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Preliminary version

FULL CONSULTANCY REPORT

Domestic Greywater Treatment System

for

AquaReuse Pty Ltd

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1 INTRODUCTION

Domestic greywater treatment systems (DGTS) are becoming a need in Australia, as residences strive to provide water for internal and external uses during significant drought conditions. There is also a growing community desire to be more environmentally conscious in their consumption decisions.

AquaReuse are responding to this need, and have worked with Ecowise Environmental Pty Ltd (Ecowise) in a consultative and applied research capacity. This Full Consultancy Report provides full details of all scientific findings from the range of cooperative projects undertaken since April 2004. This work includes monitoring associated with gaining NSW Health accreditation for the ADF1 Model, tested at Jervis Bay Cabins and Camping.

2 NATURE OF STUDIES

2.1 PILOT PLANT STUDY

A pilot scale unit of the greywater treatment system was installed at 4 Stella Street, Hill Top, and operated from February 2004 to XXX 2005. The pilot plant unit was approximately 1/11 the scale of the proposed full-scale design. This is considered to be representative to determine preliminary monitoring benefits. The system was constructed of a series of crates and included a number of process units from primary to secondary filters. Each filter had its own unique biofilter arrangement, which is commercially confident. The installation is shown in Figure 2.1.

Greywater is routinely added to the influent biofilter from the onsite residential premises at 4 Stella Street, Hill Top. This includes macerated and un-macerated kitchen waste (Figure 2.2), bath water and shower water. Approximately 4 to 10L of greywater is added to the influent biofilter at a time, representing a suitable flow rate for the given scale of 1/11. The quantity of water actually added has not been recorded, and at times significant hydraulic loads have been placed on the system. Approximately 40 to 80L/day has been supplied to the unit, and it is not believed that the system has been left 'unfed' for any period of time since February 2004.

Effluent is aerated in a final effluent chamber, and is either pumped to an inline hydroponics plant watering system, or transferred to a bucket for placement on the sites gardens.

Figure 2.1: Greywater System Pilot Plant Unit Setup

Figure 2.2: Greywater System Kitchen Waste Addition

2.2 ACCREDITATION TEST SITE STUDY

DGTS require accreditation by NSW Health via the DGTS Accreditation Guidelines (February 2005). This guideline includes residential systems treating up to ten persons per day, equivalent to 900 Ld^{-1}. AquaReuse wished to gain NSW Health accreditation of their peat based biological filter system (Model ADF1), designed for a flow of nine persons, or 810 Ld^{-1}, including kitchen sink, but not insinkerators. Ecowise were commissioned by AquaReuse to conduct the required monitoring program to gain accreditation.

A test site facility was confirmed at a caravan park and camping site in Jervis Bay, NSW (Figure 2.3). The site includes permanent resident cabins and caravans, tourist cabins and caravans, and camping sites with some amenities. This site was chosen due to its ready access to the range of greywater sources wanted for treatment (laundry, bath, sinks, shower, kitchen sink), insitu greywater sump well, ready access to greywater plumbing, guarantee of flow at desired flow rates, proximal location. Site staff were also very interested in the project, and the possibility to reuse greywater for toilet flushing (and possibly laundry use), reducing the need for tank and rainwater. The test site was setup in October 2004 and commissioned during November 2004.

Figure 2.3: Caravan Park Test Site Installation

The site was subject to weekly monitoring of effluent and fortnightly sampling of influent, in accordance with the NSW Health accreditation guidelines. Minimum parameters analysed included biochemical oxygen demand (BOD), suspended solids (SS) and thermotolerant coliforms (TC). Some samples were also analysed for additional parameters such as total nitrogen (TN), ammonia (NH_4), nitrate (NO_3), nitrite (NO_2), oxidisable nitrogen (NOx), total phosphorus (TP), ortho phosphorus (OP) and *Escherichia coli* (*E. coli.*).

This site was also used to study the ability for organisms to regrow in treated and stored greywater effluent. Stored effluent at the site, and sub-sampled and stored effluent at the laboratory, were analysed weekly for TC and *E. coli*.

A microbiological sample of the peat biofilter was submitted for analysis by the University of Queensland for determination of the diversity of species present. rRNA analysis was undertaken to develop a species list.

An ongoing monthly routine and quarterly intensive monitoring program is now continuing.

2.3 RESIDENTIAL INSTALLATION STUDIES

AquaReuse installed two test sites at residential premises, one in Katoomba, New South Wales and one in Tamborine, Queensland (Figures 2.4 and 2.5 respectively). Figure 2.6 shows the biological community within the peat biofilter from the Tamborine site.

Figure 2.4: Katoomba, NSW Test Site Installation

Figure 2.5: Tamborine, QLD Test Site Installation

Figure 2.6: Biological Community Within Peat Bed Filter at Tamborine Test Site Installation

These sites were monitored at least once weekly for over 20 weeks for a range of physical, chemical and biological parameters (temperature, BOD, SS, TC, TN, NH_4, NO_3, NO_2, NOx, TP, OP, *E coli*. Microbiological samples were collected monthly at the Tamborine facility as part of collaborative research with Ecowise and the University of Queensland (UQ). This included rRNA species analysis, fluorescent insitu hybridisation (FISH) assay to assess community changes over time, and DGGE (??).

