Final Report

SWF Project No 512-001

Quantification of pathogen removal in Australian activated sludge plants

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Executive Summary

This Smart Water Fund Project (512-001) is providing information to assist the prediction of pathogen reduction performance from activated sludge plants (ASPs) and to enable the effect of 'upset' plant conditions on pathogen reduction to be integrated into treatment plant management plans. The project outcomes can inform an approach to validation of pathogen reduction across the ASP. The information will assist with the implementation of recently updated national and state water recycling guidelines. Project objectives include:

- Conducting a literature review to assess the objectives of the experimental work and identify gaps in the broader literature and inform the research plan incorporating the pilot plant and predictive model.
- Conducting a data review to understand the link to operating parameters and pathogen $\log_{10}$ reductions achieved in Australian activated sludge plants.
- Commissioning, stabilising and demonstrating that the pilot activated sludge plant has achieved steady state conditions and is ready for experimental work.
- Understanding the possible indicators for pathogen removal.
- Pilot plant related pathogen and indicator testing, analysis and reporting of results.
- Reviewing the applicability of a predictive model for monitoring data and operating data.
- To understand the effect of variability in plant conditions on protozoan pathogen removal.
- Summarising all project and experimental work and analysing in the context of the published literature.

Literature review outcomes

The key recommendations arising from the literature review and carried forward to the experimental approach included:

- Experiments should be designed to detect 0.5 to 2.5 $\log_{10}$ reduction of pathogens and indicators across the activated sludge plant (ASP).
- Pathogen reduction processes are biphasic (not first-order kinetics) and dosing design, sampling design and data analysis should take this into consideration.
- Sample manipulation and handling should be minimized to improve recoveries.
- Coagulant use should be considered a separate condition if included in the primary treatment process.
- Plant variables that should be monitored include pH, DO, MCRT (sludge age), MLSS and turbidity.
- Pathogenic microorganisms should be monitored in addition to potential indicators to investigate possible relationships, as the assessment of indicators alone is insufficient.
- Grab rather than composite samples should preferentially be collected during steady state, continuous operation conditions when assessing log reductions.
- The spike concentration needs to be selected carefully as applying too high a spike dose may allow the demonstration of greater log reductions which are unlikely to represent realistic conditions. Applying too low a spike dose will provide a potential underestimate.
- Identified appropriate surrogates for protozoan pathogen reduction across ASPs.

Data review outcomes

For the twelve Australian wastewater treatment plants that participated in the data review survey, it was observed that only a few facilities have conducted verification studies across process units for the range of pathogens of concern. The most commonly available information was final effluent *E. coli* levels, as this is generally monitored for license and compliance purposes. Only one study reported viruses separately. Most studies used the indicator organisms for viruses and parasites with only a few samples specifically representing *Cryptosporidium* and *Giardia*.

The standard deviations were high for some data sets. The indicators and pathogens gave LRVs of 2.8 $\log_{10}$ for bacteria, 1 to 1.5 $\log_{10}$ for protozoa and 1.5 to 2.9 $\log_{10}$ for viruses.

For the detailed data review of one data set of one facility, the indicators and pathogens had LRVs of 2.8 $\log_{10}$ for bacteria, 2.2 $\log_{10}$ for protozoa and 2.5 $\log_{10}$ for viruses. The variation over time was around 0.5 $\log_{10}$ for viruses and protozoa, and around 0.4 $\log_{10}$ for bacteria. Despite the small data set, significant correlations were observed between the following microbial and physico-chemical variables and this was considered in the experimental design and predictive model development:

- Effluent Suspended Solids and *E. coli* LRV (r^2 0.95)
- Suspended Solids Mixed Liquor tank 1 and *C. perfringens* LRV (r^2 0.90)
- Suspended Solids Mixed Liquor tank 2 and *C. perfringens* LRV (r^2 0.92)
- Effluent NH_3 and *C. perfringens* LRV (r^2 -0.93).

Experimental outcomes

Table ES1 summarises the experimental data outcomes for the sludge ages trialled.

Table ES1. *Cryptosporidium* and microbiological surrogate mean $\log_{10}$ reduction during 48 h sampling regime

***Cryptosporidium* and microbiological surrogates**	**Study (sludge age in days) LRV (mean ± standard deviation)**				
	15 (1 of 3)	**15 (2 of 3)**	**15 (3 of 3)**	**20 (1 of 1)**	**10 (1 of 1)**
Average temperature	19-20°C	19-20°C	Unknown**	19-20°C	26°C*
F-RNA coliphage	0.79 ± 0.04	1.32 ± 0.03	1.68 ± 0.04	2.11 ± 0.12	1.11 ± 0.06
C. perfringens	0.43 ± 0.12	1.27 ± 0.08	1.17 ± 0.08	1.67 ± 0.06	0.84 ±0 .10
Coliforms	0.57 ± 0.10	1.84 ± 0.07	1.39 ± 0.07	3.10 ± 0.12	3.22 ± 0.04
Cryptosporidium	0.51 ± 0.17	1.05 ± 0.06	0.41 ± 0.15	2.06 ± 0.16	1.44 ± 0.14
E. coli	0.78 ± 0.07	2.08 ± 0.06	1.79 ± 0.08	3.20 ± 0.06	3.27 ± 0.06
Enterococci/Faecal Streptococci	0.63 ± 0.13	1.93 ± 0.10	1.62 ± 0.03	2.90 ± 0.09	3.07 ± 0.09
Giardia	1.20 ± 0.21	2.29 ± 0.26	1.82 ± 0.51	2.37 ± 0.17	1.52 ± 0.21
SRC	0.62 ± 0.08	1.17 ± 0.05	1.16 ± 0.08	1.65 ± 0.02	1.02 ± 0.04

* Average temperature was above the recommended temperature range.
** Recommended temperature at 19 – 20°C

Predictive model outcomes

The data analysis involved three components, namely:

1. Summarising and comparing data between trials using plots and tables.
2. Exploratory data analysis.
3. Development of predictive models for each pathogen group monitored. Predictive model development involved the use of both standard multiple regression techniques and neural networks.

The primary biological variables of interest in the study were the $\log_{10}$ reduction in pathogen and pathogen indicator concentrations between t = 0 hour influent to the reactor vessel and the effluent. 48 hour samples of influent were also monitored to assess its variability over the spiking period. The differences in pathogen LRVs measured in the effluent between trials showed:

- Low pathogen and pathogen indicator LRVs in Trial 1. This may be due to setup difficulties associated with sampling and laboratory analysis for this first trial.
- Increasing the sludge age to 20 days appeared to increase LRVs for bacteriophage, coliforms, *Cryptosporidium, E. coli* and *Enterococci*.
- Decreasing the sludge age to 10 days and increasing the average reactor temperatures by several degrees seemed to decrease the bacteriophage LRV, but increased the coliform, *E. coli* and *Enterococci* LRVs.

Predictive models for each pathogen or indicator are shown in Table ES2 along with an assessment of overall model performance. In general, model performance was good although it should be noted that no attempt was made to validate the models by partitioning and quarantining a subset of the data (say 20%) to use as a testing data set.

Table ES2. Predictive model formulas for Principal Components Regression, summary variance explained by multiple regression model and assessment of model performance

Note: the assessment is based on the training data only and the models are not validated due to insufficient data at this stage.

Pathogen / Indicator	Predictive model	Multiple R^2	Assessment of model performance
Bacteriophage	"Bacteriophage LRV"=10.406-0.2930*"POM"+0.1843*"NOx"+0.1897*"ALKBOD" -0.0400*"TIME"+0.2023*"TNH3_SL"	**91.0%**	Very Good to Excellent
Clostridium perfringens	"*Clostridium perfringens* LRV"=0.9593-0.276*"POM"-0.048*"NOx"+0.0422*"ALKBOD"+ 0.0280*"TIME"+0.0671*"TNH3_SL"	65.6%	Moderate
Coliforms	"Coliforms LRV"=20.0760-0.7100*"POM"+0.6731*"NOx"-0.2020*"ALKBOD"-0.1630*"TIME"-0.3340*"TNH3_SL"	**96.0%**	Excellent
Cryptosporidium (confirmed)	"*Cryptosporidium* (confirmed) LRV"=10.0818-0.3392*"POM"+0.4824*"NOx"-0.2119*"ALKBOD"-0.0524*"TIME"+0.0135*"TNH3_SL"	**87.4%**	Very Good
Cryptosporidium (presumptive)	"*Cryptosporidium* (presumptive) LRV"=10.2990-0.3380*"POM"+0.5420*"NOx"+0.0235*"ALKBOD"-0.059*"TIME"+0.1095*"TNH3_SL"	**92.6%**	Very Good to Excellent
E. coli	"*E. coli* LRV"=20.298-0.6944*"POM"+0.5756*"NOx"-0.0933*"ALKBOD"-0.2137*"TIME"-0.3034*"TNH3_SL"	**94.3%**	Excellent
Enterococci	"Enterococci LRV"=20.0700-0.6990*"POM"+0.5368*"NOx"-0.0820*"ALKBOD"-0.1340*"TIME"-0.3140*"TNH3_SL"	**92.9%**	Very Good to Excellent
Giardia (confirmed)	"*Giardia* (confirmed) LRV"=10.4200-0.1370*"POM"-0.2790*"NOx"+0.0821*"ALKBOD"-0.0100*"TIME"-0.1040*"TNH3_SL"	78.0%	Good
Sulphite Reducing Clostridia	"Sulphite Reducing Clostridia LRV"=10.004-0.1950*"POM"-0.0340*"NOx"+0.0187*"ALKBOD"+0.0537*"TIME"+0.0154*"TNH3_SL"	57.5%	Poor to Moderate

Key:

Factor 1	SS, Turbidity, TKN, COD	*Concentration of Particulate Organic Matter (POM)*	*POM*	Higher concentrations of fine organic particles.
Factor 2	NO2-N, NO3-N	*Oxidation state of nitrogen*	*NOx*	This axis measures the oxidation state of ionic nitrogen (NO_3 = high, NO_2 = low) in solution.
Factor 3	Alkalinity, BOD5	*Alkalinity and BOD*	*ALKBOD*	The relationship between alkalinity and BOD is likely due to the consumption of alkalinity that occurs to a limiting factor as microbes metabolise BOD.
Factor 4	Hours	*Time*	*TIME*	Time seems quite independent of other factors.
Factor 5	SS-ML (MLSS), Temperature, NH3-N	*Temperature, Sludge age and ammonia - probably confounded here*	*TNH3_SL*	Ammonia is linked to temperature across an optimal range as expected. Note the large increase in trial 5 with high temperatures. The correlation with sludge age is also expected given the optimal growth rate required for successful nitrification.

In addition to the Principal Components Regression approach described above, an alternative method, based on a Generalized Regression Neural Network (GRNN) was undertaken. Overall the nets performed well with the training data set with the exception of *Cryptosporidium* (confirmed) and *Giardia* (confirmed). With the exception of the *Clostridia* and *Cryptosporidium* (confirmed) the testing data was predicted with 100% accuracy.

Project findings

Tables ES3 summarises the pathogen and indicator LRVs noted for the various stages of the project. These could be considered as conservative but scientifically justified LRV to apply for the ASP for the given pathogen groups.

Table ES3. Summary of noted LRVs for pathogens and indicators

Study	**Log_{10} reduction value**		
	Protozoa	**Viruses and viral surrogates**	**Bacteria**
International literature review	0.5 – 1.5 *Cryptosporidium* 1 – 1.5 *Giardia*	1 - 2	1 - 2
Australian literature review	1 *Cryptosporidium* 1.9 *Giardia*	1	1.8
Data review survey	1 *Cryptosporidium* 1.5 *Giardia*	1.5 – 2.9	2.8
Detailed data review	2.2	2.8	2.5
Experimental pilot plant	0.5 – 2 *Cryptosporidium* 1.2 – 2.3 *Giardia*	1 – 2	1 – 3

Overall the Principal Components Regression approach showed promise as a predictive tool; however the superior performance of the neural network approach, the lack of any need to conduct complex (and to some, opaque) preliminary analyses and data transformations, and the ease of its application highlighted the use of neural nets as the better predictive tool. Using Palisade's Neural Tools software (Palisade Corporation 2009), live predictions can be made within a Microsoft Excel spreadsheet. Such software makes it easy to envisage a situation in which the results of on-line measurements of independent physico-chemical variables are deposited automatically into a spreadsheet and the spreadsheet plotting and predictive tools automatically plot a continuous LRV statistic for each pathogen and indicator.

While extremely encouraging, the use of Generalised Regression Neural Nets, Principal Components Regression, (both conducted here), or other statistical methods such as Bayesian networks, or other linear approaches such as multilevel linear modelling requires a larger data set with significantly more cases measured across a wider range of values of the independent variables. In particular, the data set should be augmented with trials with (i) high temperature and 20 day sludge age and (ii) low temperature 10 day sludge age. Consideration should also be given to conducting the following trials:

- 15 day sludge age trials at high/er temperatures;
- 10, 15 and 20 day sludge age trials at temperatures intermediate between those of trials 1 to 4 and trial 5;
- Maximum sludge age experienced by the ETP may be higher than the maximum sludge age trialled here.

In addition future trials should include additional sampling of influent quality over the duration of the trials. This should provide a higher quality data set from which more accurate correction factors to account for pathogen die-off in the effluent storage can be calculated.

The predictive model approach may offer the best surrogate for attributing LRV as they can be modelled to online operating data and perform as critical control points in recycled water quality management plans.

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Glossary of terms

Term	Description
ADWF	Average dry weather flow
ASP/s	Activated sludge plant/s (and/or process). In this project being limited to conventional continuous configurations. This excludes consideration of primary or tertiary treatment trains. Considered as nitrifying and not denitrifying, to be consistent with available information and pilot plant management.
BNR	Biological nutrient removal, referring only to nitrogen removal via nitrification and denitrification and not phosphorus removal mechanisms.
BOD_5	Biochemical oxygen demand (5 day)
C/G or c/g	*Cryptosporidium / Giardia*
COD	Chemical oxygen demand
Coliphages	F specific male coliphage
DO	Dissolved oxygen
EBPR	BNR plant as defined above, also achieves biological phosphorus removal using an anaerobic process unit.
HRT	Hydraulic retention time
IDAL / IDAR	Intermittent decant aeration lagoon / reactor
IDEA	Intermittent discharge and extended aeration
Indicator	Microbial 'indicator' parameter as compared to the actual pathogen being tested (for example: FRNA phage to 'indicate' viruses; C. perfringens to 'indicate' protozoa)
Intermittent	Plant that operates under intermittent operation, various configurations possible, such as SBR, IDAL / IDAR with no clarifier used.
LRV	Log_{10} reduction value
MBR	Membrane bioreactor – activated sludge process combined with membrane filtration process – either in situ or ex situ.
MCRT	Sludge age or mean cell residence time
MLE	Modified Lutzack Ettinger, configuration for achieving BNR
MLSS (SS-ML)	Mixed liquor suspended solids; SS-ML used for statistical modelling
MLVSS	Mixed liquor volatile suspended solids
N_2 gas	Nitrogen gas
PCR	Polymerase chain reaction
PFU	Plaque-forming unit
POM	Particulate organic matter
PWWF	Peak wet weather flow – generally 3 or 4 x ADWF
SBR	Sequencing batch reactor
SRC	Sulphite Reducing Clostridia
SS	Suspended solids
Steady state	Defined as operating the pilot plant for 2 to 3 sludge ages, and ensuring physico-chemical parameters are stable, with ammonia (as N) <5 mgL^{-1}
STP	Sewage treatment plant
Surrogate	Physical or chemical parameter that is a 'surrogate' for a pathogen being tested (for example: turbidity demonstrating protozoa reduction)
SVI	Sludge volume index
TKN	Total kjeldahl nitrogen

1. BACKGROUND

This project aims to develop methods to a) predict pathogen reduction across activated sludge processes and b) to contribute information to develop an accepted approach for validating pathogen reduction across activated sludge processes using microbial indicators. Both routine and 'upset' plant conditions are considered. The project evaluated and developed a process for pathogen reduction monitoring and helped provide an understanding of cause and effect of various process parameters that assist in predicting when pathogen risk may be heightened due to those conditions. The project is promoted as a response to the pathogen $\log_{10}$ reductions identified in the *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1), 2006* (AGWR) and the *Victorian EPA Guidelines for Environmental Management, Dual Pipe Water Recycling Schemes – Health and Environmental Risk Management, 2005* which require scheme proponents to assign $\log_{10}$ reductions to process units and to manage risk in accordance with those log reductions.

