
1.2.5	Algae and WSPs: a ‘love–hate’ relationship.....	13
1.2.6	WSP effluent compliance—a complex problem for a simple technology	16
1.2.7	The upgrading of WSP effluents.....	18
1.2.8	Advanced techniques for upgrading WSPs.....	19
1.2.8.1	In-pond vs. out-of-pond upgrades	22
1.2.8.2	Upgrading WSPs with aquatic macrophytes	24
1.2.8.3	Water hyacinths.....	26
1.2.8.4	Duckweed.....	27
1.2.8.5	Duckweed as advanced WSP treatment	28
1.2.8.5.1	BOD ₅ , SS, nutrient and pathogen removal in duckweed ponds.....	31
1.2.8.5.2	Advantages and disadvantages of duckweed ponds	34
1.2.8.5.3	Duckweed as an advanced in-pond upgrade for algal solids removal	38
1.2.8.6	Rock filtration as an advanced WSP upgrade	40
1.2.8.6.1	Rock filters for final effluent polishing: nutrients; BOD ₅ ; and SS abatement.....	41
1.2.8.7	Artificial attached-growth media.....	44
1.2.8.7.1	Microorganisms and biofilm processes in AGWSPs.....	45
1.2.8.7.2	AGM for final effluent polishing: SS; BOD ₅ ; and nutrients abatement	47
1.2.8.7.3	Fixed-bed horizontal-flow AGM.....	48
1.3	Local WSP systems	50
1.3.1	Bolivar WSPs.....	50
1.3.1.1	Bolivar WSP plankton ecology	53
1.3.1.2	Active management strategies for the Bolivar WSPs.....	55
1.3.2	Local community waste management (CWM) schemes.....	59
1.4	Thesis questions, objectives and research design	60
1.4.1	Thesis questions:.....	62
2	EXPERIMENTAL PILOT PLANT CONSTRUCTION, CHARACTERISATION, OPERATION	
	AND PERFORMANCE MONITORING	63
2.1	Pilot plant design and characterisation.....	63
2.1.1	Pilot plant experimental treatments.....	69
2.1.1.1	Duckweed treatment.....	71
2.1.1.2	Open Pond treatment.....	72
2.1.1.3	Rock filter treatment.....	72
2.1.1.4	Fixed-bed horizontal-flow attached-growth media	75
2.1.2	Pilot plant flow hydraulics.....	77
2.1.2.1	Hydraulic characterisation.....	78
2.1.2.2	Hydraulic balance.....	80
2.1.2.3	Hydraulic operation.....	80
2.2	Operational sampling and water quality analyses	81
2.2.1	Experimental sampling protocols.....	81
2.2.2	Field- and laboratory-based water quality analyses	84
2.2.2.1	In situ water quality monitoring	84

2.2.2.2	Total and volatile suspended solids.....	84
2.2.2.3	Turbidity.....	85
2.2.2.4	Total five-day biochemical oxygen demand	85
2.2.2.5	Total organic carbon	85
2.2.2.6	Chlorophyll a.....	86
2.2.2.7	Ammoniacal-nitrogen	86
2.2.2.8	Oxidised nitrogen (nitrate and nitrite).....	86
2.2.2.9	Soluble reactive orthophosphate	87
2.2.2.10	Indicator microorganisms.....	87
2.2.2.11	Heterotrophic microbial plate counts	87
2.2.2.12	Light–depth profiling	87
2.2.2.13	Phyto- and zooplankton quantitation and identification.....	88
2.2.2.14	Plankton community diversity	91
2.3	Data assessment, manipulation and statistical analysis	92
3	RELATIVE PERFORMANCE OF DUCKWEED PONDS AND ROCK FILTRATION FOR THE UPGRADING OF WSP EFFLUENT	94
3.1	Introduction	94
3.2	Materials and Methods	94
3.3	Results and Discussion	95
3.3.1	Pilot plant hydraulics	95
3.3.2	Pilot plant loading conditions and influent wastewater characteristics.....	99
3.3.3	Duckweed mat properties, and biomass density vs. light attenuation.....	103
3.3.4	Environmental and physicochemical parameters.....	107
3.3.5	Wastewater treatment performance: removal of particulate organics and oxygen demand.....	117
3.3.6	Wastewater treatment performance: suspended solids, turbidity and algal biomass removal .	138
3.3.7	Wastewater treatment performance: nutrient removal	174
3.3.7.1	Inorganic nitrogen dynamics.....	175
3.3.7.2	Soluble reactive orthophosphate removal	189
3.3.8	Wastewater treatment performance: indicator organism removals.....	194
3.4	General research findings and chapter summary	200
3.5	Experimental improvements and suggestions for future research	202
4	RELATIVE PERFORMANCE OF HORIZONTAL FLOW ATTACHED-GROWTH MEDIA AND ROCK FILTRATION FOR THE UPGRADING OF WSP EFFLUENT	205
4.1	Introduction	205
4.2	Materials and methods.....	206
4.3	Results and discussion.....	207
4.3.1	Pilot plant flow hydraulics	207
4.3.1.1	Pilot pond flow hydraulics: attached-growth media reactors	207
4.3.2	Pilot plant loading conditions and influent wastewater characteristics.....	210
4.3.3	Environmental and physicochemical parameters.....	213
4.3.4	Wastewater treatment performance: removal of particulate organics and oxygen demand.....	223
4.3.5	Wastewater treatment performance: suspended solids, turbidity and algal biomass removal .	236
4.3.6	Wastewater treatment performance: nutrient removal	262
4.3.6.1	Inorganic nitrogen dynamics.....	263
4.3.6.2	Soluble reactive orthophosphate removal	276
4.3.7	Wastewater treatment performance: indicator organism removals.....	280
4.3.8	Serviceable life of a rock filter and attached-growth media WSP upgrade	285

4.4	General research findings and chapter summary	291
4.5	Suggestions for future research	293
5	ECOLOGICAL CHANGES TO PHYTO- AND ZOOPLANKTON COMMUNITIES AS A CONSEQUENCE OF DIFFERENT WSP EFFLUENT UPGRADE METHODOLOGIES: RESULTS FROM PILOT-SCALE INVESTIGATIONS	295
5.1	Introduction	295
5.2	Methods	299
5.3	Results and discussion	300
5.3.1	Comparative phytoplankton ecology of the pilot plant influent and the four advanced in- pond upgrades	300
5.3.2	Comparative zooplankton ecology of the pilot plant influent and the four advanced in-pond upgrades	324
5.3.2.1	Incidence of problem zooplankton species: implications of the effluent upgrade systems for DAF/F process efficiency	375
5.4	Conclusions	379
6	PHYTOPLANKTON SURVIVAL DURING PROLONGED DARKNESS UNDER CONDITIONS OF AMBIENT AND REDUCED DISSOLVED OXYGEN—LITERATURE REVIEW AND INTRODUCTION	382
6.1	Phytoplankton and photolithotrophy	382
6.2	Photophysiological acclimation by phytoplankton to changes in light climate: a survival strategy	382
6.3	Phytoplankton and dark-survival	386
6.3.1	Dark-survival strategies	388
6.3.2	Phytoplankton cell death and dark-survival—implications for algal community ecology	390
6.3.3	Phytoplankton cell death dark-survival—implications for advanced WSP upgrade technologies	393
6.4	Analytical flow cytometry	395
6.4.1	Flow cytometry in the biological sciences	396
6.4.2	Flow cytometry in phytoplankton research: viability assessment	397
6.4.2.1	Physical light scattering	399
6.4.2.1.1	Forward-angle light scatter (FSC)	399
6.4.2.1.2	Side-angle light scatter (SSC)	400
6.4.2.2	Chlorophyll a autofluorescence	400
6.4.2.3	Population cell density	403
6.4.2.4	Biological fluorochromes and flow cytometry	404
6.4.2.4.1	Cellular metabolic activity	405
6.4.2.4.2	Cellular membrane integrity	409
6.4.2.4.3	Dual-staining for viability assessment	411
6.4.2.5	Long-term dark viability assessment—how viable is viable?	413
6.5	Experimental questions and research aims	414
6.5.1	Research aims:	415
7	PHYTOPLANKTON SURVIVAL DURING PROLONGED DARKNESS UNDER CONDITIONS OF AMBIENT AND REDUCED DISSOLVED OXYGEN—MATERIALS AND METHODS	416
7.1	Algal stock culture maintenance and experimental cultures	416
7.2	Dark-survival experimental design, sampling protocols and analyses	417
7.2.1	Experimental design rationale	417
7.2.2	Experimental sampling protocols—65 and 7 day dark-survival experiments	421
7.2.2.1	Summary of sampling protocol	423