Both the Katoomba and Tamborine sites were subject to a spiking trial using FRNA bacteriophage to assess virus removal. Each site undertook one such study, with FRNA phage spiked and analysed by Ecowise. The presence of F-specific RNA (FRNA) bacteriophages in a sample generally indicates pollution by wastewater contaminated by human or animal faeces. Their structure, limited environmental replication, disinfection resistance and retention by shellfish resemble that of human enteric viruses, for example the picornavirus and caliciviruses families which include poliovirus, Coxsackievirus, Norwalk and other small, round structured viruses, Hepatitis A and Hepatitis E viruses.

3 METHODS

3.1 SAMPLING

Table 3.1 lists the equipment generally used for the sampling programs.

Table 3.1: Summary of Sampling and Field Equipment

Equipment	Use	Details
Sample taps onsite, installed by AquaReuse at all sampling points	Collect sample from influent, secondary and effluent	None
Esky	Store and keep cool sample jars	None
500mL plastic sample jar (CHEM)	Collect sample for BOD, SS, nutrients	None
500mL plastic sterile sample jar (BACTO), preserved with thiosulphate	Collect sample for thermotolerant coliforms, *Escherichia coli*	None
Distilled water	Cleaning of equipment and collecting trip blank samples for QA	Laboratory Type 1 water
Disposable gloves	Protects hands and sample from contamination	None
Digital camera	Record site visual details	None

All samples were delivered to the laboratory within 24 hours of collection. Samples were kept on ice until delivery. Samples were accompanied by a chain-of-custody form detailing the sample ID, analysis required and delivery and receival details.

In the effluent storage project, we assessed the ability for a treated greywater effluent to support the re-growth of *E. coli*. Sampling of the Jervis Bay test facility stored greywater effluent was conducted fortnightly over around 3 months (test site facility). After six weeks of analysis, the laboratory sample was spiked with *E. coli* using the BTF 'bioball' which includes a quantifiable 30 organisms (laboratory spike A). This was placed into 1L, giving 3 organisms per 100mL. After a further 5 weeks analysis, additional bioballs were added (3) to spike the sample again. This time the spiking generated 13 organisms per 100mL (laboratory spike B).

Sampling of biofilm from the Tamborine facility for molecular analysis was conducted by University of Queensland (UQ) resources, and at times by the onsite staff trained by UQ people. Figure 3.1 illustrates this sampling method.

Figure 3.1: Sampling Biological Community (QLD)

3.2 ANALYSIS

All chemical and biological analysis was performed at the Fyshwick laboratory of Ecowise. All parameters tested were NATA accredited at this facility. Full laboratory methods procedures can be provided, however tests were in keeping with APHA Standard Methods for the Examination of Water and Wastewater. Table 3.2 summarises the analytical procedures used.

Table 3.2: Summary of Analytical Procedures

Analyte	Method
Biochemical oxygen demand	APHA 5210 B (5 day test)
Suspended solids	APHA 2540 D
Thermotolerant coliforms	APHA 9222 D (membrane filtration)
Escherichia coli	APHA 9222 D (indole test)
Total nitrogen	APHA 4500-N
Ammonia	APHA 4500-N
Nitrate	APHA 4500-N
Nitrite	APHA 4500-N
Oxidisable nitrogen	APHA 4500-N
Total phosphorus	APHA 4500-P
Ortho phosphorus	APHA 4500-P

APHA (1998) Standard Methods for the examination of water and wastewater. 20th Edition.

rRNA analysis was used to access all genomic material available in a sample matrix. The steps below were followed.

1. Utilising FastDNA SPIN Kit for Soil, DNA extraction was performed on sample 31/3/05, Cell Two, Jervis Bay to determine if genomic material was present. Two different quantities of sample were used, 250mg and 500mg to ensure sufficient DNA volume was obtained. A favourable result was gained with a large quantity of DNA existing within both peat samples.
2. PCR amplification of the above DNA 250mg sample was then performed using universal bacterial primers: 27f and 1492r in order to amplify 16S genes. Thermal profile for amplification included 30 cycles of denaturation at 94ºC for one minute, primer annealing at 48 ºC for one minute and primer extension at 72 ºC for a period of two minutes.
3. Once screened for the 16S rRNA gene, clone ligation was then performed utilizing the pGEM-TEasy vector and subsequently transformed using *Escherichia coli* cells.
4. PCR was then carried out using the plasmid specific primers of SP6 and T7. This allowed for the screening of clones in order to ascertain positive identification of 16S rRNA clones.
5. Digestion using the MspI restriction enzyme was then performed on clones positive for 16S that allowed for RFLP analysis.
6. Selected clones where then partially sequenced using primer 530f, and BLAST used to search for closely related sequenced relatives.

FISH analysis was being performed on samples from Tamborine only. Aliquots of samples were stored in 4% paraformaldehyde (PFA)/PBS mixture for approximately 1-3 hours. Following this, samples were resuspended and washed three times in PBS solution to finally be placed in a PBS/98% EtOH solution (1:1, v/v) and stored at -20°C until hybridisation was performed. As samples proved to contain a large amount of auxiliary matter, it was required that a pipette tip be cut (approximately 1cm from end) to allow adequate sample collection required for FISH.