The aims of the project include:

Economic

- Reduction in the cost of validation of process technologies;
- Reduction in the cost of ongoing monitoring programs which tend to be conservative as there is little scientific based information upon which to reduce monitoring programs;
- Ensure successful water recycling projects are approved by regulators in a timely manner.

Social

- Protection of the public from pathogens;
- Promote safety and acceptance of water recycling schemes.

Environmental

- Protection of the environment from release of pathogens;
- Minimisation of pathogen risk to the environment through scientifically justifiable management plans.

2. LITERATURE REVIEW

2.1. Overview

The purpose of the literature review was to summarise the findings of national and international studies of relevance to this Smart Water Fund project. This broader body of literature was considered when developing and progressing the detailed experimental scope of this project. Areas of focus for this review were three-fold:

- Mechanisms of pathogen and indicator reduction and relationships with surrogates and plant operating parameters that may exist;
- Pathogen and indicator reduction measured across activated sludge processes from previous studies, (as distinct from primary and tertiary process units);
- Pathogen monitoring approaches for studies of this type, including both the sampling strategies and the analytical techniques.

2.2. Key findings

The key factors found to influence pathogen reduction within activated sludge treatment processes were:

Adsorption of pathogenic microorganisms to suspended solids: Adsorption appears to be relatively rapid with the majority of adsorption complete within an hour, although parasite adsorption may increase for several hours. The rates of adsorption are not dramatically affected by the mixed liquor suspended solids (MLSS) concentrations or the pH ranges that are characteristic of activated sludge plants, however, anaerobic conditions rapidly reduce adsorption rates leading to decreased removal rates. It is therefore concluded that adsorption rates should be relatively stable within an activated sludge process under normal operating conditions. Dramatic changes in operation, such as loss of aeration (leading to anaerobic conditions), significant reductions in MLSS concentrations (i.e. < 1000 mg/L) or significant reductions in hydraulic retention times would be needed to meaningfully impact pathogen reduction performance.

Predation / loss of infectivity: As hydraulic and sludge retention time increases, predation becomes increasingly important for virus and bacterial removal / inactivation. While limited information on predation rates was found, inactivation by digestion of parasite eggs/cysts/oocysts does not appear to occur in the final effluent and did not appear to have altered infectivity. For viruses and bacteria, predation and inactivation rates would be expected to be relatively stable but would be impacted by significant changes in microorganism population characteristics in the biomass or significant reductions in hydraulic retention times (HRTs).

Efficiency of clarification: Solids removal (with adsorbed pathogens) is the key pathogen reduction step for activated sludge treatment. Therefore removal rates will be impacted in proportion to any factor that alters the efficiency of clarification. However, since the suspended solids in the clarified effluent is only a small percentage of the suspended solids that are removed through sludge wasting, pathogen reductions should be relatively insensitive to small changes in clarifier performance.

The literature overwhelmingly suggests that activated sludge plants (ASPs) readily achieve 1-2 $\log_{10}$ removal of bacteria and viruses. However, it is significant that not all references reported virus or viral indicator removals of 1 $\log_{10}$ and above. Therefore, some mechanism to quantify actual removals at a treatment facility is critical for accurate log removal predictions.

It is also critical that this mechanism is in place to address periods of potentially poor treatment plant performance. However, it does need to be acknowledged that this variability could reflect changes in virus influent concentrations, rather than treatment plant performance.

The literature also supports that activated sludge processes readily achieve a 1 to 1.5 $\log_{10}$ removal of *Giardia*. *Cryptosporidium* may have lower rates of removal of around 0.5 to 1.0 $\log_{10}$; however, it is not possible to provide a definitive ratio between the two parasites. The *Cryptosporidium* removals range was similar to the *Giardia* removals, although approximately 50% lower. The data suggests that activated sludge treatment should be able to achieve 0.5 to 1.5 $\log_{10}$ removal of *Cryptosporidium*.

Individual plants could have higher or lower levels of performance, influenced by factors such as operational control, plant flows and loadings and inclusion of primary treatment before the activated sludge process.

In summary, the key recommendations arising from the literature review and carried forward to the next phase of this project were:

- Experiments should be designed to detect 0.5 to 2.5 $\log_{10}$ reduction of pathogens and indicators across the ASP.
- Pathogen reduction processes are biphasic (not first-order kinetics) and dosing design, sampling design and data analysis should take this into consideration.
- Sample manipulation and handling should be minimized to improve recoveries.
- Coagulant use should be considered a separate condition if included in the primary treatment process.
- Plant variables that should be monitored include pH, DO, MCRT (sludge age), MLSS and turbidity.
- Pathogenic microorganisms should be monitored in addition to potential indicators to investigate possible relationships, as the assessment of indicators alone is insufficient.
- Grab rather than composite samples should be collected during steady state, continuous operation conditions when assessing log reductions.
- The spike concentration needs to be selected carefully as applying too high a spike dose may allow the demonstration of greater log reductions which are unlikely to represent realistic conditions. Applying too low a spike dose will provide a potential underestimate.

Table 2-1. Summary of $\log_{10}$ reduction values for bacterial, viral, parasitic and indicator pathogens collated from the literature

	$\log_{10}$ reduction (converted from % removal)				**$\log_{10}$ reduction**			
Pathogen /Indicator	**No. of papers**	**Mean**	**Median**	**SD**	**No. of papers**	**Mean**	**Median**	**SD**
Coliphage	Rose, 2001 Omura, 1989	1.77	1.77	0.01	Rose, 1996 Rose, 2004	1.2	1.2	0.7
Enterovirus	Rose, 2001	1.66	-	-	Rose, 1996 Rose, 2004 Ottoson, 2006	2.5	1.9	1.3
F-RNA phage	-	-	-	-	Zhang, 2007 Ottoson, 2006 Lucena, 2004 Rose, 2004	2.3	2.1	0.7
Somatic coliphage	-	-	-	-	Rose, 2004 Zhang, 2007 Ottoson, 2006 Lucena, 2004	1.9	1.6	0.8

	Log_{10} reduction (converted from % removal)				Log_{10} reduction			
Total enteric virus	Yanko, 1993 Aulicino, 1996 Rolland, 1983	1.00	0.94	0.03	Sedmak, 2005	2.4	2.4	0.5
E. coli		-	-	-	Ottoson, 2006	2.44	-	-
Faecal coliforms	Rose, 2001 Omura, 1989 Aulicino, 1996 Rolland, 1983	1.35	1.46	0.01	Rose, 1996 Rose, 2001 Zhang, 2007 Lucena, 2004	1.9	2	0.2
Entercocci	Omura, 1989	1.52	-	-	Rose, 2004 Ottoson, 2006 Lucena, 2004	2.2	2.2	0.7
C. perfringens	-	-	-	-	Rose, 2004 Ottoson, 2006 Lucena, 2004	1.7	1.7	0.6
Giardia	Rose, 1996, Robertson, 2000 Casson, 1990 Chauret, 1995 Neto, 2006 Caccio, 2003 Reinoso, 2008	0.87	1.05	0.06	Rose, 2001 Rose, 2004 Chauret, 1999 Mayer, 1996 Ottoson, 2006	2	2	0.8
Cryptosporidium	Rose, 1996 Robertson, 2000, Chauret, 1995 Neto, 2006 Reinoso, 2008	0.73	1.59	0.14	Rose, 2001 Rose, 2004 Chauret, 1999 Mayer, 1996 Ottoson, 2006 Montemayor,2005	1.7	1.5	0.6

2.3. Pathogen and indicator reduction mechanisms

This section describes the findings of studies that have investigated the underlying mechanisms for pathogen and indicator reduction within activated sludge processes.

2.3.1. Overview

Pathogen and indicator reduction through activated sludge processes may occur through three potential mechanisms, although the significance of each mechanism would be expected to alter for different organisms and with changes in plant operation:

- physical removal during the clarification stage either by direct settling as discrete particles or by adsorption to suspended solids that are then settled and removed;
- pathogen inactivation due to the adverse biophysical environment within the activated sludge process. The importance of this mechanism could vary between plants that are fully aerobic, through to plants that have alternating aerobic / anoxic, or aerobic/anaerobic/anoxic zones for nutrient removal; and
- predation by other organisms such as protozoa.

This Section describes some of the removal processes and the influencing factors for bacteria, protozoa and viruses.

2.3.2. Bacterial removal mechanism

A two-stage reduction of *E. coli* was observed by Van der Drift et al. (1977) in mixed liquor *E. coli* spiked experiments. After an initial rapid sorption of *E. coli* to the sludge flocs within the first hour, a slow rate of removal through the action of ciliated protozoa was observed. There was approximately 1 log_{10} reduction in the free *E. coli* in the mixed liquor in the first two hours, followed by another 1 log_{10} reduction in the following 6 hours.

Van der Drift et al. (1977) found that the second phase of removal can be impacted by conditions that result in reduced ciliated protozoan levels such as sludge bulking or prolonged anaerobic conditions (> 5 hours). The anaerobic conditions (caused through experimental aeration with N_2 gas) also had an apparent impact on *E. coli* adsorption, with some increases in free *E. coli* numbers post N_2 gas aeration.

2.3.3. Removal of protozoa

In comparison to the significant investigation of virus removal in activated sludge processes described in Section 2.3.4, the direct information on parasite removal mechanisms is more limited. However, there is some information that provides an understanding of the important mechanisms involved in parasite removal.

Sedimentation rates have been reported as 0.35 µm/s (0.126 cm/h) for freely suspended *Cryptosporidium* oocysts and 1.4 µm/sec (0.504 cm/h) for freely suspended Giardia cysts (Medema et al., 1998) and 2.2 to 2.8 cm/h for *Cryptosporidium* oocysts (Robertson et al., 2000). The increased rate of sedimentation is most likely due to particle association. The diameter of *Cryptosporidium* oocysts is 4-7 µm while *Giardia* cysts are generally 8x12 µm oval which may impact this removal mechanism.

Solids association occur with parasites, with Medema et al. (1998) reporting that 30% of cysts and oocysts adsorbed to solids within 1 hour of mixing with clarified effluent from an activated sludge plant (ASP). Within 24 hours, 75% of the cysts and oocysts were adsorbed. The sedimentation rate of the parasites then reflected the size of the particles onto which the particles were absorbed. These adsorption rates are expected to underestimate adsorption during the activated sludge treatment, due to the low suspended solids levels in treated effluent.

In jar tests using mixed liquor from an activated sludge plant (ASP) and seeded oocysts, the number of oocysts in the final supernatant reduced with increased duration of aeration. There was an initial rapid decrease in oocyst numbers of 1-2 $\log_{10}$ after one hour of aeration, followed by a relatively slow decrease with an additional 1 $\log_{10}$ reduction up to 8 hours. Post 8 hours, the numbers were relatively stable (Suwa and Suzuki, 2001). The authors also reported that increased MLSS concentrations resulted in higher removal rates.

Although the statistical analysis was not described, visual analysis of the information presented in the paper indicated that the higher MLSS concentration removals were 0.5 $\log_{10}$ higher. An oocyst removal equation was derived from this study as Eq. 1 (see below):

$$R = 1039\ C^{0.90}, \qquad \text{(Eq. 1)}$$

where *R = number of oocysts removed by 1 g of activated sludge (oocysts/g MLSS),*
and *C = oocyst concentration in supernatant (oocysts/L).*

Suwa and Suzuki (2001) suggested that in a municipal wastewater treatment plant that a large number of oocysts may enter the plant continuously during an outbreak, therefore, the removal capacity of activated sludge may be decreased because the returned activated sludge may no longer have a removal capacity, i.e. adsorption sites are saturated. This is however, yet to be demonstrated.

In contrast to the above findings of adsorption with secondary effluent and by MLSS, Dai and Boll (2003) found limited binding of cysts and oocysts with soil particles (undefined). The oocysts were found to be negatively charged and electrostatic forces governed their interactions with soil particles. The difference in binding between soils and secondary effluent was attributed to the organic component of the effluent.

As noted earlier, the above studies suggest that removal through adsorption to solids is a key mechanism for parasite reductions in activated sludge. The settling in clarifiers is believed to be an important component of this removal (Stadterman et al., 1995). In comparison to the cyst and oocyst sedimentation rates reported above, Department of Environment (1985) reported sedimentation rates of 100 cm/h for *Taenia saginata* ova. This would suggest that helminth ova such as *T. saginata* should be removed more efficiently than cysts and oocysts in clarification processes, most likely due to the larger size (30-40 µm) and could begin to undergo discrete particle settling in the clarification process.

There appears to be an effect of activated sludge treatment on parasite viability, however, the literature is not consistent and this is most likely due to the method used to assess viability or infectivity of the oocysts. The "Gold Standard" for determining the infectivity of *Cryptosporidium* utilizes the neonatal mouse infectivity assay. As an alternative, vital dye staining was developed for the assessment of the internal contents of the oocyst. Robertson et al. (2000) found no effect on *Cryptosporidium* viability (based on vital dye staining which is now known to overestimate infectivity) in laboratory scale studies, while Martinez et al. (1992) found some effects in a laboratory scale investigation utilising mouse infectivity assays. In the latter study, simulated activated sludge removed 80 to 84% of oocysts (0.7-0.8 $\log_{10}$). The total reduction in infection intensity (in exposed mice) was reduced by 82% to 99% (0.8-2.0 $\log_{10}$ inactivation) compared with the controls (Martinez et al., 1992). In drinking water, field studies have shown that disappearance rates of oocysts from natural waters are faster than predicted by settling theory alone, implying some additional predation effects (Hawkins et al., 2000).