7.2.2.2	Assessment of re-growth potential—experimental design and analysis	424
7.2.3	Dark-survival experimental analyses	425
7.2.3.1	Gross culture analyses	425
7.2.3.2	Optimisation of sample treatment, staining protocols, and cytometric analyses	426
7.2.3.2.1	Enumeration of population cell density	426
7.2.3.2.2	PI, FDA and cytometry bead stock solutions	428
7.2.3.2.3	PI and FDA staining optimisations	428
7.2.3.2.4	The optimal PI–FDA staining protocol	431
7.2.3.3	Flow cytometric data acquisition, analysis and presentation	432
7.2.3.4	Chlorophyll fluorimetry	435
7.2.3.5	Data treatment, statistical analyses and interpretations	436
8	RESULTS AND DISCUSSION OF FLOW CYTOMETRIC METHODOLOGICAL OPTIMISATIONS—THE IMPORTANCE OF CRITICALLY ASSESSING OPTIMAL STAINING PROTOCOLS	438
8.1	Phytoplankton enumeration	438
8.2	Flow cytometric discrimination of live vs. dead phytoplankton—optimising the dual PI–FDA assay	438
8.2.1	Flow cytometric assessment of cell membrane integrity—optimising the PI assay	439
8.2.2	Flow cytometric determination of phytoplankton metabolic activity—optimising the FDA assay	441
8.2.2.1	Determination of optimal FDA concentration and assessment of substrate FDA hydrolysis kinetics	441
8.2.2.2	Effect of culture growth phase, population cell density, and pH on staining protocol optimisation	450
8.2.2.3	Instrument drift, internal standards and data transformation	451
9	PHYTOPLANKTON SURVIVAL DURING PROLONGED DARKNESS UNDER CONDITIONS OF AMBIENT AND REDUCED DISSOLVED OXYGEN—RESULTS AND DISCUSSION.....	454
9.1	Validation of experimental design	454
9.2	Algal stock culture maintenance, standard growth curves and growth rates....	455
9.3	Effect of culture growth phase on dark-survival.....	456
9.4	Phytoplankton dark-survival kinetics: 65 versus 7 day investigations.....	458
9.5	Prolonged darkness and water quality: dissolved oxygen; pH; and dissolved inorganic carbon.....	459
9.5.1	Results from the two month dark-survival experiment	459
9.5.2	Results from the 7 day dark-survival experiment	462
9.6	Prolonged darkness: implications for population cell density; cell size; and intracellular density.....	465
9.6.1	Darkness and population cell density: results from the 65 day experiment	465
9.6.1.1	Possible sources of error in FCM-quantified population cell density—sticky cells	468
9.6.2	Darkness and population cell density: results from the 7 day experiment	469
9.6.3	Prolonged darkness and phytoplankton cell size, volume and intracellular density	471
9.6.3.1	FSC-height versus cell volume	472
9.6.3.2	The importance of cell volume for interpreting FCM data	474
9.6.3.3	Prolonged darkness and phytoplankton cell size and intracellular density: results from the 65 day experiment	476
9.6.3.4	The interrelationship between FSC and SSC signals in FCM analysis	488
9.6.3.5	Prolonged darkness and phytoplankton cell size and intracellular density: results from the 7 day experiment	489
9.6.3.6	The effects of darkness on cell volume and intracellular density—ecological implications for phytoplankton sinking velocity	492

9.7	Darkness and phytoplankton photosynthesis—photosynthetic pigments and chlorophyll <i>a</i> fluorescence activity	495
9.7.1	Dark-survival and cellular chlorophyll <i>a</i> : results from the 65 day experiment.....	495
9.7.2	Dark-survival and cellular chlorophyll <i>a</i> : results from the 7 day experiment.....	504
9.7.3	Dark-survival and <i>in vivo</i> chlorophyll <i>a</i> fluorescence: results from the 65 day experiment.....	505
9.7.3.1	Validation of flow cytometric chlorophyll <i>a</i> fluorescence measurements.....	513
9.7.4	Dark-survival and phytoplankton <i>in vivo</i> chlorophyll <i>a</i> fluorescence: results from the 7 day experiment.....	515
9.8	Prolonged darkness: phytoplankton metabolic activity and membrane integrity ..	517
9.8.1	Dual PI–FDA viability assessment: results from the 65 day dark-survival experiment.....	517
9.8.1.1	Agreement between the discrete viability assessments of the PI and FDA assays...	530
9.8.2	Dual PI–FDA viability assessment: results from the 7 day dark-survival experiment.....	532
9.8.2.1	The effects of a changing cell volume on FDA-quantified physiological activity ...	542
9.8.2.2	The importance of multiple markers for viability resolution.....	543
9.9	Phytoplankton re-growth potential following prolonged darkness	544
9.9.1	Re-growth potential of <i>C. vulgaris</i> and <i>C. reinhardtii</i> following 65 day dark-exposure ...	545
9.10	Dark-survival, dissolved oxygen concentration and dark respiration—discussion of the ‘low D.O’ treatment results	553
9.11	Light versus dark survival—light controls for dark treatments?	567
9.12	Darkness, organic substrates and heterotrophic nutrition: was the advanced dark-survival purely inorganic?	568
9.13	Prior light history and dark-survival	576
9.14	Timescales for phytoplankton acclimation during prolonged darkness—kinetics of dark-survival	578
9.15	Darkness and physiological vitality—implications for phytoplankton sinking velocity and advanced WSP upgrade process efficiency	581
9.16	The conundrum of clinical manipulations—application of laboratory results to real life scenarios	585
9.17	Research findings and experimental conclusions	587
9.18	Suggested experimental improvements and future research questions	590
9.18.1	Destructive sampling for absolute dark-control	590
9.18.2	Alternate trophic states, environmental media and dark-survival	591
9.18.3	Strict anaerobiosis and dark-survival	592
9.18.4	Axenic versus non-axenic, and uni-algal versus mixed dark-survival	592
9.18.5	Grazer interactions and dark-survival	594
9.18.6	Additional research suggestions.....	595
10	GENERAL DISCUSSION	596
10.1	Logistics of upgrading the Bolivar WSPs	604
10.1.1	Operational scale-up factors for Bolivar	606
10.2	Multiple installations of advanced WSP upgrades—a cumulative treatment effect?	608
10.3	Problems with in-pond effluent upgrades	610
10.4	WSP ecology—a management tool?	613
10.5	Wider applications of research	614

10.6	Final impact of thesis findings.....	615
APPENDICES	617
Appendix A.	Previously published data from Chapter 3:.....	617
Appendix B.	Correlation matrices for pilot plant performance data—Chapter 3.....	627
Appendix C.	Correlation matrices for pilot plant performance data—Chapter 4.....	633
Appendix D.	Zooplankton taxa most commonly observed during pilot plant operation: 2005–2006.	639
Appendix E.	Mean zooplankton body lengths, length–weight regression equations and biomass estimates for the dominant taxa observed from July 2005–August 2006. Individual dry weights were estimated either from length–weight equations or published biomass values of individuals from the same genus or species.	645
Appendix F.	Executive summary of the existing phytoplankton dark-survival literature (taxonomic classifications sourced from AlgaeBase v.3.0 http://www.algaebase.org as at 30/06/2007).	646
Appendix G.	Chemical constituents of the modified Woods Hole MBL growth medium (modified from Nichols, 1973).	649
REFERENCES	650

List of Figures

Figure 1.1. Schematic representation of daytime WSP operation (Metcalf and Eddy, 1991).	6
Figure 1.2. Cyclic ‘symbiosis’ between algae and bacteria within a WSP environment (Ramalho, 1983).	11
Figure 1.3. Schematic (left) and photographic (right) depictions of the Australian native duckweed <i>Lemna disperma</i> Hegelm.	30
Figure 1.4. Schematic cross-section of a rock filter bed showing the typical horizontal-flow configuration (modified from Powell <i>et al.</i> , 1998).	40
Figure 1.5. (a) Close-up view of a novel, horizontal-flow attached-growth media for use in WSP applications, and (b) schematic representation of the biofilm attachment and <i>in situ</i> horizontal-flow regime.	49
Figure 2.1. Schematic of the experimental pilot plant system, showing: the header tank (HT); multiple pond layout with down-the-line pond numbering format; and hydraulic configuration (dimensions given in metres).	65
Figure 2.2. Schematic representation of an individual pond inlet (a) and outlet (b) manifolds showing arrangement of the inlet and outlet hydraulic ducts.	68
Figure 2.3. Three-pond rock filter series layout showing <i>in situ</i> sampling port location with PVC covers in place, and inset, schematic detail of the <i>in situ</i> perforated sampling port design and dimensions.	75
Figure 2.4. Schematic representation of the experimental pilot plant system showing daily sampling locations (indicated by filled stars) for each of the treatment trains across all treatments: Duckweed Ponds (DW); Attached-Growth Media reactors (AGM); Open Ponds (OP); and Rock Filters (RF).	82
Figure 2.5. (a) Complete acrylic zooplankton counting wheel and base, showing: central pivot point (1); start/stop point (2); circular counting well (3); and acrylic base stand (4); and (b) cross-sectional view of the counting wheel removed from the base stand.	89
Figure 3.1. Duckweed treatment system: duplicate single pond normalised residence time distribution curves showing normalised rhodamine WT fluorescence (A.U). Tracer experiments performed under a standing duckweed plant biomass density of no less than 2kg m^{-2} (wet weight).	95
Figure 3.2. Open Pond treatment: duplicate single pond normalised residence time distribution curves showing normalised rhodamine WT fluorescence (A.U.).	96
Figure 3.3. Rock filters: duplicate single pond normalised residence time distribution curves showing normalised rhodamine WT fluorescence (A.U.).	96
Figure 3.4. Irradiance–depth PAR profiles for Duckweed (DW) and Open Pond (OP) systems, showing percent attenuation at $600\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ incident irradiation and standing duckweed plant biomass density of 8kg m^{-2} (fresh weight). Individual data points represent mean determinations from three parallel treatment ponds (± 1 S.D.).	105
Figure 3.5. Exponential fit of available duckweed biomass density vs. incident light attenuation data (broken lines represent 95% confidence intervals for the fitted line).	106
Figure 3.6. Selected mean monthly site weather conditions from July–December of 2005. Left y-axis shows average daily wind speed and monthly precipitation, and the right y-axis shows mean monthly evaporation (data courtesy of the Australian Government Bureau of Meteorology).	108