FISH was then carried out utilising the oligonucleotide probes of EUBMix (EUB338, EUB338-II and EUB338-III, specific for all *Bacteria,* Amann *et al,* 1990)*,* BET42a (*Betaproteobacteria,* Manz *et al.,* 1992), GAM42a (*Gammaproteobacteria,* Manz et al, 1992) and LGC354 (*Low G+C Gram positive bacteria,* Meier *et al,* 1999). All probes were commercially synthesized with one of the sulfoindocyanine dyes, indocarboncyanine (Cy3) or indodicarbocyanine (Cy5) by ThermoHybaid (Interactivsa Division, Ulm, Germany). After FISH, samples were observed and images collected using a BioRad Radiance 2000 confocal laser-scanning microscope (CLSM).

For FRNA analysis, 100mL volumes of sample were warmed and added to a warm solution containing a culture of the host bacterium and calcium chloride solution. This is then added to a 100mL volume of molten tryptone agar, which is then poured into 8 large petri dishes. This is allowed to solidify before being incubated overnight. Bacteriophages are visualised as zones of clearing or lysis called plaques on a lawn of a bacterial host. All plaques are counted on the plates and the total is reported as F-RNA bacteriophage concentration per 100mL sample. To confirm plaques are the result of F-RNA bacteriophages only and not from male-specific DNA, coliphages or somatic Salmonella phages, an additional 100mL sample portion is assayed with a solution containing RNase being added to the melted tryptone agar.

3.3 QA/QC

The following QA samples were collected on random sampling runs.

1. Trip Blank sample, being laboratory-grade reagent water, contained within the same type of sample jar being used for greywater samples, and maintained within the esky on ice for the duration of the sampling trip.
2. Field Duplicates, sampled in the same manner and maintained under the same sample conditions as the greywater sample. This is a blind field duplicate to the laboratory and appropriate labelling will be used to guarantee transparency.

The sampling program was generally audited around once per month. The audit included all procedures and activities in relation to sampling including the onsite Sampling Technician, the Sampling Methods Manual, site sampling work, and sample delivery to the laboratory.

The sampling audit was conducted by the Sampling Manager. The audit process included:

1. Interview of Sampling Technician.
2. Review of Sampling Methods Manual to ensure it reflects actual procedures.
3. Observation of sampling run conducted by Sampling Technician.
4. Review of chain-of-custody, field sheets and relevant database files.
5. Identification of non-compliances and making of recommendations, where required.
6. Follow-up of non-compliances to ensure appropriate and timely rectification.

4 RESULTS

4.1 PILOT PLANT SITE

Onsite monitoring of the pilot plant showed a substantial reduction in COD over the biofilter process units, even early after startup of the system. Final effluent quality continually improved over February 2004 (Figure 4.1). TCOD was substantially removed in the first biofilter (Figure 4.2), with TCOD and SCOD almost equivalent in final effluent quality. The biofilter contained a substantial worm fraction as illustrated in Figure 4.3, and the clarity of final effluent was high as shown in Figure 4.4.

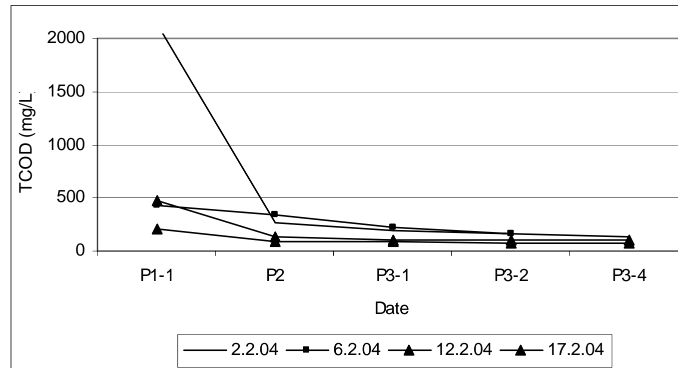

Figure 4.1: TCOD stabilisation over time, and process unit

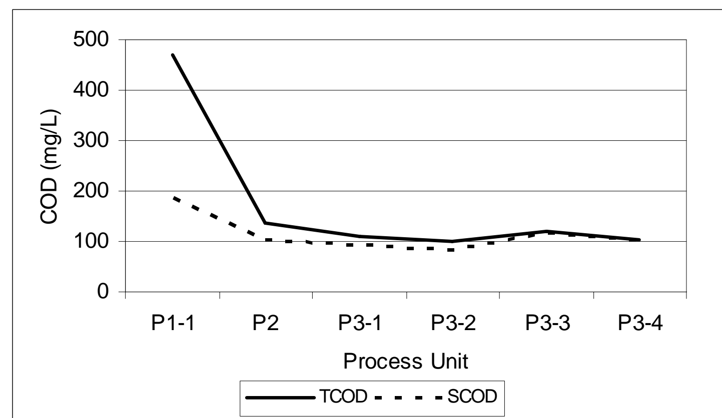

Figure 4.2: TCOD versus SCOD across process units

Figure 4.3: Worms located within biofilter

Figure 4.4: Water quality across biofilter units:
influent, primary effluent (P1-2) and final effluent (P3-3)

A series of samples were collected on 1 March 2004 and submitted for NATA accredited laboratory analysis to Ecowise. Table 4.1 summarises the results.