The above information indicates that any changes in treatment plant performance that impacts on the efficiency of suspended solids removal or adsorption of parasites to solids will impact on parasite removal performance and potential loss of infectivity. This conclusion is partially supported by Robertson et al. (2000) who examined surrogates such as BOD and SS and concluded that while the factors that influenced BOD and SS removal were important for pathogen reduction; other factors were potentially of significance.

However, they referenced a study by Hirata and Hashimoto (1997) that examined removals and typically found poor correlations between various parameters and *Giardia* removal except for a correlation between turbidity reduction and removal of *Giardia* cysts. Stadterman et al. (1995) found that although parasite removals through the primary process did not correlate with turbidity removal, removal was strongly correlated with turbidity reduction in the activated sludge treatment.

While a strong relationship between suspended solids removal rates and parasite removal rates would be expected based on a significant pathogen reduction mechanism, the absence of a relationship in the literature is not surprising. Activated sludge plants (ASPs) typically operate within a relatively narrow performance band and the more extreme operating conditions necessary to clearly demonstrate this effect would not have been trialled. Therefore, it would be difficult to establish a relationship considering the variability expected for analytical data. Further, the amount of suspended solids leaving the activated sludge process in the effluent is only a small percentage of the mass of suspended solids removed by the waste activated sludge. There is also uncertainty from the recycling of activated sludge (and potential pathogens) into the ASP.

2.3.4. Virus removal mechanisms

For viruses, the key removal mechanisms are through adsorption to suspended solids (and removal during clarification) and predation by protozoa or metazoa.

Kim and Unno (1996) investigated the mechanisms of virus removal in activated sludge systems, using poliovirus type 1 as a model virus. When the virus was added to bacterial cultures isolated from activated sludge, a rapid adsorption to the bacterial surface occurred. Within an hour approximately 30 to 40% (0.15-0.22 $\log_{10}$) of viruses were removed from the liquid phase, with floc forming bacteria causing higher removals than non-floc forming bacteria. Removals after this initial reduction in the liquid phases were relatively limited. In contrast to the effects found with bacterial cultures alone, addition to a culture of a detritus feeding microbe, a filter feeding protozoan or metazoan, resulted in ongoing reductions after the initial rapid loss from the liquid phase. The reductions were greatest for the filter feeding protozoan and metazoan, compared to the detritus feeder (50-80% or 0.3-0.69 $\log_{10}$ reduction over 7 hours).

Similar findings were also reported by Glass and O'Brien (1980), who examined the inactivation of enteric viruses (poliovirus 1, coxsackievirus B-1) and coliphage (assayed using *E. coli* A-19) in 700 L reactors to replicate an activated sludge process. They found rapid adsorption of the viruses to suspended solids, followed by a slower rate of inactivation while attached to the suspended solids. They integrated their data along with earlier published studies to develop an activated sludge removal curve. The curves indicated rapid adsorption of the viruses to the suspended solids resulting in approximately 75% (0.60 $\log_{10}$) of the viruses adsorbed to settleable solids and removed from the liquid phase. As aeration time was increased, the per cent of viruses removed was observed to increase. After 10 hours, virus removal reached 99% (2 $\log_{10}$); however, 25% (0.12 $\log_{10}$) of the original infective viruses remained infective and associated with the settleable solids. After 20 hours, the percent infectious virus adsorbed to the solids had further decreased to 10% and inactivation was 90%, leaving 10% infective virus within the settleable solids. An important observation was that until inactivation occurred, the solids adsorbed viruses remained infective, although removal was high.

The association between solids in secondary treated sewage effluent with bacteriophage (measured with *E. coli B* as the host bacterium) and animal viruses (analysed using BGM cell line) were also studied by Gerba et al. (1978). Two effluents from activated sludge plants (ASPs) were examined and it was found that the percentage of coliphages associated with solids varied between < 1 and 24%. However, the percentage of animal viruses associated with solids was 49-100%. The binding to solids was found to be reversible, as elution with pH 10 Glycine buffer resulted in the release of 67-70% of the coliphages associated with suspended solids in trickling filter plant effluent. The recovery was relatively limited for elution with pH 7 Glycine buffer (less than 25%). ASPs generally operate in the range of 6.5 to 7.5 units, consistent with this experiment.

The size distribution of the solids associated with the viruses was examined and it was concluded that the highest concentrations were found with solids of size 8 µm and larger and 0.45- 0.65 µm diameter range (Gerba et al., 1978). In considering implications for the removal of pathogens and indicators in activated sludge plants (ASPs), it should be noted that the investigation was on adsorption of microbes to particles in secondary treated effluent.

The key issue for this report is the adsorption during secondary treatment, since this is most relevant to the removals during secondary clarification. The percentages of viruses adsorbed to solids during activated sludge treatment would be expected to be higher than indicated in the Gerba et al. (1978) study due to the removal of the majority of suspended solids together with adsorbed viruses in the clarification step. Further, the high levels of suspended solids in the activated sludge process (1,000 to 4,000 mg/L) would encourage higher adsorption rates.

Consistent with this expectation, high rates of coliphages associated with solids in activated sludge flocs were found by Ketratanakul and Ohgaki (1988), with 78 to 93% (0.65-1.15 $\log_{10}$) of total coliphages adsorbed to the solids. The majority of the adsorbed phages were F-RNA specific phages. In contrast to the adsorption to solids in the activated sludge process, 12 – 30% of the phages in raw sewage were associated with solids. The suspended solids concentrations in the aeration tank ranged from 830 to 1,510 mg/L.

Moore et al. (1975) also examined the association of viruses with suspended solids in raw sewage and in secondary treated effluent from an activated sludge process. They noted previous work completed by the laboratory that showed optimal conditions for viral adsorption to organic and inorganic solids occurred in the presence of a divalent cation (e.g. Ca^{2+}) at pH 5.5-6.5. In the experiments, they seeded poliovirus and coliphages T2, T7 and f2 into the raw and treated sewage and then measured solids adsorption.

In the pH range of 6-8, the majority of the phage and poliovirus remained in solution, with 1-17% of poliovirus reported to be adsorbed to solids in secondary effluent (similar to that observed in Gerba et al., 1978) and 1-9% was adsorbed to solids in the raw sewage. Adsorption of the phages was less than 25% in raw and secondary treated effluent and at times no phages were estimated as being adsorbed to solids. The variability in the data and limited data sets make it difficult to compare adsorption between raw sewage and secondary effluent and between viruses and coliphage. Dramatic increases in adsorption were noted at a pH of 10, with greater than 90% adsorption to solids reported for viruses and phage in secondary treated effluent. In raw sewage the effect of alkaline conditions was less significant. Acidic conditions (pH 4) appeared to increase adsorption of the viruses and phage to solids, although data variability resulted in no statistically significant difference. Due to the variability in the data, the authors concluded that no single coliphage system could accurately predict the behaviour of poliovirus under either laboratory or field conditions (Moore et al., 1975). The isoelectric point of viruses is variable and there is no consistency in the isoelectric point for the enteric virus group. As such, a single virus cannot be used to assess viral removal or inactivation through the treatment processes. At a characteristic pH, defined as the isoelectric point, the virus has a zero net charge.

Viruses will be positively charged below their isoelectric point while above that pH; the virus will have a negative charge (Templeton et al., 2008). In general, high pH favours free virus and low pH favours adsorbed virus, although isoelectric points of both the virus and the surface play roles in the interaction (Gerba, 1984). As reported by Glass and O'Brien (1980), an important additional finding was that the solids associated viruses remained infective.

A pilot scale study conducted over 3 days (Arraj et al., 2005) showed that poliovirus and hepatitis A were predominantly found on solid fractions of mixed liquor, however rotavirus was only found in the liquid fraction. This study incorporated the use of ferric chloride.

Shimohara et al. (1985) investigated the removal of poliovirus 1 in batch laboratory scale experiments using activated sludge from a municipal sewage treatment plant. The scale of the experimentation enabled the authors to vary operating conditions and examine the impact on virus removal. They found that the MLSS concentration was proportional to the virus adsorption to solids and inactivation potential. This was considered a better parameter for virus removal than the BOD-SS loading rate which is more commonly used for operational control. A MLSS concentration of greater than 800 mg/L was required for a 2 $\log_{10}$ removal of the poliovirus, while a 3 $\log_{10}$ removal required a MLSS concentration of greater than 1,000 mg/L. The authors noted that these MLSS concentrations are common in conventional activated sludge processes.

Similarly Shimohara et al. (1985) found that anaerobic conditions (resulted in a 0.8 $\log_{10}$ removal) and low temperatures (< 7°C) (a 1.7 $\log_{10}$ reduction) reduced removal and settleability of the sludge flocs. The $\log_{10}$ reductions were not improved by centrifugation, indicating that the reduced efficiency of removal was most likely due to changes in the virus adsorption capacity.

Omura et al. (1989) reported increased levels of some microorganisms (compared to plant influent) in primary sedimentation effluent of an activated sludge plant (ASP). This was attributed to desorption of microorganisms from the excess sludge returned to the primary sedimentation tank from the secondary sedimentation tank, potentially caused by the low dissolved oxygen levels in the primary sedimentation tank. The authors cited Ohgaki et al. (1986) who reported that most of the coliphages associated with particulate matter returned to the liquid phase with decreasing dissolved oxygen concentrations.

Consistent with the above studies, the IAWPRC (1991) concluded that viruses and phage removal processes in activated sludge was primarily through (i) adsorption of coliphages onto the biological floc, leading to sedimentation or (ii) predation by other microbes such as bacteria, protozoa, or metazoa.

2.4. Pathogen reduction quantification

This section describes the findings of empirical studies that quantified pathogen and indicator reduction across full scale activated sludge processes.

2.4.1. Bacterial reduction

In a study based on an operating activated sludge sewage treatment plant (4 aeration-activated sludge basins) in Rome, Aulicino et al. (1996) reported 0 to 63% removal of bacteria (63% total coliforms, 35% faecal coliforms and 8% faecal streptococci) across the primary treatment process and 92-98% (1.09-1.7 $\log_{10}$) removal across secondary treatment. The removal of viruses through primary and secondary treatment was typically 90% (1 $\log_{10}$), although the values ranged from 83% to 99.7% (0.78-2.52 $\log_{10}$).

Removal of COD and total solids were approximately 90% (1 $\log_{10}$) from the raw sewage. The authors concluded that viruses were less easily removed than bacterial indicators and secondary treatment was less efficient at removing reoviruses than enteroviruses.

Lauria-Filgueiras and Hofer (1998) examined the removal of *Campylobacter* isolates in two activated sludge sewage treatment plants in Brazil (a third STP serving the local airport was not included due to the inflow of disinfection chemicals into the sewer). The frequency of *Campylobacter* detections at plant 1 was 22 biotypes detected from 35 samples of raw sewage and 7 detections from 35 samples of secondary treated effluent. At plant 2, detections were 23 biotypes in 35 samples of raw sewage, and 11 detections from 35 samples of secondary treated effluent.

A $\log_{10}$ reduction cannot be applied as the results are presence/absence with selection of a limited number of colonies. *C. jejuni* was the most prevalent species isolated (40.8%). The most common biotypes detected were *C. jejuni* biotype 1 (21.3%), *C. coli* biotype 1 (16%) and *C. jejuni* biotype 2 (14.8%).

Rose et al. (2005) investigated the microbial quality of wastewater at 6 wastewater treatment facilities in the USA. The biological treatment units tested in the project included conventional activated sludge (plants A-D), biological nitrification-denitrification (Facility E) and enhanced biological phosphorus removal (Plant F). Samples were taken from wastewater influent, followed by secondary effluent, filtration and disinfection. From the testing program, it was observed that bacterial removal through the activated sludge plants were 96-99.9% (1.39-3.0 $\log_{10}$) with *Clostridium* removed at 93.3-99.8% (1.17-2.69 $\log_{10}$). The highest removal was observed in plant F while the lowest removals were observed in Facility C followed by A (both conventional treatment plants). It is therefore concluded that a decrease in bacterial removal is proportional to increasing MLSS. The correlation coefficient (r^2) was 0.5 for the enterococci and 0.6 for the faecal coliforms.

A prior study by Rose et al. (1996) examined the removal of bacteria in an activated sludge treatment plant with nitrification in Florida. Monthly samples were taken for one year and analysed for a range of microorganisms at the following sample points; (a) preliminary treated (screening and grit removal), (b) activated sludge treatment with clarifier and with the data provided as arithmetic and geometric means. The average removal through biological treatment with clarification of total coliforms was 98.3% (1.75 $\log_{10}$), faecal coliforms was 99.1% (2.06 $\log_{10}$) and heterotrophic plate counts was 99.1% (2.06 $\log_{10}$). There was no correlation between bacterial removal across the activated sludge process and any of the physical/chemical parameters (including $CBOD_5$, organic nitrogen, total nitrogen, suspended solids or turbidity).

2.4.2. Protozoan reduction

Caccio et al. (2003) conducted an investigation in four wastewater treatment plants in Italy by sampling wastewater at each stage of the treatment process over the course of one year. The presence of parasites was assessed by immunofluorescence with a monoclonal antibody for *Giardia* and by microscopy for size, shape and the presence of a suture on the oocyst wall at 1000x magnification for *Cryptosporidium*. While *Cryptosporidium* oocysts were rarely observed, *Giardia* cysts were detected in all samples throughout the year, with peaks observed in autumn and winter. The overall removal efficiency of cysts in the treatment plants ranged from 87 to 98.4% (0.89-1.8 $\log_{10}$ removal). The removal efficiency of cysts was significantly higher when the secondary treatment consisted of active oxidation with O_2 and sedimentation instead of activated sludge and sedimentation 94.5% (1.26 $\log_{10}$) versus 72.1 to 88.0% (0.55-0.92 $\log_{10}$); $P<0.05$, analysis of variance. To characterize the cysts at the molecular level, the giardin gene was amplified by PCR, and the products were analysed by DNA sequencing or restriction fragment analysis. Cysts were typed as assemblage A or B, both of which are human pathogens, stressing the potential risk associated with the reuse of wastewater.

Bonadonna et al. (2002) investigated the occurrence of *Cryptosporidium* in secondary treated effluent samples from a municipal wastewater treatment plant, with no log removals determined. The aim was to find a correlation between oocysts, bacterial indicators (Total coliforms, faecal coliforms, *E. coli*, faecal streptococci, *Pseudomonas* sp., *Salmonella* sp. and bacteriophage), and physico-chemical parameters (turbidity, pH, redox potential, temperature, TOC, ammonia, nitrite and nitrate), using a multivariate analysis.

Data collected showed high numbers of oocysts (detected by immunofluorescence microscopy and a specific monoclonal antibody), with a high percentage of positive samples and a significant correlation with pH, redox potential and total organic carbon (t for $2\alpha < 0.05$). No correlation was found between *Cryptosporidium* numbers and that of the other microorganisms. The results showed that none of the selected microorganisms or physico-chemical parameters were a reliable predictor of the presence or removal of the parasite.