Figure 3.7. Water temperature data for pilot plant: Influent (■); Rock Filters (△); Open Ponds (○); and Duckweed Ponds (◇). For ease of interpretation, data points show only the mean temperature (± 1 S.D) averaged across each three-pond treatment series, with a line fitted only to the influent data set.....	108
Figure 3.8. Water temperature box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). The shaded ‘box’ represents the interquartile data range (IQR), the horizontal bar shows the median value, and the ‘whiskers’ show the absolute data range.....	109
Figure 3.9. Dissolved oxygen data for pilot plant: Influent (■); Rock Filters (△); Open Ponds (○); and Duckweed Ponds (◇). For ease of interpretation, data points show only the mean DO concentration (± 1 S.D.) averaged across each three-pond treatment series.....	110
Figure 3.10. Dissolved oxygen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3).	110
Figure 3.11. pH box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3).	114
Figure 3.12. Specific conductivity data for pilot plant: Influent (■); Rock Filters (△); Open Ponds (○); and Duckweed Ponds (◇). For ease of interpretation, data points show only the mean conductivity (± 1 S.D.) averaged across each three-pond treatment series, with a line fitted only to the influent data set.....	116
Figure 3.13. Specific conductivity box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed ponds 1, 2, 3 (DW-1, DW-2, DW-3).....	116
Figure 3.14. BOD ₅ box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). The shaded ‘box’ represents the IQR, the horizontal bar shows the median value, and the ‘whiskers’ show the absolute data range....	118
Figure 3.15. Box-plots showing daily percentage BOD ₅ removal performance relative to pilot plant Influent concentration for Ponds 1 and 3 of each pilot treatment system ($n \geq 12$ for all plots).....	120
Figure 3.16. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. mass removal (as a percentage of daily loading rate) for Pond 1 data only. Individual data points represent performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●).....	122
Figure 3.17. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. mass removal (as a percentage of daily loading rate) for Pond 3 data only. Individual data points represent performance data for: Duckweed Pond 3 (□); Open Pond 3 (*); and Rock Filter 3 (●).....	122
Figure 3.18. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●). Linear regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of x- and y-axis breaks.	124
Figure 3.19. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. total mass removal for Pond 3 data only. Individual data points represent performance data for: Duckweed Pond 3 (□); Open Pond 3 (*); and Rock Filter 3 (●). Linear	

regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of <i>x</i> - and <i>y</i> -axis breaks.....	124
Figure 3.20. Scatter-plot of observed vs. predicted BOD ₅ effluent concentrations for Open Pond 1 based on the model of Uhlmann (1979; 1980) for equal volume pond reactors arranged in a series. The solid line represents the fitted regression line for OP-1 observed and predicted data (\pm 95% CI's; thick broken lines), and the thin broken line shows the theoretical perfect fit line for the data.....	127
Figure 3.21. Suspended solids box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). Filled circles (●) above the INFL data represent the four extreme spike outliers $>3\times$ IQR from the 75 th percentile value.	141
Figure 3.22. Turbidity box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). Filled circles (●) above the INFL data represent the three extreme spike outliers $>3\times$ IQR from the 75 th percentile value.....	141
Figure 3.23. Box-plots showing daily percentage suspended solids removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems ($n \geq 20$ for all plots).....	143
Figure 3.24. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 1 data only (note the condensed <i>y</i> -axis scale for values below zero). Individual data points represent mean performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●). Individual data points show the mean of duplicate determinations.....	145
Figure 3.25. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 3 data only. Individual data points represent mean performance data for: Duckweed Pond 3 (□); Open Pond 3 (*); and Rock Filter 3 (●). Individual data points show the mean of duplicate determinations.....	145
Figure 3.26. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent mean performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●). Fitted lines represent best-fit lines from simple linear regression analyses. Individual data points show the mean of duplicate determinations.....	147
Figure 3.27. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. total mass removal for Pond 3 data only. Individual data points represent mean performance data for: Duckweed Pond 3 (□); Open Pond 3 (*); and Rock Filter 3 (●). Fitted lines represent best-fit lines from simple linear regression analyses. Individual data points show the mean of duplicate determinations.....	147
Figure 3.28. Relative volatile suspended solids fraction data (as a percent of total SS) for: pilot plant Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3).	149
Figure 3.29. Chlorophyll <i>a</i> box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). Filled circles (●) above the INFL data represent the three extreme spike outliers $>3\times$ IQR from the 75 th percentile value.	152

Figure 3.30. Box-plots showing percentage chlorophyll <i>a</i> removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems ($n \geq 15$ for all plots).....	153
Figure 3.31. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 1 data only (note the truncated <i>y</i> -axis scale for values below zero). Individual data points represent mean performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●). Individual data points show the mean of triplicate determinations..	155
Figure 3.32. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 3 data only (note the truncated <i>y</i> -axis scale for values below zero). Individual data points represent mean performance data for: Duckweed Pond 3 (□); Open Pond 3 (*); and Rock Filter 3 (●). Individual data points show the mean of triplicate determinations..	156
Figure 3.33. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent mean performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●). Fitted lines represent best-fit lines from simple linear regression analyses. Individual data points show the mean of triplicate determinations.....	157
Figure 3.34. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. total mass removal for Pond 3 data only. Individual data points represent mean performance data for: Duckweed Pond 3 (□); Open Pond 3 (*); and Rock Filter 3 (●). Fitted lines represent best-fit lines from simple linear regression analyses. Individual data points show the mean of triplicate determinations.....	157
Figure 3.35. Ammonia-nitrogen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). The shaded 'box' represents the IQR, the horizontal bar shows the median, and the 'whiskers' show the absolute data range.	175
Figure 3.36. Nitrite-nitrogen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3).....	176
Figure 3.37. Nitrate-nitrogen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3).....	176
Figure 3.38. Box-plots showing daily percentage ammonia removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems ($n = 11$ for all plots).....	177
Figure 3.39. Scatter-plot showing NH_4^+ -N mass loading (pilot plant Influent) vs. percent mass removal (relative to daily loading rate) for Pond 1 data only. Individual data points represent mean performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●). Individual data points show the mean of triplicate determinations.....	183
Figure 3.40. Scatter-plot showing NH_4^+ -N mass loading (pilot plant Influent) vs. percent mass removal (relative to daily loading rate) for the combined data of Ponds 2 and 3. Individual data points represent mean performance data for: Duckweed Ponds 2 and 3 (□); Open Ponds 2 and 3 (*); and Rock Filters 2 and 3 (●). Individual data points show the mean of triplicate determinations.....	183

Figure 3.41. Scatter-plot showing NH_4^+ -N mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent mean performance data for: Duckweed Pond 1 (□); Open Pond 1 (*); and Rock Filter 1 (●). Fitted lines represent best-fit lines from simple linear regression analyses, with regression slopes shown alongside the respective figure legends. Individual data points show the mean of triplicate determinations.....	184
Figure 3.42. Scatter-plot showing NH_4^+ -N mass loading (pilot plant Influent) vs. total mass removal for the combined data of Ponds 2 and 3. Individual data points represent mean performance data for: Duckweed Ponds 2 and 3 (□); Open Ponds 2 and 3 (*); and Rock Filters 2 and 3 (●). Fitted lines represent best-fit lines from simple linear regression analyses, with regression slopes shown alongside the respective figure legends. Individual data points show the mean of triplicate determinations.	185
Figure 3.43. Soluble reactive orthophosphate-phosphorous box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). The shaded 'box' represents the IQR, the horizontal bar shows the median value, and the 'whiskers' show the absolute data range.....	190
Figure 3.44. Box-plots showing percentage orthophosphate-phosphorous removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems ($n = 11$ for all plots).	191
Figure 3.45. Faecal coliform box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3). The shaded 'box' represents the IQR, the horizontal bar shows the median value, and the 'whiskers' show the absolute data range.....	194
Figure 3.46. <i>E. coli</i> box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Duckweed Ponds 1, 2, 3 (DW-1, DW-2, DW-3).	195
Figure 4.1. Duplicate single reactor normalised RTD curves for the Attached-Growth Media treatment; showing normalised rhodamine WT fluorescence (A.U.; y-axis) and time (days; x-axis).	207
Figure 4.2. Selected mean monthly site weather conditions from February–August of 2006. Left y-axis shows average daily wind speed and monthly precipitation, and the right y-axis shows mean monthly evaporation (data courtesy of the Australian Government Bureau of Meteorology).....	213
Figure 4.3. Water temperature data for pilot plant: Influent (■); Rock Filters (△); Open Ponds (○); and Attached-Growth Media (◇) Reactors. For ease of interpretation, data points show only the mean temperature (± 1 S.D.) averaged across each three-pond treatment series, with a line fitted only to the influent data set.	214
Figure 4.4. Water temperature box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3). The shaded 'box' represents the interquartile data range (IQR), the horizontal bar shows the median value, and the 'whiskers' show the absolute data range.	214
Figure 4.5. Dissolved oxygen data for pilot plant: Influent (■); Rock Filters (△); Open Ponds (○); and Attached-Growth Media (◇) Reactors. For ease of interpretation, data points show only the mean DO concentration averaged across each three-pond treatment series.	215

Figure 4.6 Dissolved oxygen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).....	216
Figure 4.7. 24 hour online dissolved oxygen data from part of monitoring <i>Period 2</i> of 2006 for pilot plant: Influent (INFL); Rock Filter 1 (RF-1); and Open Pond 1 (OP-1).....	218
Figure 4.8. 24 hour online dissolved oxygen data from part of monitoring <i>Period 2</i> of 2006 for Attached-Growth Media Reactor 1 (AGM-1) only.	219
Figure 4.9. pH box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).	220
Figure 4.10. Specific conductivity data for pilot plant: Influent (■); Rock Filters (△); Open Ponds (○); and Attached-Growth Media (◇) Reactors. For ease of interpretation, data points show only the mean conductivity (± 1 S.D.) averaged across each three-pond treatment series, with a line fitted only to the influent data set.....	221
Figure 4.11. Specific conductance box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).	222
Figure 4.12. BOD ₅ box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3). The shaded ‘box’ represents the IQR, the horizontal bar shows the median value, and the ‘whiskers’ show the absolute data range.	223
Figure 4.13. Box-plots showing percentage BOD ₅ removal performance relative to pilot plant Influent concentration for all three ponds of each pilot treatment system ($n \geq 21$ for each plot).....	225
Figure 4.14. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. mass removal (as a percentage of daily loading rate) for Pond 1 data only. Individual data points represent performance data from single determinations for: Rock Filter 1 (●); Open Pond 1 (*); and Attached-Growth Media Reactor 1 (□).....	229
Figure 4.15. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. mass removal (as a percentage of daily loading rate) for Pond 3 data only. Individual data points represent performance data from single determinations for: Rock Filter 3 (●); Open Pond 3 (*); and Attached-Growth Media Reactor 3 (□).....	230
Figure 4.16. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent performance data from single determinations for: Rock Filter 1 (●); Open Pond 1 (*); and Attached-Growth Media Reactor 1 (□). Linear regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of x- and y-axis breaks. Individual treatment regression lines are shown with corresponding slope (m).	231
Figure 4.17. Scatter-plot showing BOD ₅ mass loading (pilot plant Influent) vs. total mass removal for Pond 3 data only. Individual data points represent performance data from single determinations for: Rock Filter 3 (●); Open Pond 3 (*); and Attached-Growth Media Reactor 3 (□). Linear regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of x- and y-axis breaks. Individual treatment regression lines are shown with corresponding slope (m).	231