Table 4.1: Summary of Laboratory Monitoring Data from 1 March 2004 for Pilot Plant Unit

Analyte	Units	P1	P2	P3-2	P3-3	P3-4
Biochemical oxygen demand	mgL^{-1}				4	
TCOD	mgL^{-1}	2300	760	51	27	
SCOD	mgL^{-1}			36	27	27
Ammonia	mgL^{-1}		0.84	0.24	0.04	
Nitrite	mgL^{-1}		< 0.01	0.14	0.04	
Nitrate	mgL^{-1}		0.05	2.7	3.6	
Oxidised nitrogen	mgL^{-1}		0.05	2.8	3.6	
Total kjeldahl nitrogen	mgL^{-1}		8.2	2.3	1.4	
Total nitrogen	mgL^{-1}		8.3	5.1	5	
Total phosphorus	mgL^{-1}		4.1		0.41	0.74
Soluble phosphorus	mgL^{-1}		3		0.3	0.61

The laboratory monitoring confirms the substantial removal of COD across the primary biofilters (P1 to P2), and the high quality of final effluent (P3-4). Effluent BOD is very low at 4 mgL^{-1}, and the TCOD and SCOD in final effluent is equivalent, indicating the removal of all available COD and that remaining COD is probably biologically unavailable.

It can be seen that there were low concentrations of nitrogen compounds in the incoming influent, which is expected at most times for a greywater source. However, some nitrification may be occurring as the ammonia reduces from 0.84 to 0.04 across the treatment units, and the nitrate levels increase. In addition the TKN:TN ratio is almost 1:1 at P2, but changes to 1.4:5 in effluent (P3-3), indicating a relative reduction in organic nitrogen compared to inorganic nitrogen compounds.

The final biofilter between P3-3 and P3-4 is not contributing to phosphorus removal, and the levels double across this unit. The mechanism for this increase is not known at this stage. However, overall phosphorus removal from P2 to P3-3 is around 10 times.

4.2 ACCREDITATION TEST SITE

4.2.1 Accreditation Monitoring

The accreditation data from the Jervis Bay site monitored for six months for accreditation purposes, is summarised in Table 4.2 below. It can be seen that the AquaReuse DGTS readily meets the NSW Health effluent criteria for all forms of reuse, including toilet flushing and laundry (Table 4.3). The effluent results were stable and generally below laboratory detection.

Table 4.2: Summary of Influent and Effluent Data from the Jervis Bay Accreditation Test Site			
Sample	**BOD (mgL^{-1})**	**Suspended Solids (mgL^{-1})**	**Thermotolerant Coliforms (cfu/100mL)**
Influent (n=16)			
Maximum	550	440	5,800,000
Minimum	110	42	120,000
50%ile	245	130	1,100,000
90%ile	410	270	2,700,000
Effluent (n=28)			
Maximum	3	4	1
Minimum	0	0	0
50%ile	0	2	0
10%ile	0	0	0
10%ile represents the number whereby 90% of all samples are less than. This relates to the performance criteria required to be met by the NSW health Guidelines.			

Table 4.3: Test Site Effluent Data Compared to Compliance Criteria for Effluent Quality from DGTS According to Disposal / Utilisation Method							
	Analyte						
Disposal Method	**BOD mgL^{-1}**	**SS mgL^{-1}**	**T. coliforms cfu/100mL**	**TKN mgL^{-1}**	**TN mgL^{-1}**	**TP mgL^{-1}**	**Free Cl_2 mgL^{-1} ***
Effluent Quality from Test Site							
90% of Samples	0	0	1				
Maximum threshold	3	4	0				
Sub-surface irrigation							
90% of Samples	< 20	< 30		TBN	TBN	TBN	
Maximum threshold	< 30	< 45		TBN	TBN	TBN	
Surface irrigation							
90% of Samples	< 20	< 30	< 30	TBN	TBN	TBN	>0.2-<2.0
Maximum Threshold	< 30	< 45	< 100	TBN	TBN	TBN	<2.0
Toilet / Washing Machine reuse							
90% of Samples	< 10	< 10	< 10	TBN	TBN	TBN	>0.5-<2.0
Maximum Threshold	< 20	< 20	< 30	TBN	TBN	TBN	<2.0
TBN to be nominated, by manufacturer ** where chlorine is the disinfectant*							

Table 4.4 illustrates nutrient data for the test site, where 5 samples were submitted for testing. It can be seen that nutrient concentrations in the influent varied widely, and that the ADF1 did not contribute to significant nutrient removal from the greywater. However, there is evidence of nitrification, with ammonia levels decreasing and nitrate levels increasing. No evidence of denitrification was observed. Some phosphorus removal is also apparent, with levels reducing by around half. In addition, most effluent phosphorus was in the ortho phosphorus (or available) form, and therefore not associated with solids.