Thirteen intensive pig farms and two conventional activated sludge treatment plants for pig slurry in north-western Spain were studied by Reinoso and Becares (2008). The aim was to evaluate the presence of enteric pathogens (*Cryptosporidium*, *Giardia* and helminths) and the efficiency with which they were removed. These parasites were present on 53%, 7% and 38% of the farms studied, respectively, with slurry concentrations of 10^4–10^5 oocysts/L for *Cryptosporidium*, 10^3 cysts/L for *Giardia* and 10^2–10^3 eggs/L for helminths. The overall removal of parasites in the pig slurry treatment plants ranged from 86.7% to over 99.99% (0.88-4 $\log_{10}$ removal) (see Table 2-2). The results revealed a constant reduction at each stage of the treatment system, with the activated sludge process being the most effective treatment in reducing pathogens in pig slurry, being 78–81% (0.65-0.72 $\log_{10}$ removal) for *Cryptosporidium* oocysts and over 99.9% (>3 $\log_{10}$) for helminth eggs (Table 3.1).

Table 2-2. Pathogen occurrence in raw slurry and removal during process units (Reinoso and Becares, 2008)

Pathogen occurrence in raw slurry and removal at each step of the treatment system at two plants (Plant 1 and Plant 2) in north-western Spain

Plant	(Oo)cysts/eggs	Influent (Mean ± SD)[a]	Removal	Treatment				
				Screening	Primary clarifier	Floc/coag	Activated sludge and secondary clarifier	Complete treatment
1 ($n=9$)	*Cryptosporidium*	5.62 ± 0.29	%	8.77	25.92	44.57	78.11	93.57
			$\log_{10}$	0.05	0.15	0.28	0.73	1.24
	Giardia	0.78 ± 1.55	%	>99.99	n.d.	n.d.	n.d.	>99.99
			$\log_{10}$	–	n.d.	n.d.	n.d.	–
	Helminths	1.7 ± 0.21	%	11.11	59.38	40	>99.99	>99.99
			$\log_{10}$	0.21	0.44	0.38	–	–
2 ($n=2$)	*Cryptosporidium*	5.32 ± 0.01	%	10.89	20.97	–	81.22	86.75
			$\log_{10}$	0.05	0.1	–	0.73	0.88
	Giardia	n.d.[b]	%	n.d.	n.d.	–	n.d.	n.d.
			$\log_{10}$	n.d.	n.d.	–	n.d.	n.d.
	Helminths	1.76 ± 0.33	%	16.67	75	–	>99.99	>99.99
			$\log_{10}$	0.09	0.92	–	–	–

[a]Influent is the Mean $\log_{10}$ Influent.

Rose et al. (1996) examined the removal of parasites in an activated sludge treatment plant in Florida. Monthly samples were taken over one year and analysed for a range of microorganisms with the data provided as arithmetic means, geometric means and maxima (see Table 2-3). Arithmetic means were used to evaluate percentage removals. The average removal of *Giardia* over the 12 month sampling period was 1.19 $\log_{10}$ while *Cryptosporidium* was similarly removed at 1.14 $\log_{10}$. Helminth ova were detected in raw sewage at a maximum of 110 ova/L, an arithmetic mean of 16 ova/L and a geometric mean of 4.3 ova/L. However, the ova were not detected post activated sludge suggesting removals of greater than 2 $\log_{10}$.

Table 2-3. Information on parasite concentrations and removals in a Florida activated sludge plant (Rose et al., 1996)

Pathogen (n=12)	Raw sewage	Post activated sludge treatment
Giardia (cysts/100 L)		
Arithmetic mean	$6.9\pm3.7x´10^3$	$4.4\pm4.7x10^2$
Geometric mean	$3.9x10^3$	$8.8x10^1$
Maximum	$1.3x10^4$	$2.3x10^3$
Minimum	$1.0x10^2$	$1.4x10^1$
Log_{10} **reduction**		**1.19**
Cryptosporidium (oocysts/100 L)		
Arithmetic mean	$1.5\pm1.8x10^3$	$1.4\pm1.7x10^2$
Geometric mean	$3.7x10^2$	$3.5x10^1$
Maximum	$1.2x10^4$	$1.1x10^3$
Minimum	$<6.1x10^1$	$2.5x10^1$
Log_{10} **reduction**		**1.14**
Helminths (ova/L)		
Arithmetic mean	$1.6\pm1.7x10^1$	-
Geometric mean	4.3	-
Maximum	$1.1x10^2$	<17
Minimum	<2	-
Log_{10} **reduction**		**>2.0**
Turbidity reductions	(from raw of 126 NTU)	97.5%
Total suspended solids	(from raw of 231 mg/l)	97.8%

A study by Rose et al. (2004), also examined the impact of loading conditions, process design, and operating parameters on the removal of protozoa. The biological treatment units in operation tested in the project included conventional activated sludge (plants A-D), biological nutrient removal (Facility E), and enhanced biological phosphorus removal (Plant F). Samples were wastewater influent followed by secondary effluent. Increased parasite removal was associated with enhanced nitrification, MCRT of 8.7-13.3 days and variations in *Giardia* in the effluent were attributed to differences in each of the six treatment plants. In general, it was found that the removal of *Giardia* cysts ranged from 97.7-99.8% (average 2 log_{10} removal) while *Cryptosporidium* oocyst removal was variable (0-99.4%, average 1.5 log_{10} removal) by the biological treatment process. Cysts and oocysts were removed least effectively by Facilities D and F respectively (conventional and enhanced biological phosphorus removal facilities respectively with MCRTs of 3-5 days and 8-16 days respectively) and most effectively by Facility E (biological nutrient removal facility with MCRT of 8.7-13.3 days). Evaluation of the process variables included MCRT and MLSS and demonstrated no correlation with pathogen and indicator removals.

In a prior study, Rose et al. (2001) examined pathogen reduction at the Upper Occoquan Sewage Authority activated sludge plant (ASP). Raw sewage and activated sludge (secondary treated) effluent was collected monthly for a year. Mean averages of 96.9% (1.51 log_{10}) and >79% (0.68 log_{10}) removals for *Giardia* and *Cryptosporidium* oocysts respectively were achieved. Limited details of the plant characteristics or plant specific removal data were provided.

Gennaccaro et al. (2003) monitored the removal of *Cryptosporidium* in six United States water reclamation facilities. Limited details of the plant characteristics or plant specific removal data were provided. However, secondary treatment was reported to reduce total oocyst numbers from an arithmetic mean of 6,910±7,731/100 L (range <5.6 to 26,300) in influent to 112 (±153) oocysts/100 L (range <12.8 to 345/100 L). The levels of infectious oocysts were investigated using the focus infectivity assay and were reduced by secondary treatment from an arithmetic mean of 993(±1,277)/100 L in influent to 37(±28) oocysts /100 L (1.43 log_{10}, or 96% inactivation based on the arithmetic means).

Robertson et al. (2000) investigated parasite analytical method recoveries during a three-year study of the removal of *Cryptosporidium* and *Giardia* in UK (Scotland) sewage treatment plants (STP). The methods trialled were typically found to achieve 30-50% recovery of *Giardia* cysts and 30-36% recovery of *Cryptosporidium* when seeding into sewage influent samples with low (120 oocysts/L) and high seeding rates (5,000 oocysts/L), although a method with centrifugation and no purification had recoveries of >80%. Composite samples of influent and effluent taken over 24 hours were used for calculating removals. The majority of removal was through the secondary treatment processes with primary sedimentation having a limited effect. The removal data for the individual components of the three activated sludge plants (ASPs) is provided in Table 2-4. The removals were based on sample sizes of 15 to 44. Plants C and D incorporated primary settlement and activated sludge and Plant E utilized no primary treatment with activated sludge. The range for removals at the majority of the STPs was reported as 0 to 100% and on some occasions more parasites were detected in the effluent than the influent impacted by the limits of detection and analytical capability. Key findings of Robertson et al. (2000) were that the removal of *Giardia* was significantly higher ($P<0.05$) than for *Cryptosporidium*, removal was highly variable between treatment plants and *Cryptosporidium* removal was more variable than *Giardia.*

Table 2-4. Removal of *Cryptosporidium* and *Giardia* in three UK (Scotland) activated sludge sewage treatment plants (Robertson et al., 2000)

STP	*Giardia* cyst removal through primary treatment (Mean ± SD)	*Giardia* cyst removal through secondary treatment (Mean ± SD)	*Cryptosporidium* oocyst removal through primary treatment (Mean ± SD)	*Cryptosporidium* oocyst removal through secondary treatment (Mean ± SD)
C	42 ± 33%	94 ± 11%	22 ± 38 %	98 ± 35%
D	24 ± 32%	66 ± 28%	4 ± 22 %	53 ± 47%
E	No primary treatment	66 ± 35%	No primary treatment	28 ± 34%

Robertson et al. (2000) also reviewed the literature on parasite removal by primary and secondary treatment noting potential issues with analytical methodologies and some authors assuming high removals due to low number of parasites being detected in the effluent. Low numbers may not correspond to high removal if insufficient numbers were present in the influent. Robertson et al. (2000) referenced Parker et al. (1993) as reporting 80-98% (0.7-1.7 $\log_{10}$) removal for *Cryptosporidium* and 98-99% (1.7-2.0 $\log_{10}$) removal for *Giardia* by an activated sludge process. Recovery of oocysts from wastewater samples can be highly variable.

Average removals of 2.96 $\log_{10}$ and 1.40 $\log_{10}$ for *Cryptosporidium* and *Giardia*, respectively, were reported in an Ottawa treatment facility treating approximately 500 ML/d of sewage through an activated sludge plant (ASP) with primary treatment (Chauret et al., 1999). This high rate of removal for *Cryptosporidium* is relatively unusual, however, only a small sample number was used ($n = 4$). Primary treatment was shown to have a limited effect on protozoan removals being 0.1 $\log_{10}$. Less overall reduction was observed for both *C. perfringens* spores and vegetative cells (0.89 $\log_{10}$ and 0.96 $\log_{10}$ respectively). The other bacteria tested (including total coliforms, faecal coliforms, *Enterococcus sp.* and *Aeromonas sp.*) were reduced by $\geq$ 3.50 $\log_{10}$.

High removals were reported by Mayer and Palmer (1996) at a US treatment facility. The activated sludge process removed 2.3 $\log_{10}$ and 0.81 $\log_{10}$ of *Giardia* and *Cryptosporidium* respectively. However, variability was reported as being high. Primary treatment made a further contribution to removal, with 0.69 $\log_{10}$ and 1.13 $\log_{10}$ reductions for cysts and oocysts respectively. However, it should be noted that the primary treatment included chemical dosing which would result in higher removals than traditional primary processes.

The removal of *Giardia* at a Californian activated sludge plant through primary treatment ranged from 98% to greater than 99% (1.7 - >2.0 $\log_{10}$) (Casson et al., 1990).

Chauret et al. (1995) examined parasite removal across the 430 ML/d Pickard activated sludge plant with *Cryptosporidium* oocyst and *Giardia lamblia* removals reported as 96.8% (1.49 $\log_{10}$) and 99.3% (2.15 $\log_{10}$), respectively. However, sample numbers were low with only 2-3 samples taken from the raw sewage and treated effluent for analysis.

Martinez et al. (1992) undertook a laboratory scale investigation of *Cryptosporidium parvum* oocyst removal by activated sludge. Primary clarifier effluent was seeded with oocysts and subjected to simulated activated sludge treatment involving 3 hours of aeration followed by 35 minutes of settling. The activated sludge simulation was found to remove 80 to 84% (0.7-0.8 $\log_{10}$) of the oocysts.

In another laboratory scale activated sludge process (with a design flow of 17 mL/min and detention time of 6 hours), Stadterman et al. (1995) estimated 83% (0.76 $\log_{10}$) and 91% (1.05 $\log_{10}$) removal of seeded *C. parvum* oocysts (2,000 oocysts/mL) by primary and secondary treatment respectively. The overall removal was 98% (1.69 $\log_{10}$). From the five sampling events, the removals through secondary treatment ranged from 79 to 98% (0.68-1.69 $\log_{10}$).

Madore et al. (1987) examined the levels of *Cryptosporidium* in the effluents from 9 activated sludge plants in Arizona. The average number of oocysts found in raw and treated sewage were 5.18×10^3 (range 890 to 13,700/L) and 1.30×10^3/L (range 4 to 3,960/L), respectively. While removal calculations were not reported, the secondary effluent levels are relatively high suggesting limited removal. Details of plant operating conditions were not reported, limiting the usefulness of this data.

The $\log_{10}$ reduction of *Cryptosporidium* and *Giardia* were studied in four full scale Swedish activated sludge plants (ASPs), all consisting of chemical precipitation as primary treatment, followed by a conventional activated sludge process. At Klangshamn, the effluent passed through a rapid sand filter and in Ryaverket, additional nitrogen removal was included (Ottoson, 2006). Paired samples were taken at the inlet and outlet of each treatment plant. These studies showed an average reduction at all four full-scale plants for *Cryptosporidium* and *Giardia* of 1.30 ± 0.46 $\log_{10}$ and 3.32 ± 0.46 $\log_{10}$ and very similar log removals in the pilot plant of 1.58 ± 1.30 $\log_{10}$ and 3.52 ± 0.87 $\log_{10}$ respectively. Log_{10} reductions were only measured from influent to effluent, thus, the effectiveness of the activated sludge process cannot be measured directly and independently.

Sewage influent from 40 Norwegian wastewater treatment plants, of which 15 incorporated activated sludge biological treatment, was monitored for *Cryptosporidium* and *Giardia* oocysts between March and July 2004. The analyses showed 80 and 93% of the influents were positive for *Cryptosporidium* and *Giardia*, respectively (Robertson et al., 2006). Of these 40 plants, effluent samples were taken every two weeks from two of the plants over18 months and at one plant over 8 months. However, no specific process details were provided. Of these three plants, reductions of 50% (0.3 $\log_{10}$) and 85-95% (0.82-1.3 $\log_{10}$) in *Cryptosporidium* and *Giardia*, respectively, were observed for two of the plants while a 5% increase in *Cryptosporidium* and *Giardia* levels was detected following treatment in the third plant (Robertson et al., 2006). Due to a lack of process information, it is difficult to link protozoa removal data to the activated sludge process.

Five sewage treatment plants studied in north-east Spain showed higher levels of *Cryptosporidium* oocysts in raw sewage during the spring months, with lowest levels during summer, an observation that was considered to be due to holiday load times (Montemayor, 2005). Three of the activated sludge plants (C, D and E) were studied for *Cryptosporidium* removal with LRV geometric means from raw sewage to secondary effluent of 1.71, 1.89 and 1.37 (mean 1.66±0.27) respectively.

The ability of primary treatment and the activated sludge process to remove *Cryptosporidium* and *Giardia* oocysts from a wastewater treatment plant in Campinas, Brazil was investigated over two years using 53 samples (Neto, 2006). Removals of 99.7% (2.52 $\log_{10}$) and 98.8% (1.92 $\log_{10}$) for *Cryptosporidium* and *Giardia*, respectively, were achieved. In contrast to many other studies, removal of *Cryptosporidium* was higher than *Giardia* with removal of the former being comparatively high.