Figure 4.18. Scatter-plot showing BOD₅ mass loading (pilot plant Influent) vs. total mass removal for the combined three-pond data of each treatment train (excluding the single extreme outlying loading event across all treatments). Individual data points represent performance data from single determinations for: Rock Filters (●); Open Ponds (*); and Attached-Growth Media Reactors (□).....234

Figure 4.19. Suspended solids box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3). The shaded ‘box’ represents the IQR, the horizontal bar shows the median value, and the ‘whiskers’ show the absolute data range. Filled circles (●) above the INFL data represent the two extreme spike outliers >3×IQR from the 75th percentile value.....238

Figure 4.20. Turbidity box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3). Filled circles (●) above the INFL data represent the two extreme spike outliers >3×IQR from the 75th percentile value.....239

Figure 4.21. Box-plots showing percentage suspended solids removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems ($n \geq 32$ for all plots).....240

Figure 4.22. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 1 data only (note the reduced y-axis scale for values below zero). Individual data points represent mean performance data from duplicate determinations for: Rock Filter 1 (●); Open Pond 1 (*); and Attached-Growth Media Reactor 1 (□).242

Figure 4.23. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 3 data only (note the reduced y-axis scale for values below -50). Individual data points represent mean performance data from duplicate determinations for: Rock Filter 3 (●); Open Pond 3 (*); and Attached-Growth Media Reactor 3 (□).242

Figure 4.24. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent mean performance data from duplicate determinations for: Rock Filter 1 (●); Open Pond 1 (*); and AGM Reactor 1 (□). Linear regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of x- and y-axis breaks. Individual treatment regression lines are shown with corresponding slope (m).244

Figure 4.25. Scatter-plot showing suspended solids mass loading (pilot plant Influent) vs. total mass removal for Pond 3 data only. Individual data points represent mean performance data from duplicate determinations for: Rock Filter 3 (●); Open Pond 3 (*); and AGM Reactor 3 (□). Linear regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of x- and y-axis breaks. Individual treatment regression lines are shown with corresponding slope (m).244

Figure 4.26. Relative volatile suspended solids fraction data (as a percent of total SS) for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).....247

Figure 4.27. Chlorophyll *a* box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-

<p>Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3). The filled circle (●) above the INFL data represents the single extreme outlying spike $>3 \times \text{IQR}$ from the 75th percentile value.</p>	250
<p>Figure 4.28. Box-plots showing percentage chlorophyll <i>a</i> removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems ($n \geq 34$ for all plots).</p>	251
<p>Figure 4.29. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 1 data only. Individual data points represent mean performance data from triplicate determinations for: Rock Filter 1 (●); Open Pond 1 (*); and Attached-Growth Media Reactor 1 (□).</p>	253
<p>Figure 4.30. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 3 data only (note the reduced <i>y</i>-axis scale for values below zero). Individual data points represent mean performance data from triplicate determinations for Rock Filter 3 (●); Open Pond 3 (*); and Attached-Growth Media Reactor 3 (□).</p>	253
<p>Figure 4.31. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent mean performance data from triplicate determinations for: Rock Filter 1 (●); Open Pond 1 (*); and Attached-Growth Media Reactor 1 (□). Linear regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of <i>x</i>- and <i>y</i>-axis breaks.</p>	255
<p>Figure 4.32. Scatter-plot showing chlorophyll <i>a</i> mass loading (pilot plant Influent) vs. total mass removal for Pond 3 data only. Individual data points represent mean performance data from triplicate determinations for: Rock Filter 3 (●); Open Pond 3 (*); and Attached-Growth Media Reactor 3 (□). Linear regression lines were fitted to the entire data set, but for ease of presentation are shown only to the point of <i>x</i>- and <i>y</i>-axis breaks.</p>	255
<p>Figure 4.33. Ammonia-nitrogen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).</p>	263
<p>Figure 4.34. Nitrite-nitrogen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).</p>	263
<p>Figure 4.35. Nitrate-nitrogen box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).</p>	264
<p>Figure 4.36. Box-plots showing percentage ammonia removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems ($n = 11$ for all plots).</p>	266
<p>Figure 4.37. Scatter-plot showing NH_4^+-N mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for Pond 1 data only. Individual data points represent mean performance data from triplicate determinations for: Rock Filter 1 (●); Open Pond 1 (*); and Attached-Growth Media Reactor 1 (□).</p>	269
<p>Figure 4.38. Scatter-plot showing NH_4^+-N mass loading (pilot plant Influent) vs. percentage mass removal (relative to daily loading rate) for the combined data of Ponds 2 and 3. Individual data points represent mean performance data from</p>	

triplicate determinations for: Rock Filters 2 and 3 (●); Open Ponds 2 and 3 (*); and Attached-Growth Media Reactors 2 and 3 (□).....	270
Figure 4.39. Scatter-plot showing NH ₄ ⁺ -N mass loading (pilot plant Influent) vs. total mass removal for Pond 1 data only. Individual data points represent mean performance data from triplicate determinations for: Rock Filter 1 (●); Open Pond 1 (*); and AGM Reactor 1 (□). Fitted lines represent best-fit lines from simple linear regression analyses, with regression slopes (<i>m</i>) shown alongside the respective figure legends.....	271
Figure 4.40. Scatter-plot showing NH ₄ ⁺ -N mass loading (pilot plant Influent) vs. total mass removal for the combined data of Ponds 2 and 3. Individual data points represent mean performance data from triplicate determinations for: Rock Filters 2 and 3 (●); Open Ponds 2 and 3 (*); and AGM Reactors 2 and 3 (□). Fitted lines represent best-fit lines from simple linear regression analyses, with regression slopes (<i>m</i>) shown alongside the respective figure legends.	271
Figure 4.41. Entire Rock Filter train mass ammonia-nitrogen removals showing observed versus predicted performance (predicted values calculated based on Rock Filter regression performance analyses from Section 3.3.7.1). Fitted regression line (solid line) shown with 95% CI's (broken lines).	274
Figure 4.42. Soluble reactive orthophosphate-phosphorous box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3). The shaded 'box' represents the IQR, the horizontal bar shows the median value, and the 'whiskers' show the absolute data range.	277
Figure 4.43. Box-plots showing percentage orthophosphate-phosphorous removal performance relative to pilot plant Influent concentration for all ponds and across all 3 pilot treatment systems (<i>n</i> = 10 for all plots).	278
Figure 4.44. Faecal coliform box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3). The shaded 'box' represents the IQR, the horizontal bar shows the median value, and the 'whiskers' show the absolute data range.	281
Figure 4.45. <i>E. coli</i> box-plot data for pilot plant: Influent (INFL); Rock Filters 1, 2, 3 (RF-1, RF-2, RF-3); Open Ponds 1, 2, 3 (OP-1, OP-2, OP-3); and Attached-Growth Media Reactors 1, 2, 3 (AGM-1, AGM-2, AGM-3).....	281
Figure 4.46. Theoretical serviceable life of a Bolivar-based rock filter (based on a void volume of 55.86%, HLR of 1.0m ³ m ⁻³ d ⁻¹ , mean influent SS of 27.4g m ⁻³ , mean VSS of 52.5%, mean SS removal efficiency of 76% and a sludge water content of 85%)......	289
Figure 4.47. Theoretical serviceable life of a Bolivar-based AGM upgrade installation (based on a void volume of 95.7%, HLR of 1.0m ³ m ⁻³ d ⁻¹ , mean influent SS of 27.4g m ⁻³ , mean VSS of 52.5%, mean SS removal efficiency of 71% and a sludge water content of 85%)......	290
Figure 5.1. Pilot plant influent phytoplankton population dynamics during both monitoring <i>Period 1</i> of 2005 and <i>Period 2</i> of 2006 showing relative temporal abundance of the dominant genera (left <i>y</i> -axis) as well as total abundance (log ₁₀ cells ml ⁻¹ ; broken line; right <i>y</i> -axis).....	302
Figure 5.2. Pilot plant influent phytoplankton population dynamics during both monitoring <i>Period 1</i> of 2005 and <i>Period 2</i> of 2006 showing relative percentage	

temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.	302
Figure 5.3. Box-plot showing total phytoplankton abundance for: the 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Abundance data sourced from the entire monitoring duration from July 2005–August 2006.....	303
Figure 5.4. Duckweed Pond 1 phytoplankton population dynamics for a limited duration during the 2005 <i>Period 1</i> showing relative temporal abundance of the dominant genera (left y-axis) as well as total phytoplankton abundance (\log_{10} cells ml^{-1} ; broken line; right y-axis).	307
Figure 5.5. Duckweed Pond 1 phytoplankton population dynamics during the 2005 <i>Period 1</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.	307
Figure 5.6. Duckweed Pond 3 phytoplankton population dynamics during the 2005 <i>Period 1</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total phytoplankton abundance (\log_{10} cells ml^{-1} ; broken line; right y-axis).....	308
Figure 5.7. Duckweed Pond 3 phytoplankton population dynamics during the 2005 <i>Period 1</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.	308
Figure 5.8. Open Pond 1 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total phytoplankton abundance (\log_{10} cells ml^{-1} ; broken line; right y-axis).	309
Figure 5.9. Open Pond 1 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.	309
Figure 5.10. Open Pond 3 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total phytoplankton abundance (\log_{10} cells ml^{-1} ; broken line; right y-axis).	310
Figure 5.11. Open Pond 3 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.	310
Figure 5.12. Rock Filter 1 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total phytoplankton abundance (\log_{10} cells ml^{-1} ; broken line; right y-axis).	311
Figure 5.13. Rock Filter 1 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.	311
Figure 5.14. Rock Filter 3 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total phytoplankton abundance (\log_{10} cells ml^{-1} ; broken line; right y-axis).	312
Figure 5.15. Rock Filter 3 phytoplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.	312