Table 4.4: Summary of Influent and Effluent Nutrient Data from the Jervis Bay Accreditation Test Site (all units mgL^{-1})								
Sample	**TN**	**TKN**	**NO_3**	**NO_2**	**NOx**	**NH_4**	**OP**	**TP**
Influent (n=5)								
Maximum	28	28	0.01	0.01	0.02	11	12	17
Minimum	12	12	0.01	0.01	0.01	0.95	2.2	3
50%ile	14	19.5	0.01	0.01	0.01	8.8	5.2	8.9
90%ile	26.8	27.1	0.01	0.01	0.01	10.6	9.3	16.1
Effluent (n=5)								
Maximum	38	6	31	0.62		4	6.2	6.4
Minimum	2.5	0.5	2	0.01		0.02	3.4	3.6
50%ile	11	1	9.6	0.045		0.05	5.6	5.7
90%ile	28.4	4.4	23.4	0.50		2.52	6.1	6.4

4.2.2 Effluent Storage Testing

Table 4.5 summarises the data for *E. coli* from the test site and laboratory samples. It can be seen that significant growth of *E. coli* was not supported by the stable treated greywater effluent generated by the AquaReuse process. When the laboratory samples were spiked with organisms, their growth was not supported, probably due to the low nutrient and carbon content of the effluent, and its biologically stable nature.

Table 4.5: Summary of *E. coli* data from Effluent Storage Trials (cfu/100mL)					
Test Site facility		**Laboratory Spike A**		**Laboratory Spike B**	
Week 0	0	Week 0	3	Week 0	13
Week 2	0	Week 1	0	Week 1	0
Week 4	0	Week 2	0	Week 2	0
Week 6	0	Week 3	0	Week 3	0
Week 8	0	Week 4	0	Week 4	0
Week 10	0	Week 5	0	Week 5	0
Week 12	16	Week 6	0		

4.2.3 rRNA Species Study

rRNA data is summarised in Table 4.6 below for the NSW site. It is expected that this is representative of the clone library that would be found at most AquaReuse installations. Overall, a very diverse community was observed with 86 ordered taxonomic units (OTUs) being recognised. It was noted that a few dominant groups were present within the clone library, however, when sequenced and entered into BLAST, each of the selected clones within the particular OTU groups proved to be dissimilar organisms.

Table 4.6: rRNA Findings for the NSW Site

Clone Number, Plate	Organism	Origin OR Journal focus
PLATE A		
2A	*Betaproteobacterium, Zoogloea ramigera*	Batch reactor
9A	Uncultured unidentified bacterium	Equine contamination
10A	Unidenified *Betaproteobacterium*	PAO study
16A	Unidentified *Gammaproteobacterium*	Biological foaming, activated sludge
17A	Unidentified *Gammaproteobacterium*	Acidic uranium-contaminated aquifer
19A	Unidentified *Deltaproteobacterium*	Coastal marine sediment
20A	Uncultured unidentified bacterium	Biological removal of P batch reactor
22A	*Gammaproteobacterium, Pseudomonas putida*	Equine contamination
26A	Uncultured unidentified bacterium	Arctic lake sediment
28A	*Betaproteobacterium, Variovorax sp.*	Soil
32A	Unidentified *Actinobacterium*	Subsurface microbial community structure
34A	*Betaproteobacterium, Comamonas testosteroi*	Plant pathogenic bacteria
36A	Uncultured *Eubacterium*	Travertine depositional facies
44A	Unidentified *Betaproteobacterium*	Underground oil storage cavity
49A	Uncultured unidentified bacterium	Bacterial community shifts in compost
51A	*Gammaproteobacterium, Pseudomonas putida*	Pseudomonas putida genome analysis
53A	Unidentified *Betaproteobacterium*	Paddy field soil
59A	Uncultured proteobacterium	Activated sludge
PLATE B		
1B	Uncultured *Eubacterium*	Travertine depositional facies
3B	*Betaproteobacterium*	Paddy field soil
13B	*Bacteroidetes*	Geothermal bacterial community
18B	*Deltaproteobacterium*	Marine communities
26B	Uncultured unidentified bacterium	Hawaiian archipelago
39B	Uncultured unidentified bacterium	Groundwater contaminated with uranuim w
PLATE C		
6C	*Betaproteobacterium, Zoogloea ramigera*	Molecular systematics of Zoogloea
7C	Unidentified *Alphaproteobacterium*	Acid and pristine sub alpine stream sedimer
8C	Unidentified *Deltaproteobacterium*	Soil community in New Caledonia
16C	*Betaproteobacterium, Variovorax sp.*	Soil community
17C	*Betaproteobacterium, Ralstonia solanacearum*	Plant pathogen
24C	Unidentified *Planktomycete*	River biofilms

4.3 RESIDENTIAL SITES

4.3.1 Chemical Data

Table 4.7 summarises the data for the Katoomba facility. This installation suffered a failure which generated a hydraulically overloaded biofilter layer, resulting in anaerobic conditions. The biological process was severely diminished, however the fault was rectified and effluent data improved. This site does not include disinfection, and therefore effluent coliform levels are relatively high. The site has not performed as well as other AquaReuse sites, probably due to the initial failure. It is anticipated that the process will become more stable as monitoring continues, as is shown for the last sample dated 16 June 2005.