In pilot plant studies receiving municipal wastewater (Suwa and Suzuki, 2003), a 2 $\log_{10}$ removal of *Cryptosporidium* through the activated sludge process with a HRT of 8 hours and MLSS concentration of 2,000 mg/L was achieved. Further treatment by coagulation with continuous dosing of polyaluminium chloride (PAC) increased *Cryptosporidium* removal by 1 $\log_{10}$.

A review of the literature by Parkinson and Roddick (2004) summarised the LRVs after each step in the wastewater treatment process (see Table 2-5), however, minimal detail on plant operating conditions, analysis methods or details of organisms analysed were provided.

Table 2-5. Summary of LRV for protozoa (Parkinson and Roddick, 2004)

Treatment Process	***Giardia***	***Cryptosporidium***	**Helminths (*Ascaris* spp., *Taenia* spp.)**
Primary sedimentation	0-1	0-1	0-1
Trickling filter	0-1	0-1	0-1
Activated sludge (primary, secondary sedimentation)	1-2	1-2	0-1

A critical review of the literature (Crockett, 2007) summarising *Cryptosporidium* removal by conventional activated sludge based secondary wastewater treatment indicated an average of 1.4 $\log_{10}$ removal can be achieved (ranging from 0.7 to 2.0 $\log_{10}$). The average level of *Cryptosporidium* oocysts in secondary wastewater effluents, based on studies from 1987 to 2005, was 389.3 oocysts/L (minimum = 0.031 oocysts/L, maximum = 3,963 oocysts/L).

A significant concern with many of the studies reviewed above is the wide range reported for *Cryptosporidium* removals at individual treatment plants (e.g. 0-100% in extreme cases). The likely explanation for this apparent high variability lies in the oocyst analysis. The methods used to detect *Cryptosporidium* have never been consistent or reproducible and most are developed for drinking water purposes rather than wastewater applications. The standard method in use in Australia is based on United States Environmental Protection Agency Method 1623 (U.S. EPA 1999). In recent times, the development and incorporation of Colorseed™ containing 100 inactivated *Cryptosporidium* and *Giardia* oocysts, all permanently labelled with red fluorescent dye (BTF, Australia), has allowed enumeration of the internal control standard being incorporated into every sample being analysed for protozoa. The red dye allows differentiation between Colorseed oocysts and naturally occurring oocysts which are not red. Prior to the development of Colorseed™, quality control for sample recovery was established in different water types and results adjusted according to the recovery. The variability of oocyst recovery within samples was high leading to a variation in the results. A second grab sample was spiked with a known number of live oocysts to determine a % recovery for each sample.

By incorporating Colorseed^{TM} internal control, greater accuracy in the LRV's should now be achievable. As noted by Clancy et al. (2004), the recovery of oocysts tends to improve with higher levels of treatment leading to a situation where treated effluent can have higher rates of positive samples for *Cryptosporidium* oocysts than raw sewage. This is due to improved recoveries in cleaner, treated waters and poorer recoveries observed in raw, primary and secondary treated wastewaters. The low levels of *Cryptosporidium* oocysts often reported in raw sewage also increase the variability observed in the literature as it is understood that low influent numbers can offer concentrations below statistically significant detection in the effluent.

2.4.3. Virus reduction

Payment et al. (1986) undertook a study to determine if viruses were selectively eliminated during wastewater treatment. Human enteric viruses were detected at all steps of treatment in a conventional activated sludge wastewater treatment plant. Liquid overlays and large volume sampling with multiple passages on BGM cells permitted the detection of poliovirus (serotypes 1, 2 and 3), coxsackievirus B (serotypes 1,2,3,4 and 5) and echovirus (serotypes 3, 14 and 22) as well as reoviruses. The mean virus concentration was 95.1 most probable number of infectious units per litre (MPN IU/L) in raw sewage, 23.3 in settled sewage, 1.4 in effluent after activated sludge treatment and 40.3 MPN IU/L in sludge samples. All samples of raw sewage and settled water, 79% of secondary treated effluent, and 94% of sludge samples contained viruses. The mean reduction was 75% (0.6 $\log_{10}$) after primary settling and 98% (1.7 $\log_{10}$) after combined primary settling and activated sludge treatment.

Activated sludge treatment alone resulted in removal of 94% (1.22 $\log_{10}$) of the viral content of the water. Poliovirus type 3 was rarely isolated after the activated sludge treatment but was still detected in about one-third of the sludge samples. Reoviruses and coxsackieviruses were detected at similar rates from all samples and appear to be more resistant to the activated sludge treatment than poliovirus type 3, whether this is due to lack of adsorption to particulates, predation or inactivation is not clear. Poliovirus types 1 and 2 were present in almost every sample of raw sewage and settled water and still found in about half of the effluent and sludge samples indicating a level of persistence similar to reoviruses and coxsackieviruses.

The fate of coliphages in a wastewater treatment process in Japan was reviewed by Tanji et al. (2002). The abundance of coliphages was monitored at 7 sampling locations from influent through primary settling, secondary treatment and tertiary disinfection using chlorine. The secondary treatment plant included anaerobic and aerobic zones. Influent numbers across 3 host strains of *E. coli* were found to be 10^3 to 10^4 pfu/mL and did not vary seasonally. Approximately 0.5 to 1 $\log_{10}$ removal was achieved across primary treatment, with a further 1 to 1.5 $\log_{10}$ removed during secondary treatment. Coliphages in influent and primary settled sewage samples were detected as suspended forms rather than settled particles. Anaerobic-aerobic treatment enhanced adsorption of the phage to the solid particles with almost no phage detected in the effluent. Few coliphages were found in the final effluent samples which had been chlorinated. The RNA phage was more stable than the DNA phage against aerobic treatment using activated sludge.

In a study examining the performance of reclamation plants under the Californian guidelines, Yanko (1993) found relatively high removals of enteric virus in three activated sludge treatment plants with an average of 99.8% (2.7 $\log_{10}$) reduction in enteric virus concentrations. Data was presented as the median virus concentrations for primary effluent and secondary effluent for three activated sludge treatment plants (see

Table 2-6). Note that all activated sludge aeration systems were changed from coarse to fine bubble diffusers for the trials.

Table 2-6. Removal of enteric viruses in three Californian activated sludge treatment plants (modified from Yanko, 1993)

Treatment plant	Median virus concentrations in primary effluent (PFU/378.5 L)	Median virus concentrations in secondary effluent (PFU/378.5 L)	LRV across ASP process
Pomona	$2.3x10^4$ (n=13)	$6.5x10^2$ (n=12)	1.55
San Jose Creek	$1.6x10^5$ (n=8)	$5.5x10^1$ (n=14)	3.46
Whittier Narrows	$7.6x10^4$ (n=8)	$5.6x10^1$ (n=9)	3.13

In an investigation of an activated sludge plant in France (Rolland et al., 1983), influent and secondary effluent samples were taken over 24, 48 and 72 hour intervals using 24 hour composite samples and 2-3 hour grab sampling. They found that based on grab samples, enteric virus removals on the four sampling days were 83% (0.77 $\log_{10}$), 98% (1.7 $\log_{10}$), 83% (0.77 $\log_{10}$) and 87% (0.89 $\log_{10}$) respectively, average removal reported was 84% (0.8 $\log_{10}$), while faecal coliform removals were 92% (1.09 $\log_{10}$), 99% (2.0 $\log_{10}$), 96% (1.4 $\log_{10}$) and 99% (2.0 $\log_{10}$). The plant involved grit removal, primary settling, aeration and secondary settling with a theoretical hydraulic detention time of approximately 6 hours. The authors also reported that there were limited correlations between virus levels in final effluent and other parameters such as COD, SS or faecal coliforms (calculated using the linear regression model for all flux parameters, $0.05<P<0.10$). Virus removals exceeded the SS, COD and turbidity removals. The virus removals estimated from grab samples closely aligned with the removals from automatic composite samples.

The fate of coliphage in a Japanese activated sludge treatment plant treating 28 ML/d was investigated over 10 months incorporating primary settling and activated sludge treatment (anaerobic tank, aerobic tank and secondary clarifier) (Tanji, 2002). The assay utilised 3 types of *E. coli* host cells to determine the number of somatic bacteriophage and F-specific bacteriophage while incorporating a ribonuclease (RNase) treatment to determine the numbers of DNA phage and RNA phage using the *E. coli* K12 HfrH F^+ strain. Removal of the combined phage across the activated sludge process was estimated at 3.26 ± 0.62 $\log_{10}$. General trends in the fate of phages were almost identical. Numbers remained almost constant in the influent and primary settling tank. A slight decrease was observed in the anaerobic tank and a significant decrease was observed in the aerobic tank and settling tank with only a few phages detected in the supernatant of the secondary settling tank. The majority of the phage present in the aerobic tank was RNA phages demonstrating the RNA phage was more stable than the DNA phage during aerobic treatment in the activated sludge process. Primary treatment produced an additional 0.08 ± 0.09 $\log_{10}$ removal.

Havelaar et al. (1993) assessed the relationship between F-specific RNA bacteriophages and enteric viruses in a range of water sources including raw and treated sewage. The study reported that the median levels of enteroviruses in raw sewage were 200 PFU/L (range <0.1 to 570) and enteric viruses (combined enterovirus and reoviruses detected) 1,100 PFU/L (range 400 to 8,700). With secondary treatment (not detailed) median numbers of enteroviruses were reduced to 1.3 PFU/L (range <0.1 to 15) and enteric viruses to 18 PFU/L (range <0.1 to 1900). It is unclear whether secondary clarification was included in the treatment process as samples were also taken after coagulant dosing. Data from two plants were combined for the analysis of the secondary treated wastewater.

A total of 11 samples of raw sewage and 29 secondary effluents were analysed, indicating that the samples were not matched pairs. The log reduction of approximately 2$\log_{10}$ for the median levels of enteroviruses and enteric viruses was estimated based on the data provided. Therefore, the log reductions are at best indicative.

The reductions in F-RNA phage were comparable to the virus data (1.66 $\log_{10}$ based on median values) with reductions from 1,800 PFU/mL (range 360 – 3,100) in raw sewage to 39 PFU/mL (range 3.1 – 170). However, there were poor correlations between the actual F-RNA phage levels, enteroviruses levels (r^2=-0.072) and enteric virus levels (r^2=0.504) in the raw sewage and in secondary effluent (r^2=-0.118, r^2=0.250 respectively) while phage were present at 1,000 fold higher numbers than enteric viruses. The lack of a correlation was attributed to virus levels being dependent on a small number of infected people within the population while many more release phage into the sewage.

Metcalf and Eddy (2003) present distribution curves for the removal of MS-2, the F-specific RNA bacteriophage, across a sewage treatment plant. The removal curves showed a consistent 1.83 $\log_{10}$ mean reduction across the primary and secondary treatment process at the Tillman Wastewater Reclamation Plant, Los Angeles.

A nine year study (August 1994 to July 2003) of virus removal from a 380 ML/d wastewater treatment plant consisting of primary, secondary (activated sludge) and tertiary (phosphorus removal, chlorination and dechlorination) at Milwaukee's Jones Island was undertaken by Sedmak et al. (2005). Sampling from the plant consisted of raw sewage (influent) and plant effluent (entire treatment process). The results demonstrated that of the culturable viruses, reovirus dominated the influent followed by enterovirus (28.6%) and adenovirus (3.1%). The titres were generally highest for reovirus ranging from 0 to 12,027 MPN/L. The enterovirus titre detected ranged from 0-3,347 MPN/L. Adenoviruses were detected at lower frequency and were detected in the range of 0-250 MPN/L. The average total viral removals from influent to effluent of the entire process train for each month ranged from 1.165 to 3.188 $\log_{10}$ with a yearly average $\log_{10}$ removal of 2.410. Typically, virus influent levels and removals were lowest during the cooler months.

The removal of bacteriophage (somatic coliphage (ϕX174) and F-RNA phage (MS2)) and enteric virus (poliovirus, rotavirus, Hepatitis A) in a pilot plant simulating a full-scale activated sludge plant (ASP) was measured in both the liquid and solid phases (Arraj, 2005). After 3 days, the estimated mean $\log_{10}$ viral reductions were 9.2 ± 0.4 $\log_{10}$ for rotavirus, 6.6 ± 2.4 $\log_{10}$ for poliovirus and 5.9 ± 3.5 $\log_{10}$ for Hepatitis A. The bacteriophages were less efficiently removed with mean $\log_{10}$ reductions of 3.2 ± 1.2 $\log_{10}$ and 2.3 ± 0.5 $\log_{10}$ for the F-RNA phage and somatic coliphage respectively.

Based on this, it was suggested that somatic coliphage (ϕX174) and F-RNA phage (MS2) would be a suitable indicator of the enteric viruses studied (poliovirus, rotavirus, Hepatitis A). Poliovirus and Hepatitis A were predominantly found on solid fractions of mixed liquor, however, rotavirus was only found in the liquid fraction and the spiked MS-2 bacteriophage was found predominantly in the liquid fraction. Viral numbers on the solid phase remained fairly stable throughout the 3-day sampling period indicating that absorption to the solid phase was not a reversible process.

2.4.4. Combined pathogens

Payment et al. (2001) investigated pathogens and faecal indicator bacteria occurrence and removal for a period of 6 months at the Montreal Urban Community Wastewater Treatment Facility. With a capacity of approximately 7.57 cubic metres per day, it is the largest primary physico-chemical

treatment plant in America. Samples were collected for a period of 6 months and they demonstrated that the plant was not efficient at removing indicator bacteria and the pathogens tested. Faecal coliforms were the most numerous of the indicator bacteria and their removal averaged 24% (0.12 $\log_{10}$). Faecal streptococci removal was 28% (0.14 $\log_{10}$), while *E. coli* removal was 13% (0.06 $\log_{10}$).

In untreated sewage, faecal coliforms, *E. coli*, and human enteric viruses were more numerous in summer and early autumn. Faecal streptococci counts remained relatively similar throughout the period. *C. perfringens* removal averaged 51% (0.31 $\log_{10}$). *Giardia* cyst numbers were not markedly different throughout the study period and 76% (0.62 $\log_{10}$) of the cysts were removed by treatment. *Cryptosporidium* oocyst counts were erratic, possibly due to the method used for oocyst recovery and enumeration and variability in the recovery, however, removal was 28% (0.14 $\log_{10}$). Human enteric viruses were detected in all samples of raw and treated wastewater with no removal observed (0%). Overall, the plant did not perform well for the removal of faecal indicator bacteria, human enteric viruses or parasite cysts. Supplementary treatment and disinfection were recommended to protect public health.