Figure 5.16. Attached-Growth Media Reactor 1 phytoplankton population dynamics during the 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left <i>y</i> -axis) as well as total phytoplankton abundance (\log_{10} cells mL^{-1} ; broken line; right <i>y</i> -axis).....	313
Figure 5.17. Attached-growth media Reactor 1 phytoplankton population dynamics during the 2006 <i>Period 2</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.....	313
Figure 5.18. Attached-growth media Reactor 3 phytoplankton population dynamics during the 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left <i>y</i> -axis) as well as total phytoplankton abundance (\log_{10} cells mL^{-1} ; broken line; right <i>y</i> -axis).....	314
Figure 5.19. Attached-growth media Reactor 3 phytoplankton population dynamics during the 2006 <i>Period 2</i> showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.....	314
Figure 5.20. Mean percentage contributions of the four major phytoplankton groups (greens, diatoms, cyanobacteria and cryptophytes; (a)) and the three problem phytoplankton genera (<i>Chlamydomonas</i> , <i>Chlorella</i> and <i>Euglena</i> ; (b)) to the total algal population. Data shown for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF 1&3), Open Ponds 1 and 3 (OP 1&3), Duckweed Ponds 1 and 3 (DW 1&3) and Attached-Growth Media Reactors 1 and 3 (AGM 1&3). Average values for the RF and OP treatments were calculated from the combined Pond 1 and 3 data of the 2005–2006 monitoring duration.....	321
Figure 5.21. Pilot plant influent zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left <i>y</i> -axis) as well as total zooplankton abundance (individuals L^{-1} ; broken line; right <i>y</i> -axis).....	326
Figure 5.22. Pilot plant influent zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative temporal abundance of the dominant zooplankton groups.....	326
Figure 5.23. Pilot plant influent zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant genera (left <i>y</i> -axis) as well as total zooplankton biomass (mg L^{-1} ; broken line; right <i>y</i> -axis).....	327
Figure 5.24. Pilot plant influent zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant zooplankton groups.....	327
Figure 5.25. Rock Filter 1 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left <i>y</i> -axis) as well as total zooplankton abundance (individuals L^{-1} ; broken line; right <i>y</i> -axis).....	339
Figure 5.26. Rock Filter 1 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant zooplankton groups.....	339
Figure 5.27. Rock Filter 1 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant genera (left <i>y</i> -axis) as well as total zooplankton biomass (mg L^{-1} ; broken line; right <i>y</i> -axis).....	340

Figure 5.28. Rock Filter 1 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant zooplankton groups.	340
Figure 5.29. Rock Filter 3 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total zooplankton abundance (individuals L ⁻¹ ; broken line; right y-axis).	341
Figure 5.30. Rock Filter 3 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant zooplankton groups.	341
Figure 5.31. Rock Filter 3 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant genera (left y-axis) as well as total zooplankton biomass (mg L ⁻¹ ; broken line; right y-axis).	342
Figure 5.32. Rock Filter 3 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant zooplankton groups.	342
Figure 5.33. Open Pond 1 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total zooplankton abundance (individuals L ⁻¹ ; broken line; right y-axis).	343
Figure 5.34. Open Pond 1 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant zooplankton groups.	343
Figure 5.35. Open Pond 1 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant genera (left y-axis) as well as total zooplankton biomass (mg L ⁻¹ ; broken line; right y-axis).	344
Figure 5.36. Open Pond 1 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant zooplankton groups.	344
Figure 5.37. Open Pond 3 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total zooplankton abundance (individuals L ⁻¹ ; broken line; right y-axis).	345
Figure 5.38. Open Pond 3 zooplankton population dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant zooplankton groups.	345
Figure 5.39. Open Pond 3 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant genera (left y-axis) as well as total zooplankton biomass (mg L ⁻¹ ; broken line; right y-axis).	346
Figure 5.40. Open Pond 3 zooplankton biomass dynamics during the 2005 <i>Period 1</i> and 2006 <i>Period 2</i> showing relative percentage temporal biomass of the dominant zooplankton groups.	346
Figure 5.41. Duckweed Pond 1 zooplankton population dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total zooplankton abundance (individuals L ⁻¹ ; broken line; right y-axis).	347

Figure 5.42. Duckweed Pond 1 zooplankton population dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal abundance of the dominant zooplankton groups.....	347
Figure 5.43. Duckweed Pond 1 zooplankton biomass dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal biomass of the dominant genera (left y-axis) as well as total zooplankton biomass (mg L ⁻¹ ; broken line; right y-axis).....	348
Figure 5.44. Duckweed Pond 1 zooplankton biomass dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal biomass of the dominant zooplankton groups.....	348
Figure 5.45. Duckweed Pond 3 zooplankton population dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total zooplankton abundance (individuals L ⁻¹ ; solid white line; right y-axis).....	349
Figure 5.46. Duckweed Pond 3 zooplankton population dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal abundance of the dominant zooplankton groups.....	349
Figure 5.47. Duckweed Pond 3 zooplankton biomass dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal biomass of the dominant genera (left y-axis) as well as total zooplankton biomass (mg L ⁻¹ ; broken line; right y-axis).....	350
Figure 5.48. Duckweed Pond 3 zooplankton biomass dynamics during the 2005 monitoring <i>Period 1</i> showing relative percentage temporal biomass of the dominant zooplankton groups.....	350
Figure 5.49. Attached-growth media Reactor 1 zooplankton population dynamics during the 2006 monitoring <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total zooplankton abundance (individuals L ⁻¹ ; broken line; right y-axis).....	351
Figure 5.50. Attached-growth media Reactor 1 zooplankton population dynamics during the 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant zooplankton groups.....	351
Figure 5.51. Attached-growth media Reactor 1 zooplankton biomass dynamics during the 2006 monitoring <i>Period 2</i> showing relative percentage temporal biomass of the dominant genera (left y-axis) as well as total zooplankton biomass (mg L ⁻¹ ; broken line; right y-axis).....	352
Figure 5.52. Attached-growth media Reactor 1 zooplankton biomass dynamics during the 2006 monitoring <i>Period 2</i> showing relative percentage temporal biomass of the dominant zooplankton groups.....	352
Figure 5.53. Attached-growth media Reactor 3 zooplankton population dynamics during the 2006 monitoring <i>Period 2</i> showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total zooplankton abundance (individuals L ⁻¹ ; broken line; right y-axis).....	353
Figure 5.54. Attached-growth media Reactor 3 zooplankton population dynamics during the 2006 <i>Period 2</i> showing relative percentage temporal abundance of the dominant zooplankton groups.....	353
Figure 5.55. Attached-growth media Reactor 3 zooplankton biomass dynamics during the 2006 monitoring <i>Period 2</i> showing relative percentage temporal biomass of the dominant genera (left y-axis) as well as total zooplankton biomass (mg L ⁻¹ ; broken line; right y-axis).....	354

Figure 5.56. Attached-growth media Reactor 3 zooplankton biomass dynamics during the 2006 monitoring <i>Period 2</i> showing relative percentage temporal biomass of the four zooplankton groups.....	354
Figure 5.57. Box-plot showing zooplankton abundance data for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data sourced from the entire pilot plant monitoring period from July 2005–August 2006.....	356
Figure 5.58. Box-plot showing zooplankton biomass data for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data sourced from the entire pilot plant monitoring period from July 2005–August 2006.....	356
Figure 5.59. Box-plot of zooplankton community Shannon diversity indices (H') for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data sourced from the entire pilot plant monitoring period from July 2005–August 2006.	362
Figure 5.60. Box-plot showing the relative population densities of problem zooplankton for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data from the entire pilot plant monitoring period of July 2005–August 2006.	377
Figure 5.61. Box-plot showing the relative biomass densities of problem zooplankton for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data from the entire pilot plant monitoring period of July 2005–August 2006.	377
Figure 6.1. Laser excitation and detection optics layout for a standard bench-top (FACSCalibur, Becton Dickinson, USA) flow cytometer (modified from Campbell, 2001). Forward-angle light scatter, FSC; side-angle light scatter, SSC; red fluorescence, RFL; orange fluorescence, ORFL; green fluorescence, GRFL.....	396
Figure 6.2. Three-dimensional flow cytometric scatter plot from a theoretical mixed (50/50 live/dead) phytoplankton population showing: live 'healthy' cells (low PI–high FDA–high chlorophyll <i>a</i> fluorescence); dead cells (high PI–low FDA–low chlorophyll <i>a</i> fluorescence); and instrumental signal 'noise'.	412
Figure 7.1. Graphical depiction of the three-marker system used for differentiating the fluorescence activity states of the total algal cell population during prolonged dark-exposure. The cellular FDA-fluorescence histogram of a hypothetical mixed population depicts: the normal or 'healthy' fluorescence state (S3); a cell population with a reduced fluorescence state (S2); and an FDA-negative 'non-viable' cell population (S1). The <i>y</i> -axis shows the number of counted cell events and <i>x</i> -axis shows \log_{10} cellular FDA-fluorescence activity (A.U.) (Figure adapted from Franklin <i>et al.</i> , 2001a).....	433
Figure 8.1. (a) Overlaid frequency distribution plots depicting live: dead <i>C. vulgaris</i> FCM discrimination in various PI-stained (1.5 μ M) mixtures of live: dead (heat-killed) cultures: (A) 100% live; (B) 75% live; (C) 50% live; (D) 25% live; (E) 0% live (primary <i>x</i> -axes show \log_{10} PI fluorescence yield (A.U.), <i>y</i> -axes show number	