Table 4.7: Summary of Influent and Effluent Data for the Katoomba Facility

	Influent			Effluent		
Sample Date	**BOD (mg/L)**	**SS (mg/L)**	**Coliforms (cfu/100mL)**	**BOD (mg/L)**	**SS (mg/L)**	**Coliforms (cfu/100mL)**
23.2.05	420	84	540000	45	27	<10000
3.3.05	52	170	340000	58	33	48000
10.03.05	260	66	22000	55	33	<10000
16.03.05	220	32	370000	50	35	100000
21.03.05	250	68	360000	52	29	51000
29.03.05	730	240	320000	40	32	79000
06.04.05	540	98	260000	92	46	24000
12.04.05	310	120	260000	53	38	20000
19.04.05	490	130	220000	160	48	190000
16.6.05	130	450	49000000	14	17	490

Figures 4.5 to 4.7 illustrate the excellent BOD, SS and coliform removal achieved at the Tamborine facility, which includes UV disinfection. Effluent quality was stable and of a high quality throughout the monitoring program, which is continuing to some extent.

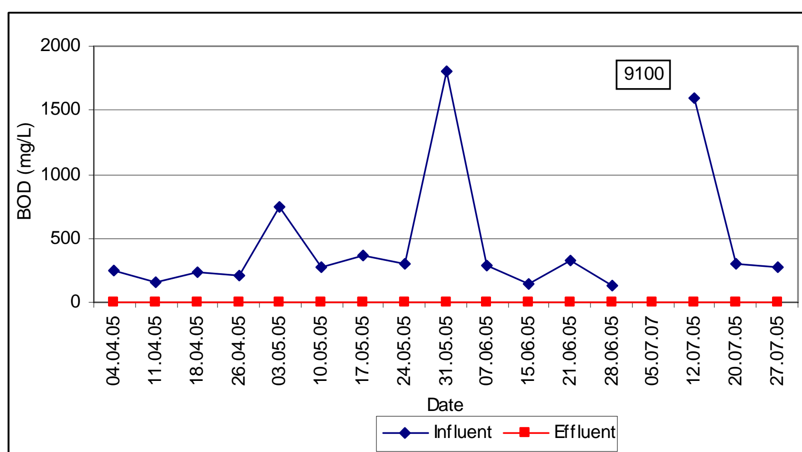

Figure 4.5: Influent and Effluent BOD for Tamborine Facility

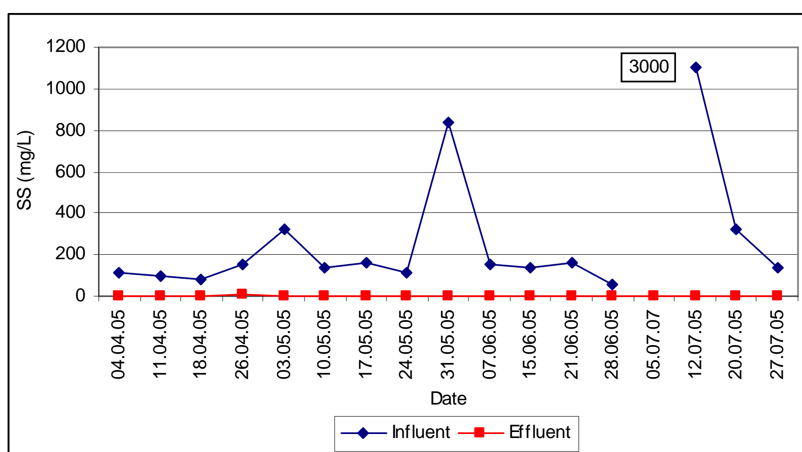

Figure 4.6: Influent and Effluent SS for Tamborine Facility

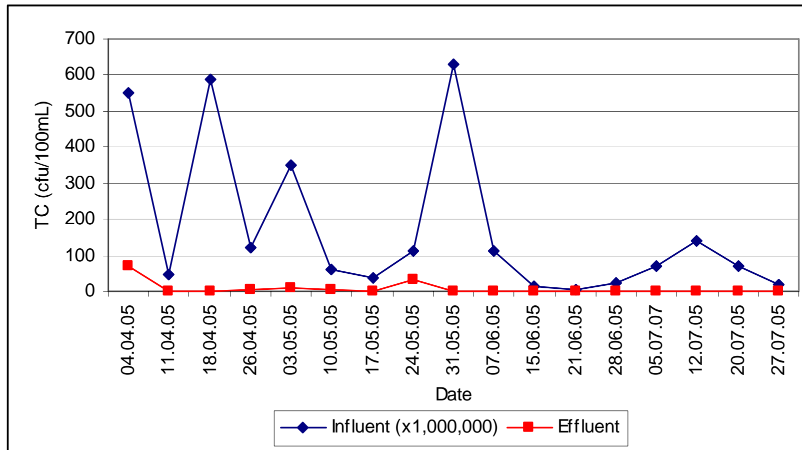

Figure 4.7: Influent and Effluent Coliforms for Tamborine Facility

Figure 4.8 shows in detail the process unit performance of coliform removal. Significant reduction occurs during the biological process unit, with the tertiary UV disinfection generally providing a low effluent concentration. Some spikes appear larger than the data, due to the log scale of the y axis.