Graczyk et al. (2007) investigated pathogen loads in activated sludge and their corresponding sludge end products. The activated sludge samples originated from urban wastewater treatment plants: Collooney, Strandhill, Grange and Keadue, Ireland. All plants used primary treatment by coarse screening. The Collooney, Strandhill, and Grange plants employed secondary treatment using activated sludge. The Keadue plant uses a rotating filter and settling tank. The sewage effluent was then polished using a reedbed (wetland filtration system) with effluent discharging directly to Lake Meelagh. The activated sludge samples (2 litres) were collected directly from the top wastewater layer during the activation process. *Giardia lamblia* was found at a significantly higher concentration in activated sewage sludge at Collooney, Strandhill, and Grange than *Cryptosporidium* or microsporidia (Table 2-). However, the highest concentrations of *G. lamblia* (1,540 cysts/L) and *Enterocytozoon bieneusi* (371 spores/L) were found in the final effluent from the wetland filtration system at Keadue. Removal efficiency was not estimated as raw waters were not analysed.

Table 2-7. Pathogen concentrations in final effluent from various facilities (Graczyk et al., 2007)

Site	***Cryptosporidium parvum/C. hominis* (oocysts/L)**	***Giardia lamblia* (cysts/L)**	***Enterocytozoon bieneusi* (spores/L)**
Collooney	320	1100	245
Strandhill	43	540	31
Grange	650	890	247
Keadue	0	1540	371

Measurement by culture measures only those that are infective in the culture system provided. The PCR method will detect all of the particular virus whether potentially infective or not (provided the DNA/RNA is intact). Many of the viruses are not culturable in the current systems available so PCR has been utilised to quantify the number of genomes present. The limitation of the method is that both infectious and non-infectious virus particles will be detected, possibly providing an overestimate of the health risk. In contrast, culture based enumeration using cytopathic effect (or plaque formation) can fail to detect a large number of viruses as suitable culture systems do not exist and as a result can potentially underestimate the number of infectious virus particles present (Lee and Jeong, 2004). Gantzer et al. (1998) found no correlation between the presence of infectious enterovirus and enterovirus genomes. Choi and Jiang (2005) found no correlation for the direct detection of adenovirus or enterovirus genomes with infectious virus.

Ozaki et al. (2005) monitored water quality associated with activated sludge and lagoon plants across monsoonal Asia. Table 2- below shows the removal of pathogens across process units. Removal of total coliforms and norovirus G2 was highly variable, ranging from 0-99.6% (0-2.40 $\log_{10}$) and 0-100% (0 – complete log removal), respectively at Khon Khan Plant. Consistent removals of Norovirus G1 and *Giardia* were observed at the plant ranging from 82.4-100% (0.75 $\log_{10}$-complete removal) and 99.3-100% (2.15 $\log_{10}$-complete removal), respectively. Average removal of total coliforms was 83.2% (0.77 $\log_{10}$), 95.2% (1.32 $\log_{10}$) of Norovirus G1, 89% (0.95 $\log_{10}$) Norovirus G2 and 99.9% (3 $\log_{10}$) of *Giardia*. No *Cryptosporidium* was detected in any of the 16 samples collected. The second treatment plant (i.e., AIT Campus WWTP) demonstrated different trends (some higher some better) for different pathogens and their removal when compared to Khon Khan Plant.

Total coliforms were consistently removed through treatment achieving an average of 99.6% (2.40 $\log_{10}$) removal (range 98.1-99.9%). Norovirus G1 removal was highly variable 0-99.9% (0-3 $\log_{10}$ removal) with an average of 74.1% (0.59 $\log_{10}$). Norovirus G2 removal was variable as previously observed with the range of 56.2-100% (0.36 $\log_{10}$ -complete log removal) and an average removal of 92.1% (1.1 $\log_{10}$). *Giardia* cysts were consistently removed 94.6-100% (1.26 $\log_{10}$-complete log removal) with an average removal of 99.4% (2.22 $\log_{10}$). *Cryptosporidium* oocysts were detected in the second plant and were 100% removed.

Table 2-8. Removal of pathogens across two treatment plants (Ozaki et al., 2005)

		Total coliform (cfu or MPN/ml)	Norovirus G1 (copies/L)	Norovirus G2 (copies/L)	Giardia (cysts/L)	Cryptosporidium (oocysts/L)
	Sampling Time	June 2004～Feb, March 2005	June 2004 ～ March 2005		June 2004 ～ March 2005	
Khon Kaen Municipal WWTP	influent	4.4E+2～2.3E+5	8.6E+3～9.7E+5	4.1E+2～1.6E+5	ND～1.5E+2 〔13/16〕	ND 〔0/16〕
	aerated	4.0E+1～3.0E+4	ND～1.7E+4	ND～1.7E+4	ND～2.2E+1 〔4/16〕	ND 〔0/16〕
	effluent	3.8E+1～2.2E+4	ND～6.9E+3	ND～1.0E+4	ND～1.0E+0 〔1/16〕	ND 〔0/16〕
	(range removal %)	0～99.6	82.4～100	0～100	99.3～100	—
	(ave. removal %)	83.2	95.2	89.0	99.9	—
AIT Campus WWTP	influent	2.1E+4～9.0E+6	2.7E+4～1.8E+7	1.1E+4～2.0E+6	2.6E+2～1.8E+4 〔21/21〕	ND～1.6E+1 〔14/21〕
	effluent 1	4.6E+3～1.6E+6	6.2E+4～2.2E+6	1.3E+4～1.3E+6	1.1E+1～4.1E+2 〔13/13〕	ND～2.0E+0 〔2/13〕
	effluent 2	7.0E+1～5.0E+3	3.6E+1～4.2E+5	ND～6.0E+4	ND～5.6E+1 〔20/21〕	ND 〔0/21〕
	(range removal %)	98.1～99.9	0～99.9	56.2～100	94.6～100	100
	(ave. removal %)	99.6	74.1	92.1	99.4	100

The efficacy of a conventional activated sludge wastewater treatment process and the membrane bioreactor (MBR) technology in removing microbial pathogens was investigated by Zhang and Farabaksh (2007). Treatment consisted of screening and grit removal, primary sedimentation, activated sludge (aeration tank), secondary sedimentation, sand filtration and chlorine based disinfection. Total and faecal coliforms and somatic and F-specific coliphages were used as indicators of pathogenic bacteria and viruses. Removals of up to 5.7 $\log_{10}$ of coliforms and 5.5 $\log_{10}$ of somatic coliphages were observed in the conventional activated sludge process with advanced tertiary treatment. This was divided across primary treatment (being 0.33 to 1.4 $\log_{10}$), secondary treatment (1.5 to 2.3 $\log_{10}$) and the remainder achieved with tertiary disinfection. Addition of chemical coagulants improved the efficacy of primary and secondary treatment for pathogen reduction for coliphages but this was not observed for the bacteria. Log removal values for F-specific coliphages were 0.4-1.9 $\log_{10}$ for primary treatment, 0.8-2.2 $\log_{10}$ for secondary treatment and 0.3-2.8 $\log_{10}$ for tertiary treatment. A significant removal of somatic and F-specific coliphages in the secondary treatment was observed with an average LRV of 1.6 and 1.9 $\log_{10}$, respectively. Complete removal of faecal coliforms and up to 5.8 $\log_{10}$ removal of coliphages were observed in the MBR system indicating that the system achieves better pathogen reduction in fewer steps than conventional activated sludge process with advanced tertiary treatment.

Figure 2-1. F-specific coliphages removal across process steps (Zhang and Farabaksh, 2007)
show the $\log_{10}$ removal achieved across process steps for F-specific coliphages and somatic coliphages respectively. It can be seen that F-specific and somatic coliphages behaved similarly across process steps and over the sampling period (Zhang and Farabaksh, 2007).

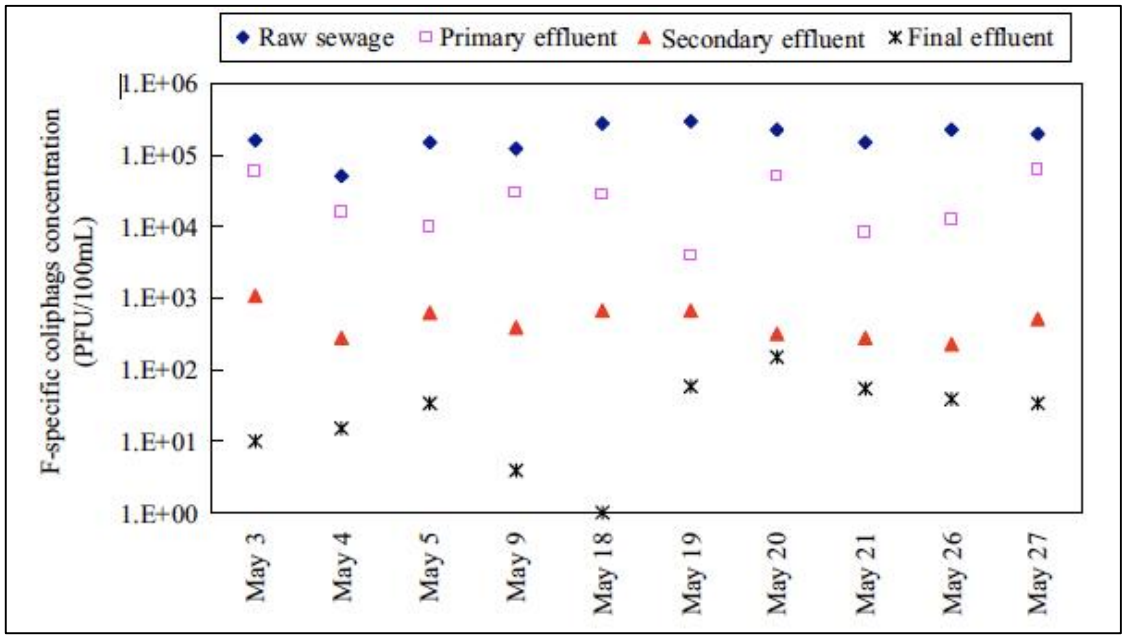


Figure 2-1. F-specific coliphages removal across process steps (Zhang and Farabaksh, 2007)

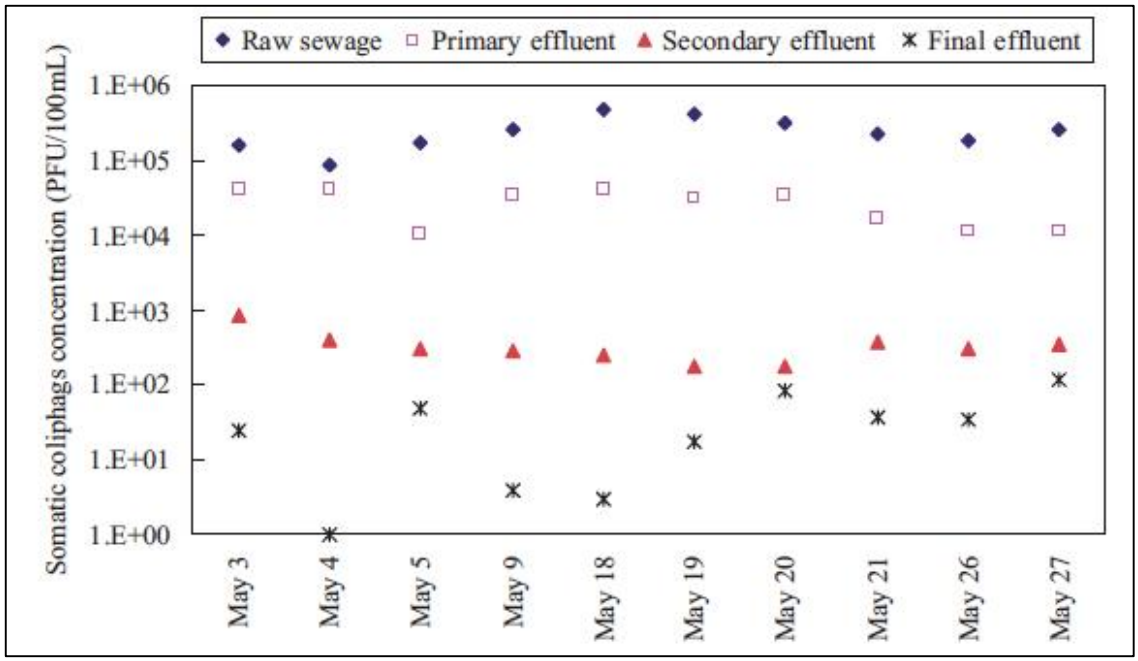


Figure 2-2. Somatic coliphages removal across process steps (Zhang and Farabaksh, 2007)

Pathogen reduction credits were established for an operational activated sludge secondary treatment plant based on eighteen months monitoring of removal efficiency for key pathogenic organisms; *Cryptosporidium* oocysts, *Giardia* cysts, and the enteric viruses: adenoviruses, enteroviruses and reoviruses (H. Reid, pers. comm., 2009). The removals of indicator organisms *E. coli*, somatic coliphages and F-RNA bacteriophages were also monitored. This information was coupled with

operational performance and underpinned by a literature review of pathogen reduction mechanisms in activated sludge. Across the entire treatment plant, the median log_{10} removals were 1.2 log_{10} for *Cryptosporidium* oocysts, 1.8 log_{10} for *Giardia* cysts and greater than 3.8 log_{10} for the enteric viruses while adenovirus were removed at 3.8 log_{10}, enteroviruses were removed at 2.87 log_{10} and reoviruses were removed at 1.68 log_{10} (Table 2-).

Log_{10} removal values for indicator organisms were 3.0 log_{10} for *E. coli*, 2.2 log_{10} for somatic coliphages and 2.3 log_{10} for F-RNA bacteriophages. Median log_{10} removal values across the activated sludge process alone were 1.07 log_{10} for *Cryptosporidium* oocysts, 1.92 log_{10} for *Giardia* cysts 1.13-1.86 log_{10} for all viruses. Log_{10} removal values for indicator organisms across the activated sludge process alone were 1.79 log_{10} for *E. coli*, 1.06 log_{10} for somatic coliphages and 1.89 log_{10} for F-RNA bacteriophages. The activated sludge process was found to provide the majority of the pathogen reductions. The key control points for managing pathogen reduction efficiency were identified as hydraulic flow rate, aeration demand and the turbidity of clarified effluent.