of cells counted); (b) stacked histogram depicting the accurate discriminatory capacity for the various mixtures of live: dead PI-stained (1.5 μ M) <i>C. vulgaris</i> ($n \geq 20,000$ cells for each live: dead mixture).....	440
Figure 8.2. (a) Overlaid histogram plot depicting live: dead <i>C. reinhardtii</i> discrimination via FCM and PI (1.5 μ M) staining of mixed ratio live: dead (heat-killed) cultures: (A) 100% live; (B) 75% live; (C) 50% live; (D) 25% live; (E) 0% live (primary x -axis shows \log_{10} PI fluorescence yield (A.U.), y -axis shows number of cells counted); (b) stacked histogram depicting the accurate discriminatory capacity for the various mixtures of live: dead PI-stained (1.5 μ M) <i>C. reinhardtii</i> ($n \geq 20,000$ cells for each live: dead mixture).....	440
Figure 8.3. FDA uptake and hydrolysis kinetics dot-plots (with plotted mean fluorescence kinetics line) for FCM staining protocol optimisation of <i>C. vulgaris</i> : (a) 2.4 μ M; (b) 12 μ M; (c) 24 μ M and (d) 36 μ M.	442
Figure 8.4. (a) Live <i>C. vulgaris</i> FDA (24 μ M) hydrolysis kinetics and fluorescence yield, y -axis shows relative FDA (fluorescein) fluorescence yield ($n = 8 \times 10^4$ cells). (b) Heat-killed negative control <i>C. vulgaris</i> FDA (24 μ M) hydrolysis kinetics and fluorescence yield, y -axis shows relative FDA fluorescence yield ($n = 3 \times 10^5$ cells). NB. >99.9% of algal cells in Figure 8.4(b) are below the solid 10^1 fluorescence cut-off line.	443
Figure 8.5. Parameters describing the time dependence of intracellular FDA hydrolysis: v = initial velocity, t_1 , t_2 = relaxation times, ρ = plateau level, or the time at which the extracellular substrate concentration gradient is made zero (modified from Sengbusch <i>et al.</i> , 1976).	444
Figure 8.6. Graphical plot of the initial linear portion of the Phase 1 FDA hydrolysis curve for <i>C. vulgaris</i> at: 2.4; 12; 24; and 36 μ M; with associated linear regression (r^2) coefficients given in parentheses.	445
Figure 8.7. FDA (24 μ M) hydrolysis and fluorescein-fluorescence kinetics for <i>C. vulgaris</i> (fitted curves are derived from single- and two-phase exponential associations).	447
Figure 9.1. Standard 8 day algal growth curves for <i>C. vulgaris</i> and <i>C. reinhardtii</i> (individual data points are the result of duplicate determinations).	456
Figure 9.2. Aqueous dissolved oxygen and pH for <i>C. vulgaris</i> over the 65 day experiment for all 4 treatments: x -axis represents the experimental duration (days); left y -axis (in black) shows dissolved oxygen concentration (mg L $^{-1}$); and the right y -axis (in blue) depicts aqueous pH. Data points shown mean values ± 1 S.D from triplicate treatment cultures.	459
Figure 9.3. Aqueous dissolved oxygen and pH for <i>C. reinhardtii</i> over the 65 day experiment for all 4 treatments: x -axis represents the experimental duration (days); left y -axis (in black) shows dissolved oxygen concentration (mg L $^{-1}$); and the right y -axis (in blue) depicts aqueous pH. Data points shown mean values ± 1 S.D from triplicate treatment cultures.	460
Figure 9.4. Aqueous dissolved oxygen and pH for <i>C. vulgaris</i> over the course of the 7 day experiment for all 4 treatments (x -axis represents the experimental duration (days); left y -axis (in black) shows DO concentration (mg L $^{-1}$); and the right y -axis (in blue) depicts aqueous pH). Data points shown mean values ± 1 S.D from triplicate treatment cultures.	463
Figure 9.5. Aqueous total dissolved inorganic carbon (DIC) levels for <i>C. vulgaris</i> over the course of the 7 day experiment for all 4 treatments (x -axis represents individual sampling intervals (days); and the y -axis shows DIC concentration (mg L $^{-1}$). Data points shown mean values ± 1 S.D from triplicate treatment cultures.	465

Figure 9.6. Two month <i>C. vulgaris</i> population cell density for all treatments (data points show the mean of three replicate cultures \pm 1 S.D.).....	466
Figure 9.7. Two month <i>C. reinhardtii</i> population cell density for all treatments (data points show the mean of three replicate cultures \pm 1 S.D.).....	466
Figure 9.8. Seven day <i>C. vulgaris</i> cell density following incubation under experimentally-manipulated light and dissolved oxygen conditions (data points show mean of three replicate cultures \pm 1 S.D.)	470
Figure 9.9. Graphical representation of published data showing the analytical linearity between FSC signal amplitude and cell volume: (a) modified from Shalapyonok <i>et al.</i> (2001); and (b) modified from Demers <i>et al.</i> (1989). Axial markings indicate the approximated relevant regions of the fitted curves ($10\text{--}1000\mu\text{m}^3$) pertaining to the cell size ranges used in this research.	472
Figure 9.10. 65 day <i>C. vulgaris</i> population mean FSC signal amplitude for all four experimental treatments (data points represent the mean of triplicate algal cultures \pm 1 S.D).	477
Figure 9.11. 65 day <i>C. vulgaris</i> population average cell volume for all four treatments. Cell volumes calculated according to the predefined equations of Section 9.6.3.1 (data points represent the mean of three replicate cultures \pm 1 S.D).....	477
Figure 9.12. 65 day <i>C. reinhardtii</i> population mean FSC signal amplitude for all four experimental treatments (data points represent the mean of triplicate algal cultures \pm 1 S.D).	478
Figure 9.13. 65 day <i>C. reinhardtii</i> population average cell volume for all four experimental treatments. Cell volumes calculated according to the pre-stated equations of Section 9.6.3.1 (data points represent the mean of three replicate cultures \pm 1 S.D).....	478
Figure 9.14. 65 day <i>C. vulgaris</i> population mean SSC signal amplitude for all four experimental treatments (data points represent the mean of triplicate algal cultures \pm 1 S.D).....	482
Figure 9.15. 65 day <i>C. reinhardtii</i> population mean SSC signal amplitude for all four experimental treatments (data points represent the mean of three replicate algal cultures \pm 1 S.D).....	483
Figure 9.16. <i>C. vulgaris</i> 65 day FSC vs. SSC signal amplitudes from: 'light / aerobic' (■); 'light / low D.O.' (□); 'dark / aerobic' (●); and 'dark / low D.O.' (○) treatments. Both regression slopes were significantly non-zero ($p < 0.0001$), with fitted regression lines shown \pm 95% CI's (broken lines).	486
Figure 9.17. 7 day <i>C. vulgaris</i> FSC signal amplitude for all 4 experimental treatments (data points show the mean of 3 replicate cultures \pm 1 S.D).....	489
Figure 9.18. 7 day <i>C. vulgaris</i> cell volume (μm^3) for all 4 treatments (data points show the population average of triplicate cultures \pm 1 S.D).....	490
Figure 9.19. 7 day <i>C. vulgaris</i> SSC signal amplitude for all 4 treatments (data points show the mean of 3 replicate cultures \pm 1 S.D).....	491
Figure 9.20. <i>C. vulgaris</i> 7 day FSC vs. SSC signal amplitudes from: 'light / aerobic' (■); 'light / low D.O.' (□); 'dark / aerobic' (●); and 'dark / low D.O.' (○) treatments. Fitted regression line (solid) shown with 95% CI's (broken lines). Pooled regression slope was significantly non-zero ($p < 0.0001$).	492
Figure 9.21. Two month aqueous chlorophyll <i>a</i> concentration for <i>C. vulgaris</i> (a) and <i>C. reinhardtii</i> (b) across all experimental treatments (data points show the mean of triplicate culture determinations \pm 1 S.D.)	496

Figure 9.22. Two month chlorophyll <i>a</i> per-cell dynamics for <i>C. vulgaris</i> across the four experimental treatments (data points show the mean of triplicate algal cultures \pm 1 S.D.).	497
Figure 9.23. Two month chlorophyll <i>a</i> per-cell dynamics for <i>C. reinhardtii</i> across all four experimental treatments (data points show the mean of triplicate cultures \pm 1 S.D.).	497
Figure 9.24. Cytograms from FCM analyses showing <i>C. reinhardtii</i> cultures at: day zero (a); Day 64 of 'dark / aerobic' treatment (b); and at Day 64 of 'light / aerobic' treatment (c). Figures on the left hand side show 2-D FCM scatter plots, and figures on the right show 2-D contour plots depicting relative cell population proportions from high (central) to low (outer) cell numbers (<i>x</i> and <i>y</i> axes show log ₁₀ forward and side-scatter respectively).	502
Figure 9.25. 7 day <i>C. vulgaris</i> chlorophyll <i>a</i> per-cell for all experimental treatments (data points show mean of three replicate cultures \pm 1 S.D.).	504
Figure 9.26. Two month <i>C. vulgaris</i> chlorophyll <i>a</i> fluorescence (FCM-quantified) per-cell (note the broken <i>y</i> -axis scale). Data points show the mean of three replicate cultures (\pm 1 S.D.).	506
Figure 9.27. Two month <i>C. reinhardtii</i> chlorophyll <i>a</i> fluorescence (FCM-quantified) per-cell (note the broken <i>y</i> -axis scale). Data points show the mean of three replicate cultures (\pm 1 S.D.).	506
Figure 9.28. Two month <i>C. vulgaris</i> chlorophyll <i>a</i> fluorescence (FCM-quantified) per unit cellular volume (μm^3). Data points show the mean of triplicate cultures (\pm 1 S.D.).	508
Figure 9.29. Two month <i>C. reinhardtii</i> chlorophyll <i>a</i> fluorescence (FCM-quantified) per unit cellular volume (μm^3). Data points show the mean of triplicate cultures (\pm 1 S.D.).	508
Figure 9.30. Relative rate of photosynthesis in continuously illuminated ageing cultures of <i>C. vulgaris</i> over various culture ages (modified from Pratt, 1943).	509
Figure 9.31. 65 day cell volume vs. chlorophyll <i>a</i> fluorescence for <i>C. vulgaris</i> (a) and <i>C. reinhardtii</i> (b) for 'dark / aerobic' (●); and 'dark / low D.O.' (○) treatments (showing Pearson's correlation coefficient (<i>r</i>) and fitted regression lines \pm 95% CI's).	512
Figure 9.32. 7 day <i>C. vulgaris</i> cellular chlorophyll <i>a</i> fluorescence (note the truncated <i>y</i> -axis). Data points show the mean of three replicate cultures (\pm 1 S.D.).	515
Figure 9.33. One week <i>C. vulgaris</i> chlorophyll <i>a</i> fluorescence (FCM-quantified) normalised per unit cell volume (μm^3). Data points show the mean of triplicate cultures (\pm 1 S.D.).	515
Figure 9.34. Two month <i>C. vulgaris</i> (a) and <i>C. reinhardtii</i> (b) mean PI fluorescence for all four treatments. The horizontal <i>y</i> -axis line (red) indicates the pre-determined lower 'cut-off' limit of the PI-positive 'non-viable' regional marker (i.e. cells below the line are PI-negative 'viable', and cells above the line are PI-positive 'non-viable'). Both <i>y</i> -axes represent cellular PI fluorescence (A.U.) and <i>x</i> -axes show elapsed time (days). Data points show the mean of 3 replicate cultures (\pm 1 S.D.).	518
Figure 9.35. Two month <i>C. vulgaris</i> (a) and <i>C. reinhardtii</i> (b) PI cellular fluorescence (normalised to cell volume; μm^3) for all treatments. Both <i>y</i> -axes represent cellular PI fluorescence (μm^{-3}) and <i>x</i> -axes show elapsed time (days). Data points show the mean of 3 replicate cultures (\pm 1 S.D.).	519