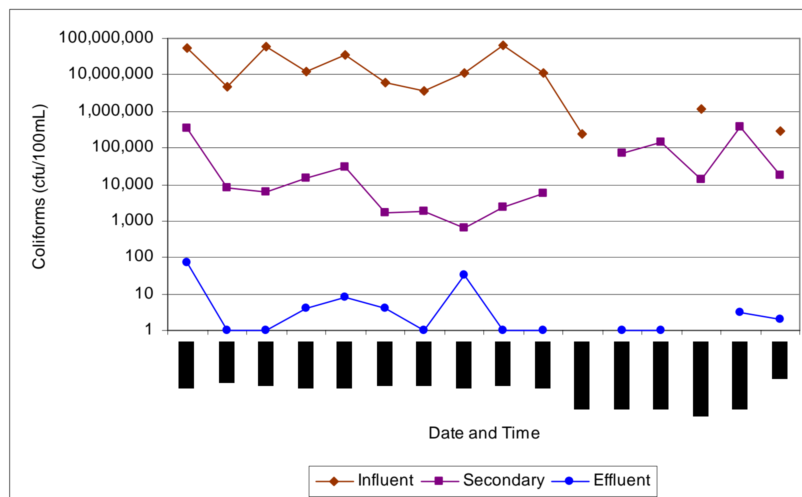

Figure 4.8: Thermotolerant Coliform Levels Over Process Units for Tamborine Facility

4.3.2 FRNA Phage Spike Study

A FRNA phage spiking trial was undertaken to assess the removal of phage (surrogate for viruses) over process units. Figures 4.9 (no UV disinfection) and 4.10 (with UV disinfection) show phage removal by process units at the NSW and QLD facilities respectively. Phage was initially spiked at a level of around 120 billion pfu per 100mL. Once placed into the influent sump, this gave a feed concentration of around 17 million pfu per 100mL. Effluent levels are very low, demonstrating model virus reduction across the AquaReuse approach, however complete removal was not achieved.

Figure 4.9 also shows that the phage spike occurs at around 8 hours, suggesting the hydraulic residence time of the process. Note that these levels in the effluent are elevated as there is no disinfection process unit at this site.

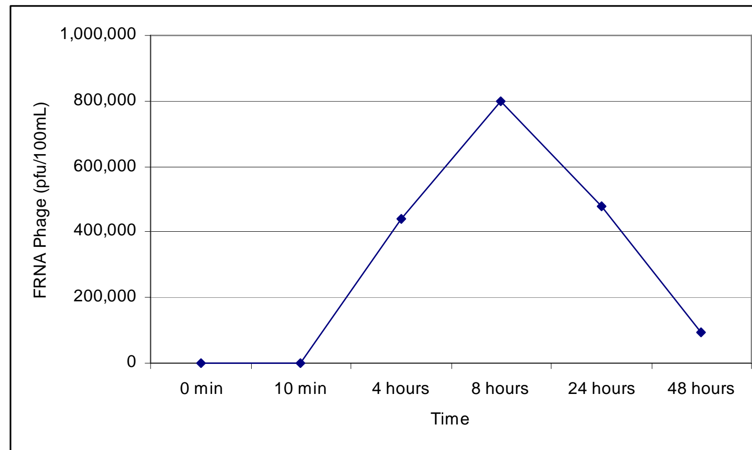

Figure 4.9: FRNA Phage Levels In Effluent Over Time (NSW – no UV)

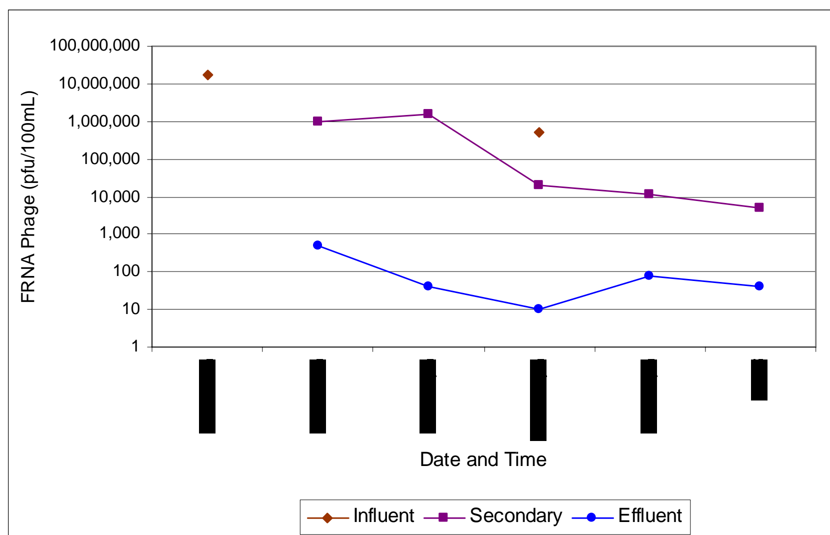

Figure 4.10: FRNA Phage Levels Over Process Units (QLD – with UV)

4.3.3 FISH Study at QLD Facility

FISH analysis was performed on two samples at the time of this report, including 29/4/05 and 14/6/05. It was noted that the dominant organisms observed in April 2005 were that of the *Alphaproteobacteria*. They were present within cells 1-3 in large abundance. However, alpha cell quantities decreased within cell 4. This was also the case for *Betaproteobacteria*, however, the latter were found not to be present in cell 3. Minimal numbers of *Gammaproteobacteria* were observed within cells 1-4, while no low G+C gram positive (LGC) classified bacteria were detected. This all suggest a very immature ecology present.