Table 2-9. Pathogen concentrations and log reduction across process steps (Reid et al., in press)

	Raw sewage	Primary treatment	Secondary treatment	Tertiary treatment	Combined log_{10} reduction values (LRV)
E. coli Median ± sdv*** (Orgs/100ml) Median LRV	$9.8\times10^6 \pm 1.7\times10^8$	$7.0\times10^6 \pm 2.2\times10^7$ 0.04±0.42	$2.2\times10^5 \pm 2.7\times10^5$ 1.79±0.71	$1.7\times10^4 \pm 1.1\times10^5$ 1.05±0.93	Median 3.0 5%ile 2.1 Min. 1.8
Enteric viruses Median ± sdv (PFU/L) Median LRV	$7.3\times10^3 \pm 1.6\times10^4$	$8.3\times10^2 \pm 8.3\times10^2$ 0.94±0.24	67 ± 88 1.13±0.36	<1 >1.83±0.37	Median >3.8 5%ile N/A Min. >3.4
Adenoviruses Median LRV	-	0.90±0.21	1.12±0.30	1.73±0.28	3.80±0.32
Enteroviruses Median LRV	-	1.01±0.31	1.22±0.75	NA	2.87±0.42*
Reoviruses Median LRV	-	1.59±0.35	1.86±0.07**	NA	1.68±0.45*
Somatic coliphage Median ± sdv (PFU/100ml) Median LRV	$9.0\times10^5 \pm 1.7\times10^6$	$3.3\times10^5 \pm 8.9\times10^5$ 0.44±0.65	$3.3\times10^4 \pm 4.3\times10^4$ 1.06±0.66	$4.7\times10^3 \pm 7.7\times10^3$ 0.69±0.42	Median 2.2 5%ile 1.3 Min. 0.95
F-RNA phage Median ± sdv (PFU/100ml) Median LRV	$1.2\times10^6 \pm 1.1\times10^7$	$5.2\times10^5 \pm 9.5\times10^5$ 0.40±0.61	$6.8\times10^3 \pm 1.5\times10^4$ 1.89±0.78	$6.6\times10^3 \pm 8.8\times10^3$ 0.18±0.26	Median 2.4 5%ile 1.8 Min. 1.6
***Cryptosporidium* oocyst** Median ± sdv (oocysts/L) Median LRV	250 ± 268	80 ± 450 0.25±0.50	10.4 ± 40.8 1.07±0.50	5.7 ± 76.3 0.14±0.22	Median 1.2 5%ile - Min. 0.5
***Giardia* cysts** Median ± sdv (cysts/L) Median LRV	$2.6\times10^3 \pm 1.6\times10^3$	749 ± 1281 0.24±0.43	16.3 ± 18.6 1.92±0.50	17.4 ± 32.9 -0.22±0.44	Median 1.8 5%ile 1.4 Min. 1.4
Helminth ova Median ± sdv (eggs/L) Unit process LRV	19 ± 8.7	9 ± 5.8 0.35±0.23	<1 (77% of values below detection limit) >0.85±0.28	<1 100% of values below detection limit	Median >1.26

** Value is an underestimate due to levels reduced below detection limits at an intermediate treatment step. Therefore, removals for later treatment process stages are not calculated (NA).*

*** Value is based on only two samples with detectable reoviruses in secondary effluent.*

**** Sdv (standard deviation)*

A comprehensive study of six full scale treatment facilities (comprising four non-nitrifying activated sludge, one nitrification activated sludge and one enhanced biological phosphorus removal) by Rose et al. (2005) examined the impact of loading conditions, process design and operating parameters on the removal of nine microbial species (bacteria, coliphages, enteric viruses and protozoa, see Section 3.3) expanding on traditional indicators. Six samples were taken from each treatment facility over a 12 month period with samples taken across the treatment train for each treatment process. The combined removal efficiencies of primary and secondary treatment were assessed rather than considering them as separate removal processes. The study found that higher levels of MLSS and longer MRCTs in biological treatment resulted in increased removal of microbial indicators and pathogens. Furthermore, increased levels of virus removal were associated with both nitrification and enhanced biological phosphorus removal. Effluent levels of bacteria and coliphage from each of the treatment plants varied (a finding that can only be a result of each plant process) as influent levels of these organisms were not statistically different ($P > 0.05$).

In general, it was found that the biological treatment processes removed 96-99.9% (1.39-3 $\log_{10}$) of bacteria and viruses, 93.3-99.8% (1.17-2.7 $\log_{10}$) of *Clostridium* and 87-99.9% (0.89-3 $\log_{10}$) of somatic coliphages with a mean average $\log_{10}$ reduction value of 2 $\log_{10}$ for enterovirus (Table 2-7). No direct correlations were observed between combination of indicator(s) and pathogen(s) for the data collected throughout the study, however, correlations were observed among the indicators for total coliforms and faecal coliforms (provided both were above the detection limit) throughout each of the treatment steps. When faecal coliforms and enterococci were codetected, it demonstrated a consistent relationship between detected numbers. It was suggested that coliphage may be a useful predictor of the absence of enteric viruses when less than 10 coliphage /100mL were indicative of effluents with no detectable culturable enteric virus.

Table 2-7. Log_{10} reduction values of bacterial and viral indicators from influent to secondary effluent in six activated sludge wastewater treatment plants (Rose et al., 2004)

Indicator organism	**Plant A** (Non-nitrifying, 0.9-2.6 MGD*)	**Plant B** (Non-nitrifying, 13.9-16.2 MGD)	**Plant C** (Non-nitrifying, 9.6-10.3 MGD)	**Plant D** (Non-nitrifying, 11-25 MGD)	**Plant E** (Nitrification, 8.7-13.3 MGD)	**Plant F** (EBPR**, 8-16 MGD)
Faecal coliforms (CFU/100mL) Average LRV Geometric mean LRV	1.77 1.92	1.76 1.74	1.41 1.62	2.31 3.11	2.48 2.33	2.81 2.81
Enterococci (CFU/100mL) Average LRV Geometric mean LRV	2.32 2.32	2.09 1.95	1.48 1.60	2.28 2.84	2.33 2.28	2.91 2.93
C. perfringens (CFU/100mL) Average LRV Geometric mean LRV	1.46 2.09	1.74 1.54	2.00 2.23	1.16 1.50	2.40 1.86	2.79 2.73
Coliphage 15597 host (PFU/100mL) Average LRV Geometric mean LRV	1.85 2.14	2.04 1.98	-0.95 0.69	1.97 2.35	1.88 2.06	3.60 3.60
Coliphage F-amp host (PFU/100mL) Average LRV Geometric mean LRV	2.63 2.56	2.63 2.77	3.41 2.27	1.81 1.88	3.26 2.36	3.64 3.69
Enterovirus (MPN/100L) Average LRV Geometric mean LRV	1.85 2.04	2.54 2.30	2.05 1.85	2.61 2.44	2.04 2.19	1.43 2.21

***** MGD – million gallons per day
** EBPR – enhanced biological phosphorous removal

Rose et al. (2001) also found high removal rates for enteric viruses and coliphages by activated sludge at the Upper Occoquan Sewage Authority Reclamation Plant. Raw sewage and secondary treated effluent samples were collected each month for a year. The numbers and removals of various organisms are described in Table 2-8. Of all the indicators (bacteria and coliphage), *Clostridium* best reflected the removal of enteroviruses for secondary treatment.

Table 2-8. Removal of pathogens through activated sludge treatment at the Upper Occoquan Sewage Authority Reclamation Plant, California. Mean values are given (Rose et al., 2001)

Pathogen	Raw sewage	Secondary treated effluent	Removal	LRV
Faecal coliforms (CFU/100 mL)	9.0×10^5	7.8×10^3	99.1%	2.05
Coliphage (PFU/100 mL)	3.8×10^5	1.8×10^3	99.5%	2.30
Enteroviruses (PFU/100 mL)	1.1×10^3	24	97.8%	1.65
C. perfringens (CFU/100 mL)	3.7×10^4	4.5×10^3	88%	0.92

The removal of coliforms, enterococcus and coliphages was examined over a 12 month period at a Japanese activated sludge plant (Omura et al., 1989). The plant was considered to be well operated as indicated by the mean BOD removal of 92.8% (1.14 $\log_{10}$). Monthly samples were taken of influent and primary and secondary sedimentation tank effluents. Good removals were reported from the raw sewage to the secondary treated effluent with the average removals being 92% (1.10 $\log_{10}$) for coliforms, 97% (1.52 $\log_{10}$) for enterococcus and 97% (1.53 $\log_{10}$) for coliphages. Over the 12 sampling intervals, the minimum coliphage removal was approximately 90% (1 $\log_{10}$). Minimum coliform removal was approximately 75% (0.60 $\log_{10}$) which occurred on 3 of the 12 sampling intervals. Based on the data presented, no relationship between the coliphage and bacterial removal rates was apparent (Omura et al., 1989).

Four full scale treatment plants located in Sweden and operated with chemical precipitation followed by activated sludge treatment (Ottoson, 2006) were studied to investigate plant variations in the removal of enterovirus, norovirus and faecal indicators from influent to effluent (Table 2-12). The combined average $\log_{10}$ removals of enteroviruses and noroviruses from the four plants were 4.44±0.76 and 3.29±0.26, respectively. Unfortunately, this study did not measure removals through each of the treatment processes, thus, it is difficult to determine the effectiveness of the activated sludge process alone.

Statistical analysis of the data (Table 2-12) provided evidence that bacterial indicators are very limited in assessing the log removal and hence risk from viruses. Since viruses persist longer in the environment than many enteric bacteria, bacterial indicators can lead to an underestimation of the risk of viral gastroenteritis. A lack of correlation between virus removal and phage indicators also limits their usefulness; however, total coliphage provides a more conservative indicator than F-specific phages. *Clostridium perfringens* spore removal overestimated the risk of cyst occurrence in activated sludge plant (ASP) wastewater effluents.

An investigation of pathogen reduction from twelve wastewater treatment plants located in Argentina, France and Spain showed no significant difference in removals among the plant treatment processes (including primary sedimentation, flocculation-aided sedimentation with Fe_2Cl_3, activated sludge, activated sludge plus precipitation and upflow anaerobic sludge blanket) or among indicator organisms (P>0.05) (Lucena, 2004). The data given for the five activated sludge plants is based on a minimum of six duplicate samples taken from raw sewage and secondary effluent following activated sludge treatment. Hence, removal during primary treatment was also included.

The average removals and 95% confidence intervals of the five plants for each indicator were approximated from the following profiles: faecal coliform (1.6 $\log_{10}$±0.33), faecal enterococci (1.42 $\log_{10}$±0.28), Sulphite Reducing Clostridia (SRC) (1.0 $\log_{10}$±0.4), somatic coliphage (1.55 $\log_{10}$±0.2), F-RNA phage (1.62 $\log_{10}$±0.28) and *Bacteroides fragilis* bacteriophage (1.4 $\log_{10}$±0.53). Reductions of SRC seemed to show lower reductions although the differences were not significant.

Removal of these indicators during primary sedimentation from five of the plants (two of which were activated sludge plants described here) were found to range from 0.3 to 0.5 $\log_{10}$ with no significant difference between plants (P>0.05). This suggests that the activated sludge process provided approximately 1 $\log_{10}$ removal for each indicator except *Clostridium* which was 0.5 $\log_{10}$.

Table 2-9. Summary of bacterial and viral log removals of four full scale secondary wastewater treatment plants (Ottoson, 2006)

Plant details	*E. coli*	Enterococci	*C. perfringens*	Somatic coliphage	F-RNA phage	Entero-virus	Noro virus
Klagshamn Chemical precipitation and activated sludge with rapid sand filtration (70,000 people)	2.38±0.39	2.1±0.51	1.95±0.26	1.04±0.21	2.19±0.63	nd	nd*
Sjölunda Chemical precipitation and activated sludge (270,000 people)	2.84±0.35	2.43±0.34	1.84±0.23	1.08±0.15	1.73±0.91	nd	nd
Ryaverket Chemical precipitation and activated sludge with N removal (610,000 people)	2.25±0.88	1.78±0.76	1.09±0.36	1.10±0.57	1.64±0.42	nd	nd
Ön Chemical precipitation and activated sludge (120,000 people)	2.30±0.35	2.20±0.50	1.19±0.16	0.84±0.19	1.54±0.78	nd	nd

*nd = not determined

2.5. Sampling methodologies – Additional studies

This short section considers the monitoring of pathogens in activated sludge and the approaches that can be used.

Traditional methods for the detection of waterborne viruses rely on concentration of viruses from large volumes of water followed by application to a range of cell lines to determine the levels of infective virus present and the types of viruses present. The complex nature of the wastewater matrix makes recovery of viruses from the water samples highly variable. When coupled with cell culture detection of viruses from wastewaters, the levels of detection can be an underestimate of the total viral load.

The viral content of wastewaters was evaluated with an immunoperoxidase methodology by Payment and Trudel (1987). By this method, the average viral content of raw sewage was 900 MPN IU/L, 1056 MPN IU/L in primary effluent, and 106 MPN IU/L in secondary effluent (approximately 1 $\log_{10}$ reduction). With a cytopathic effect assay on BGM cells, values of 85, 56, and 2 MPN IU/L (approximately 2 $\log_{10}$ reduction) were respectively observed, providing an underestimation of the viral content of secondary effluents.

Payment and Pinter (2006) provided a critical assessment of the methods, results and data analysis of waterborne pathogens. They concluded that the most critical gap was the lack of validation of methods used in environmental microbiology for detecting pathogens and that data generated by various laboratories are difficult to compare, making it difficult to serve as the basis for risk assessment or management. Informed communication of the risks of waterborne pathogens, the training of highly qualified personnel and the standardisation of methods are viewed as necessary for water safety and public health protection.

Robertson et al. (2000) conducted a three-year study on *Cryptosporidium* oocysts and *Giardia* cysts in sewage with emphasis upon the assessment of techniques for sampling, a field study of six sewage treatment works and assessment of the effect of sewage treatment on survival/destruction of *Cryptosporidium* oocysts. In assessing sampling techniques, it was found that grab samples were superior to continuously filtered samples and concentrated samples and that concentration and clarification of samples was best achieved where there was the least sample manipulation. Low numbers of viable oocysts were detected at both influent and effluent. In wastewater samples, the enumeration of oocysts in minimally manipulated sample recoveries may be affected due to shielding from particles within the wastewater. In Australia, water testing laboratories performing *Cryptosporidium* and *Giardia* analysis follow the USEPA 1622 and 1623 methods that allow standardization of the method. Incorporating Colorseed™ positive control material to the test allows for determination of the recovery efficiency within every sample.

Assavasiliavansukul et al. (2008) conducted a study into pathogen reduction across conventional drinking water treatment processes as a consequence of the initial pathogen dose rate. This report is of interest to this project in terms of the methods, monitoring and philosophical approach, although applied to conventional drinking water treatment, rather than activated sludge treatment. Findings of relevance to this project include:

- pathogen $\log_{10}$ reduction achieved depended on the initial spike dose, with higher doses yielding higher $\log_{10}$ reductions while lower initial pathogen concentrations yielded lower removals;
- 1 to 5 $\log_{10}$ pathogen reduction was achieved across various water treatment plants, depending on raw water quality, treatment operations and the influent concentration of pathogens;
- continuous sampling better demonstrated log reductions than grab samples since the latter often reported non-detect data;
- microscopy and flow cytometry provided similar results in samples other than settled sewage (where particles interfered); and
- Giardia and Cryptosporidium showed similar log reductions for sedimentation but Giardia showed higher LRV's for filtration processes.

The Assavasiliavansukul et al. (2008) study favoured continuous sampling which was in contrast to the observations from the Robertson et al. (2000) study that identified potential problems when using continuous samplers. It is likely that for filtration processes and conventional water treatment, the continuous samplers are preferred to allow the effects of pathogen build up on filters and break through spikes to be captured across a longer sampling period (Assavasiliavansukul et al. 2008). However, for wastewater, the active biological nature of the matrix and the different reduction mechanism means that grab samples are preferred over continuous sampling (Robertson et al. 2000).