Figure 9.36. Two month <i>C. vulgaris</i> PI assay viability over the four treatments: (a) Light / aerobic; (b) Light / low D.O.; (c) Dark / aerobic; (d) Dark / low D.O. (x-axis represents time (days) and vertical bars show daily average live vs. dead cell populations (%) from 3 replicates \pm 1 S.D.).....	521
Figure 9.37. Two month <i>C. reinhardtii</i> PI assay viability over the four treatments: (a) Light / aerobic; (b) Light / low D.O.; (c) Dark / aerobic; (d) Dark / low D.O. (x-axis represents time (days) and vertical bars show daily average live vs. dead cell populations (%) from 3 replicates \pm 1 S.D.).....	521
Figure 9.38. Two month <i>C. vulgaris</i> FDA fluorescence per-cell. Data points show the mean of three replicate cultures (\pm 1 S.E.M.).....	523
Figure 9.39. Two month <i>C. reinhardtii</i> FDA fluorescence per-cell. Data points show the mean of three replicate cultures (\pm 1 S.E.M.).....	524
Figure 9.40. Two month <i>C. vulgaris</i> cellular FDA fluorescence (per unit cell volume; μm^{-3}). Data points show mean of three replicate cultures (\pm 1 S.E.M.).....	524
Figure 9.41. Two month <i>C. reinhardtii</i> cellular FDA fluorescence (per unit cell volume; μm^{-3}). Data points show mean of three replicate cultures (\pm 1 S.E.M.).....	525
Figure 9.42. Two month <i>C. vulgaris</i> FDA metabolic activity states (S1 – non-viable; S2 – compromised; S3 – viable) over the four treatments: (a) Light / aerobic; (b) Light / low D.O.; (c) Dark / aerobic; (d) Dark / low D.O. (x-axes represent elapsed time (days) and vertical bars (y-axes) show daily average percentage of the total population in each FDA fluorescence activity state \pm 1 S.D. from 3 replicates cultures)).....	529
Figure 9.43. Two month <i>C. reinhardtii</i> FDA metabolic activity states (S1 – non-viable; S2 – compromised; S3 – viable) over the four treatments: (a) Light / aerobic; (b) Light / low D.O.; (c) Dark / aerobic; (d) Dark / low D.O. (x-axes represent elapsed time (days) and vertical bars (y-axes) show daily average percentage of the total population in each FDA fluorescence activity state \pm 1 S.D. from 3 replicates cultures)).....	529
Figure 9.44. Sensitivity analyses of dual PI–FDA staining for viability (live vs. dead) discrimination of: (a) <i>C. vulgaris</i> and; (b) <i>C. reinhardtii</i> for all four experimental treatments: 'light / aerobic' (■); 'light / low D.O.' (□); 'dark / aerobic' (●); and 'dark / low D.O.' (○). Both y-axes reflect the percentage of FDA-positive cells in a given sample, whilst x-axes show the percentage of PI-negative cells from the same sample. Individual linear regression coefficients shown (with slope) for the fitted regression lines (broken lines represent 95% CI's for the fitted line).....	531
Figure 9.45. (a) Seven day <i>C. vulgaris</i> cellular PI fluorescence for all treatments (note the reduced y-axis scale). (b) Same data as for (a) but with an expanded y-axis scale. The horizontal line indicates the pre-determined lower 'cut-off' limit of the PI-positive 'non-viable' regional marker (i.e. cells below the line are PI-negative 'viable', and cells above the line are PI-positive 'non-viable'). Both y-axes reflect the relative PI cellular fluorescence (A.U.), whilst x-axes show the elapsed time at each sampling interval. Data points show the mean of 3 replicate cultures (\pm 1 S.D.).....	533
Figure 9.46. 7 day <i>C. vulgaris</i> PI-assay viability for the four experimental treatments: (a) Light / aerobic; (b) Light / low D.O.; (c) Dark / aerobic; (d) Dark / low D.O. (x-axis represents time (days) and vertical bars show daily average live vs. dead cell populations (%) from 3 replicate cultures \pm 1 S.D.).....	534
Figure 9.47. (a) Seven day <i>C. vulgaris</i> FDA fluorescence per-cell, and (b) FDA fluorescence per-cell normalised to cell volume (μm^{-3}). The y-axes show respective	

cellular FDA fluorescence, whilst *x*-axes show the elapsed time at each sampling interval. Data points show mean of triplicate cultures (± 1 S.E.M.).....535

Figure 9.48. 7 day *C. vulgaris* FDA metabolic activity states (S1 – non-viable; S2 – compromised; S3 – viable) over the four treatments: (a) Light / aerobic; (b) Light / low D.O.; (c) Dark / aerobic; (d) Dark / low D.O. (*x*-axis represents time (days) and vertical bars show daily average percentage of the total population in each FDA fluorescence activity state ± 1 S.D. from 3 replicates).537

Figure 9.49. 10 day algal re-growth curves for (a) *C. vulgaris* and (b) *C. reinhardtii* (data points show mean of three replicate cultures ± 1 S.D.)546

List of Plates

Plate 1.1. Photograph of an established duckweed surface mat being contained by a floating containment grid network.	36
Plate 1.2. An aerial view of the expansive Bolivar WSP system, located north of Adelaide, South Australia (photograph courtesy of Keremane and McKay, 2006).	51
Plate 2.1. Aerial view of the Bolivar WWTP (top left) showing the pilot plant location, and inset, an up-close aerial view of the Bolivar DAF/F plant, inlet sump and pilot plant location (photographs courtesy of United Water International and Google Earth; http://earth.google.com).	64
Plate 2.2. (a) 2mm stainless steel passive influent screen, and (b) detail of the pilot plant influent feed piping under the initial 'Phase 1' configuration.	66
Plate 2.3. Detail of the pilot plant influent feed piping under the modified 'Phase 2' configuration (broken arrows show the direction of flow).	67
Plate 2.4. Posterior view of a pilot pond (2 nd in series), showing the polyethylene pond liner, supporting steel frame, and outlet piping configuration.	69
Plate 2.5. Elevated view of the experimental pilot plant operating under experimental <i>Period 1</i> (from left to right): Duckweed, Open Pond, and Rock Filter treatment configuration.	70
Plate 2.6. Elevated view of the experimental pilot plant operating under experimental <i>Period 2</i> (from left to right): Attached-Growth Media, Open Pond, and Rock Filter treatment configuration (picture taken during a filamentous algal bloom in the Open Pond series).	70
Plate 2.7. Photograph of the established <i>L. disperma</i> surface mat on a pilot Duckweed Pond, and inset, a more detailed view of the floating duckweed mat structure.	71
Plate 2.8. Detail of the Rock Filter mixing chamber design, showing the placement of the retaining lattice and PVC supports.	74
Plate 2.9. Up-close and structural views of the TKP-319 horizontal-flow attached-growth media (pictures courtesy of 2H plastics; http://www.2h.com.au).	76
Plate 3.1. Detail of the relatively 'clean' biofilm-free internal rock media surfaces of RF-1, showing non-attached accumulations of flocculated materials. Broken lines indicate the water surface level.	151
Plate 3.2. Detail of the relatively 'clean' biofilm-free internal rock media surfaces of RF-3, showing non-attached accumulations of flocculated materials. Broken lines indicate the water surface level.	151
Plate 3.3. Photograph showing the highly developed root network of a low-density duckweed (<i>Lemna</i>) surface mat.	160
Plate 3.4. Aerial view looking down into one of the Open Ponds; showing the high densities of both pelagic (suspended) and substrate-grazing zooplankton populations (note the heavy grazing on pond wall biofilms).	164
Plate 3.5. Aerial view looking down into another of the Open Ponds; once again showing the high densities of both pelagic (suspended) and substrate-grazing zooplankton populations (note again the dense congregation of zooplankton close to the pond wall biofilm).	164
Plate 3.6. Photograph of the periodic filamentous green (Chlorophyceae; <i>Cladophora</i> and <i>Hydrodictyon</i>) algal blooms experienced within the OP series.	165
Plate 5.1. Detail of the internal rock media surfaces of RF-1 showing accumulations of flocculated detrital materials and a number of resident snails (circles). Broken lines	

indicate the water surface level. Scale bar (bottom–left) approximately 2cm in length.....	361
Plate 7.1. Photograph showing the internals of the illuminated orbital incubator and the randomized arrangement of the experimental treatment flasks for both algal species in all four treatments.	419
Plate 7.2. Double aluminium foil wrapped and sealed flask used for ‘dark / low D.O.’ treatments (broken line represents the positioning of the opaque aluminium foil cap).....	420