Figure 4.11 graphically compares this data with that found for the more mature sample dated June 2005, showing bacteria type across time and cell unit. It can be seen that a more diverse ecology has developed during the 6 weeks between sampling events, with less *Alphaproteobacteria* and more *Betaproteobacteria* and *Gammaproteobacteria* present. Figure 4.12 shows a confocal microscope image using FISH, where blue cells are all bacteria, and magenta represent *Alphaproteobacteria*.

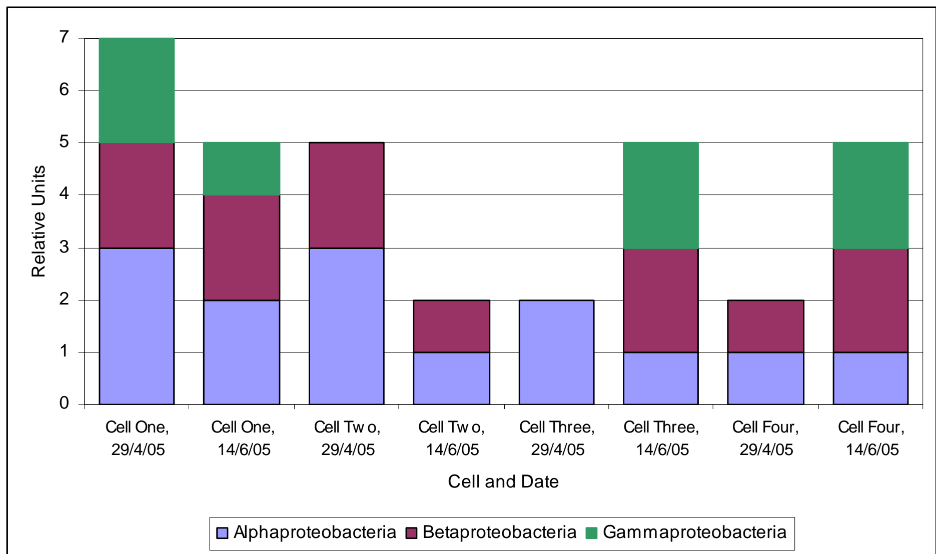

Figure 4.11: Biological Community Changes Across Cell Unit and Time, Using FISH (QLD)

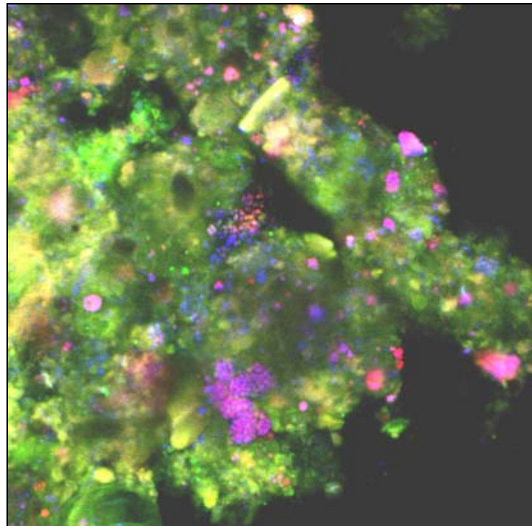

Figure 4.12: FISH Image - *Blue Cells Bacteria, Magenta cells Alphaproteobacteria*

5 CONCLUSIONS

The AquaReuse DGTS operates reliably, producing a high quality effluent. The test sites have provided a range of high quality data which allows AquaReuse to both optimise and prove its technology.

The data shows the wide variability of influent greywater, and the stable high quality of effluent generally produced. Storage of this stable effluent seems plausible for more than 24 hours, as low levels of coliforms were still apparent after 4 months of testing.

The rRNA phylogeny study of the NSW test site has indicated that the closest matches to the organisms identified are all involved in nutrient transformations in the environment. After ongoing phylogenetic analysis is completed, we will likely gain a better picture of their function. The largest outcome is that the biological community identified by rRNA, has a tremendous diversity, with a domination from the *Betaproteobacteria* and *Gammaproteobacteria*. This is to be expected considering the facility has been in operation since October 2004, and is therefore a very mature ecology.

In contrast, the FISH results from Tamborine has shown a dominance of *Alphaproteobacteria*. This has highlighted a potential difference between a new community (operating since March 2005), and the more mature community at the NSW test site (operating since October 2004).

FRNA phage monitoring has demonstrated the successful spiking and removal of the model virus through the process units of the AquaReuse system. Even with very high levels of phage spiked (at 120 billion pfu/100mL), the secondary biological process units successfully removed much of the phage (10 log), with tertiary disinfection at the Tamborine facility using UV, removing much of the remainder (down to 10 pfu/100mL).

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