List of Tables

Table 1.1. Regional WSP effluent quality upper limits for discharge with respect to BOD ₅ and SS. Data sourced from Meiring and Oellermann (1995), Mara (1996), and SAEPA (2003).	17
Table 1.2. Summary of the most commonly reported advantages and disadvantages of duckweed for the upgrading of WSP effluent (Lewis and Bender, 1961; Culley Jr. and Epps, 1973; Dale and Gillespie, 1976; Reddy, 1983; Zirschky and Reed, 1988; Brix and Schierup, 1989; Edwards <i>et al.</i> , 1992; Reed <i>et al.</i> , 1995; Bonomo <i>et al.</i> , 1997; van der Steen <i>et al.</i> , 2003).	38
Table 1.3. Listing of the most commonly reported advantages and disadvantages of rock filtration for the upgrading of WSP effluent (USEPA, 2002a; Middlebrooks, 1995).	43
Table 1.4. Summary of the most commonly reported advantages and disadvantages of using attached-growth media for the upgrading of WSP effluent (Shin and Polprasert, 1987; Lessel, 1991; Nambu <i>et al.</i> , 1991; Polprasert and Sookhanich, 1995; Zhao and Wang, 1996; McLean, 1999).	47
Table 1.5. Typical phytoplankton species found in the Bolivar WSPs (modified from Buisine and Oemcke, 2003; Herdianto, 2003; and Martyn <i>et al.</i> , 2004).	54
Table 2.1. Pilot plant operational calendar for monitoring <i>Period 1</i> and <i>2</i> for all four experimental treatments: Duckweed (DW); Rock Filters (RF); Open Pond (OP); and Attached-Growth Media (AGM). Shading indicates treatment configuration during each monitoring period.	71
Table 2.2. Physical characteristics of individual Rock Filters (RF), Attached-Growth Media reactors (AGM) and Open Ponds (OP).	77
Table 3.1. Hydraulic characterisation of individual pilot ponds for the three treatment systems: Duckweed (DW); Open Pond (OP); and Rock Filter (RF). Individual parameter values represent the mean of duplicate tracer determinations.	98
Table 3.2. Summary of the hydraulic and organic loading characteristics of the individual pilot-scale WSP upgrade treatment reactors during operational <i>Period 1</i> .	100
Table 3.3. Pilot plant loading conditions and influent water quality for the first pond reactor of each three-pond treatment series.	101
Table 3.4. Summary of BOD ₅ performance data across all pilot plant treatments for Pond 1 and 3 data only.	121
Table 3.5. Pearson's correlation matrix for pilot plant Influent water quality parameters: suspended solids (SS); turbidity; chlorophyll <i>a</i> ; and BOD ₅ .	139
Table 3.6. Summary of suspended solids performance data for all pilot plant treatments for Ponds 1 and 3 only.	144
Table 3.7. Summary of chlorophyll <i>a</i> performance data across all pilot plant treatments for Ponds 1 and 3 only.	154
Table 3.8. Summary of ammonia removal performance across all pilot plant treatments for Pond 1 and 3 data only.	178
Table 3.9. Summary of orthophosphate-phosphorous performance data for all three treatments for Pond 1 and 3 only.	192
Table 3.10. Summary of indicator organism removals across all pilot plant treatments for Pond 1 and 3 data only.	196
Table 4.1. Hydraulic characterisation of individual pilot pond reactors for the three treatment systems: Rock Filter (RF); Open Pond (OP); and Attached-Growth Media	

(AGM). Individual parameter values represent the mean of duplicate tracer determinations.	209
Table 4.2. Summary of hydraulic and organic loading characteristics of the individual pilot-scale WSP upgrade treatment reactors during operational <i>Period 2</i>	210
Table 4.3. Pilot plant loading conditions and pilot plant Influent water quality for the first pond reactor of each three-pond treatment series.	212
Table 4.4. Summary of BOD ₅ performance data across all three pilot plant treatments for Ponds 1 and 3 only.	226
Table 4.5. Spearman’s correlation matrix for pilot plant influent water quality parameters: suspended solids (SS); turbidity; chlorophyll <i>a</i> ; and BOD ₅	237
Table 4.6. Summary of suspended solids removal performance across all pilot plant treatments for Pond 1 and 3 data only.	241
Table 4.7. Summary of chlorophyll <i>a</i> removal efficiencies across all three pilot plant treatments for Pond 1 and 3 data only.	252
Table 4.8. Summary of ammonia removal performance across all pilot plant treatments for Pond 1 and 3 data only.	267
Table 4.9. Summary of orthophosphate-phosphorous performance data for all three treatments for Pond 1 and 3 only.	279
Table 4.10. Summary of indicator organism removals across all pilot plant treatments for Pond 1 and 3 data only.	283
Table 5.1. List of all phytoplankton taxa encountered in the pilot plant influent during monitoring from July 2005–August 2006.	301
Table 9.1. FSC vs. SSC signal regression slope comparisons for both algal species during the two-month dark-survival experiment. Significant differences between treatments were identified via ANCOVA, with level of significance indicated by shading intensity: no shading signifies no difference ($p > 0.05$); light shading indicates a difference at $p < 0.05$; intermediate shading is significantly different at $p < 0.01$; and black shading is different at $p < 0.001$	487
Table 9.2. Statistical significance tables for two month chlorophyll <i>a</i> cellular fluorescence (normalised to cell volume; μm^3) of <i>C. vulgaris</i> and <i>C. reinhardtii</i> for all treatments (1-way RM-ANOVA with Tukey’s multiple comparisons). Shading shows level of significant difference between treatment means: no shading signifies no difference ($p > 0.05$); medium shading shows significance at $p < 0.01$; and black shading indicates a significant difference at $p < 0.001$	510
Table 9.3. Statistical significance tables for two month PI cellular fluorescence (normalised to cell volume; μm^3) of <i>C. vulgaris</i> and <i>C. reinhardtii</i> for all treatments (1-way RM-ANOVA with Tukey’s multiple comparisons). Shading shows level of significant difference between treatment means: no shading signifies no difference ($p > 0.05$); medium shading shows significance at $p < 0.01$; and black shading indicates a significant difference at $p < 0.001$	520
Table 9.4. Statistical significance tables for two month FDA cellular fluorescence (normalised to cell volume; μm^3) of <i>C. vulgaris</i> and <i>C. reinhardtii</i> for all treatments (1-way RM-ANOVA with Tukey’s multiple comparisons). Shading shows the level of significant difference between treatment mean FDA fluorescence: no shading signifies no difference ($p > 0.05$); light shading represents a significant difference at $p < 0.05$; medium shading shows significance at $p < 0.01$; and black shading indicates a significant difference at $p < 0.001$	526
Table 10.1. Executive summary of selected advantages and disadvantages for the three investigated advanced WSP upgrade systems.	603

Abbreviations and nomenclature

τ	calculated mean hydraulic residence time
τ_{th}	theoretical hydraulic residence time
$^{\circ}\text{C}$	degrees Celsius
μg	microgram
μl	microlitre
μm	micrometre
μM	micromolar
mM	millimolar
μmol	micromole
$\mu\text{S cm}^{-1}$	microsiemens per centimetre
χ^2	Kruskal–Wallis test statistic (Chi-square approximation)
ABS	absorbance
AGM	attached-growth media
AGWSP	attached-growth waste stabilisation pond
ANCOVA	analysis of covariance
ANOVA	analysis of variance
A.U.	arbitrary units
BOD_5	five day biochemical oxygen demand
Chl. <i>a</i>	chlorophyll <i>a</i>
CI	confidence interval
cm	centimetre
CO_2	carbon dioxide
COD	chemical oxygen demand
CV	coefficient of variation
CWM	community waste management
d	day
DAF/F	dissolved air flotation/filtration
DMSO	dimethyl sulfoxide
DO	dissolved oxygen
DOC	dissolved organic carbon
DIC	dissolved inorganic carbon
DW	pilot-scale duckweed pond

F	analysis of variance F ratio
FACScan	Becton Dickinson brand flow cytometer
FC	faecal coliforms
FCM	flow cytometry
FDA	fluorescein diacetate (diacetyl fluorescein)
FSC	forward-angle light scatter
F_0	minimum <i>in vivo</i> chlorophyll <i>a</i> (PS-II) fluorescence yield (relative units) induced by a weak initial probing flash in dark-adapted cells
F_m	maximum <i>in vivo</i> chlorophyll <i>a</i> (PS-II) fluorescence yield (relative units) induced by a weak initial probing flash in dark-adapted cells
g	gram
H'	Shannon diversity index
h	hour
ha	hectare
HDPE	high-density polyethylene
HRT	hydraulic residence time
HLR	hydraulic loading rate
INFL	pilot plant influent
IQR	interquartile data range
K_1	BOD ₅ removal rate coefficient (per day)
kg	kilogram
L	litre
m	metre
MBL	Woods Hole algal culture medium
MJ	megajoule
ml	millilitre
mm	millimetre
mg	milligram
mA	milliamp
mW	milliwatt
MPN	most probable number of organisms
n	sample size
$\text{NH}_4^+\text{-N}$	ammonia nitrogen
nm	nanometre

NO ₃ ⁻ -N	nitrate nitrogen
NO ₂ ⁻ -N	nitrite nitrogen
OLR	organic loading rate
OP	pilot-scale open pond
NTU	nephelometric turbidity units
PAR	photosynthetically active radiation (400–700nm wavelength)
PFD	photon flux density (light intensity)
pg	picogram
pH	potential hydrogen
PI	propidium iodide
PO ₄ ³⁻ -P	orthophosphate phosphorous
PS-I / PS-II	chlorophyll <i>a</i> Photosystems I and II
PVC	polyvinyl chloride
Q _{sc}	short-circuiting flow rate
r	Pearson's correlation coefficient
R	flow cytometer sample injection flow rate
RF	pilot-scale rock filter
ROS	reactive oxygen species
<i>r</i> _s	Spearman's rank correlation coefficient
RTD	residence time distribution
s	second
S1	FDA-negative 'non-viable' fluorescence state
S2	reduced 'compromised' FDA fluorescence state
S3	normal 'viable' FDA fluorescence state
S.D.	standard deviation
SS	suspended solids
SSC	side-angle light scatter
TOC	total organic carbon
TC	total carbon
UV	ultraviolet
V _d	dead volume
VSS	volatile suspended solids
WSP	waste stabilisation pond
WWTP	wastewater treatment plan