3. DATA REVIEW

A full data review report has been submitted as a component of this project and can be provided upon request. The data review was conducted to develop a holistic understanding of national data and to explore correlations amongst process parameters and effluent quality parameters from full-scale activated sludge plants (ASPs) demonstrating pathogen reduction. A particular focus of the data review was the detection of correlations between pathogen reduction values and indicators as well as surrogates and plant operating parameters. The objective of the data review was to inform the design of the pilot plant facility and experimental monitoring program.

Twelve wastewater treatment facilities from across Australia were surveyed to capture their operating data and pathogen monitoring information. A cross section of activated sludge plant (ASP) configurations, operating regimes and climate zones were represented. The names and details of the surveyed facilities are held in confidence. Not all data sets were fully utilized in this study as most were incomplete for pathogen data with many focusing on *E. coli* levels in the final effluent in accordance with compliance testing regimes. Preliminary statistics were used for these sites and only general observations could be made for these plants. Only 7 out of the 12 plants studied had data that was of significant use to this study.

A detailed statistical analysis was conducted for one facility by Ecos Environmental P/L to review the options for statistical power in the current monitoring program for that plant.

3.1. General data review results

Table 3-1 represents a summary of the average data obtained across all surveyed facilities. That is, the 50th percentile (%ile) of reported average data. The reported average data was used as this was the most common statistic across the entire data set. Raw data was not provided for interrogation. It may be more prudent to view the data set for 95%ile to be more indicative of performance but this would also be less conservative. It can be seen that the $\log_{10}$ reductions vary both across and within the organism types, with some having high standard deviations reported.

Table 3-1. Summary of indicator and pathogen $\log_{10}$ reduction values across all surveyed sites

	Coli-forms	***E. coli***	**FRNA**	**Somatic phage**	***C. perf***	**Enteric virus**	**Reovirus**	**Entero-virus**	**Adeno-virus**	***Crypto-sporidium***	***Giardia***
50%ile of reported averages	2.28	2.80	3.91	2.93	2.24	1.90	1.60	1.50	1.70	0.90	1.44
95%ile of reported averages	3.19	3.51	6.02	3.23	2.78	1.90	1.60	1.50	1.70	0.99	1.92
Std Dev of reported averages	0.55	0.52	1.87	0.29	0.61	-	-	-	-	0.06	0.76
Number of reported averages	7	13	7	11	13	1	1	1	1	3	2

It was observed that few facilities have conducted verification studies across process units for the range of pathogens of concern. The most commonly available data was final effluent *E. coli* levels as this is generally monitored for license and compliance purposes. Only one study reported viruses separately. Most studies used the indicator organisms for viruses and also for parasites, with only a few samples specifically representing *Cryptosporidium* and *Giardia* data. The standard deviations were high for some data sets.

3.2. Detailed statistical review results

In this statistical review of one activated sludge plant (ASP), a short term data set that included information on concentrations of various microbial indicator organisms and physico-chemical factors was examined to identify operational parameters that may be predictive in some fashion for pathogen removal, i.e. represent a 'surrogate'.

The objective was to examine representative data from one ASP and conduct exploratory analyses to determine if there is a plausible relationship between operational parameters (surrogates) and pathogen log removal rates. The aim was to understand what could be achieved from the data obtained, and in future, to assist design of the monitoring and statistical program of the pilot plant.

To determine relationships between one set of variables (e.g. microbial indicators) and another (e.g. physico-chemical parameters), each group of parameters should be sampled at the same time. Since the microbial parameters were sampled infrequently and some of the physico-chemical data was sampled frequently (e.g. NH_3), averages were taken of the more intensively sampled data for periods that were representative of the time at which the microbial data was collected. Processing of the data resulted in a data set consisting of 5 valid cases of microbial and physico-chemical variables. A Pearson Correlation matrix and calculation of summary statistics was undertaken. The results of these analyses are reported below.

Log_{10} reduction values (LRVs) for the microbial parameters are shown in Table 3-2 along with the physico-chemical data. The parameters indicate LRVs of 2.8 for bacteria, 2.2 for protozoa and 2.5 for viruses. The variation over time was around 0.5 log_{10} for viruses and protozoa, and around 0.4 log_{10} for bacteria.

Table 3-2. Microbial and physico-chemical data for the selected ASP

Parameter	Units	Mean ± 95%CI
E. coli LRV	n/a	2.8 ± 0.06
C. perfringens LRV	n/a	2.2 ± 0.22
Somatic Coliphage LRV	n/a	2.5 ± 0.27
Effluent BOD	mg/L	4.50 ± 0.98
Effluent pH	pH units	7.86 ± 0.12
Effluent Suspended Solids	mg/L	4.00 ± 1.20
Effluent Total Nitrogen	mg/L	2.64 ± 0.69
Total Phosphorus Effluent	mg/L	3.58 ± 1.92
Effluent Turbidity	NTU	1.36 ± 0.27
Suspended Solids Mixed Liquor tank 1	mg/L	4920 ± 358
Suspended Solids Mixed Liquor tank 2	mg/L	4940 ± 423
Effluent NH_3	mg/L	2.23 ± 3.13
Dissolved oxygen 1	mg/L	1.80 ± 0.09
Dissolved oxygen 2	mg/L	1.82 ± 0.09
Influent Flow rate	L/s	475 ± 78

n = 5, sampling dates: 17/07/2008, 27/07/2008, 14/08/2008, 21/08/2008, and 24/08/2008.
Abbreviations: LRV = log reduction value, BOD = biochemical oxygen demand, Dissolved Oxygen 1 and 2 refer to separate locations in the effluent stream

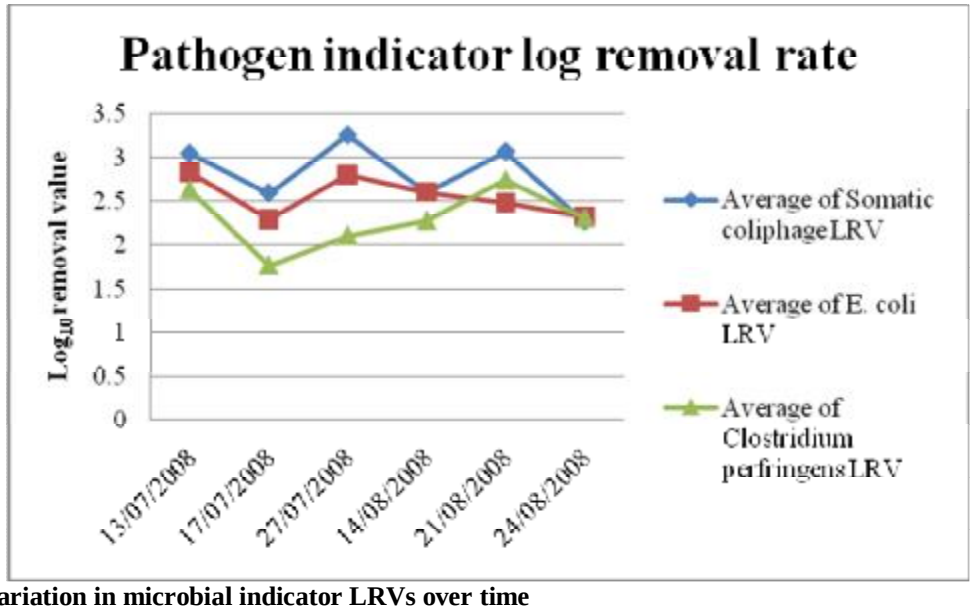


Figure 3-1. Variation in microbial indicator LRVs over time

Despite the small data set, significant correlations were observed between the following microbial indicators and physico-chemical variables:

1. Effluent Suspended Solids and *E. coli* LRV (0.95).
2. Suspended Solids Mixed Liquor 1 and *C. perfringens* LRV (0.90).
3. Suspended Solids Mixed Liquor 2 and *C. perfringens* LRV (0.92).
4. Effluent NH_3 and *C. perfringens* LRV (-0.93).

Further correlations observed amongst the physico-chemical variables were:

1. Effluent BOD and Effluent NH_3 (-0.99).
2. pH and Suspended Solids Mixed Liquor 1 & 2 (0.93, 0.88).
3. Influent Flow Rate and Effluent Suspended Solids (-0.89).
4. Effluent Total Nitrogen and Effluent Turbidity (0.98).
5. Suspended Solids Mixed Liquor 1 & 2 (0.98).
6. Dissolved Oxygen 1 & 2 (1.00).

With the low statistical power and small data set, only preliminary conclusions may be drawn on the true variability and the nature of the relationship between each parameter. The data set indicates the likelihood of strong correlations between some physico-chemical variables and pathogen indicator microorganisms and on that basis justifies the proposed pilot scale experiments.

4. MATERIALS, METHODS AND CHALLENGE STUDIES

4.1. Pilot plant

The activated sludge pilot plant design and operation was based on an intermittent discharge extended aeration (IDEA) configuration with a working volume of 150 litres. A primary logic controller (La Trobe University, Bendigo, Victoria) was used to operate the pilot plant. Primary effluent from the Eastern Treatment Plant (Carrum Victoria, Australia) was provided as influent for the pilot plant.

The cycle time was 4 hours, with 6 cycles being completed over a 24 hour hydraulic residence time (HRT). Each cycle consisted of the following stages (refer to Figure 4-1 and Figure 4-2):

1. Aeration and influent feed (2 hours): 25 litres of influent was fed into the reactor via a peristaltic pump ('influent pump') during the first 25 minutes of aeration. Mixed liquor was wasted 10 minutes prior to settling to control sludge age ('sludge waste unit', 'WAS').
2. Settling (1 hour): The 'air pump' was turned off.
3. Decant (1 hour): With the 'air pump' still off, treated effluent was removed over a 1 hour period via a peristaltic pump ('effluent pump').

Air was supplied by an aquarium air pump (Resun LP100, 150 L min^{-1}) to a diffuser (Nopol PRK 300) that produced a fine bubble. Airflow of *ca.* 1.2 m^3 h^{-1} (20 L min^{-1}) was set via a rotameter at a pressure of 12 kPa. A dissolved oxygen (DO) probe (Global Water, WQ401) and on/off air pump controller (La Trobe University, Bendigo, Victoria) maintained a DO level of 1.5 mgO_2L^{-1}. Liquor temperature (resistance thermometer) was maintained at ≥20°C by a heating jacket (model HJD, IBC Solutions, Wayville, South Australia, Australia) but could not be cooled. Redox (Global Water, WQ600) and pH (Global Water, WQ201) were monitored. Values from each of these four probes were recorded on a data logger (Onset U12-006).

Primary effluent was drawn via a submersible pump into a 950 L insulated storage tank fitted with a mixer that filled and emptied during each cycle. However, during *Cryptosporidium* challenge testing the primary effluent was stored in the 950L storage tank to allow spiking of a known volume with *Cryptosporidium* oocysts.

Sludge waste and effluent from the pilot plant were returned to ETPs primary effluent channel.

Figure 4-1. Pilot plant setup shown from three points of view

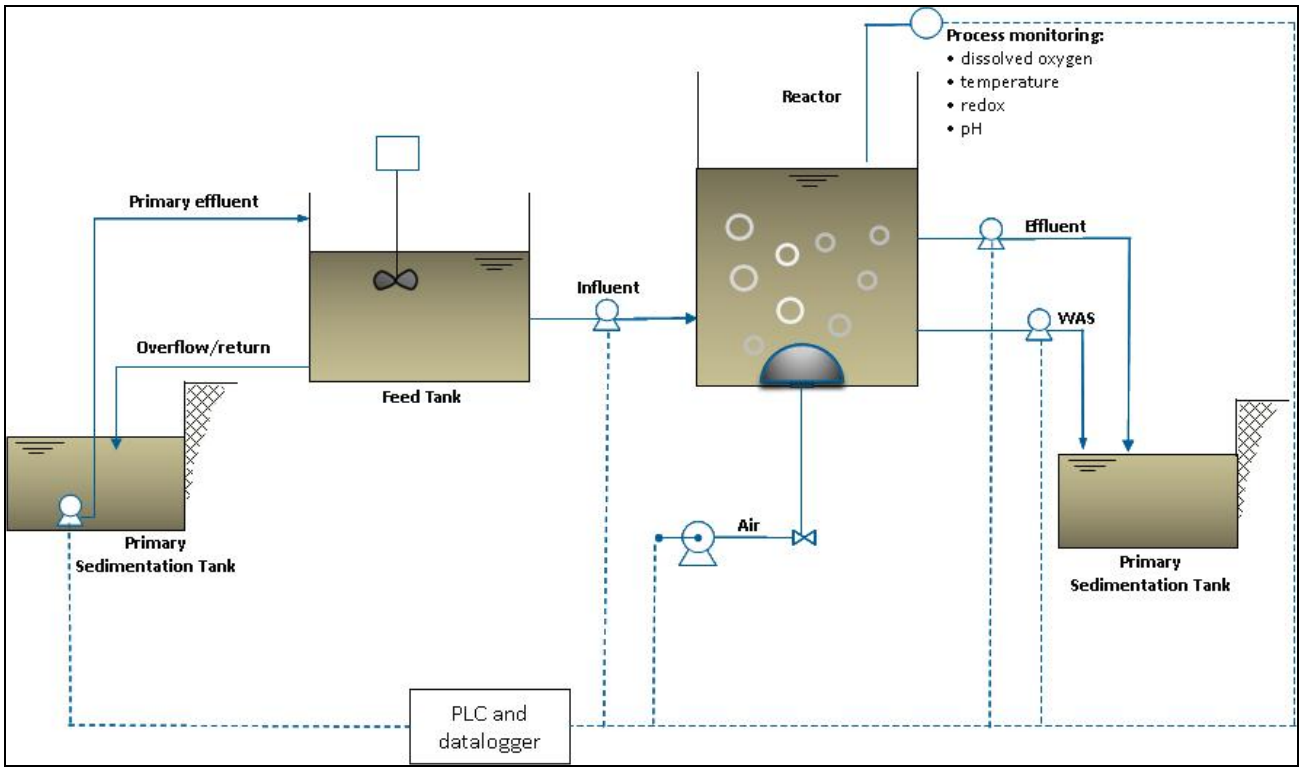


Figure 4-2. Pilot plant schematic

4.2. Experimental approach and analyses

The key outcomes from the literature review were applied to the experimental program to optimise identification of a suitable *Cryptosporidium* surrogate/s. The key objectives of the pilot plant trial were to:

1. Identify an appropriate surrogate or indicator for *Cryptosporidium* by operating the pilot plant at a standard set of conditions (15 day sludge age, 24 h HRT).
2. Investigate the effect of changing sludge age to 10 and 20 days on the *Cryptosporidium*/surrogate or indicator relationship/s compared to 15 day sludge age.

The *Cryptosporidium* indicators selected for this study based on literature review findings included *C. perfringens*, *E. coli*, coliforms, enterococci, F-RNA coliphage and particle profiling. All physico-chemical and pilot plant operational data were also considered as potential surrogates (see

Table 4-1).

A standard operating procedure (SOP) for manual microbiological sample collection from the pilot plant facility was supplied by the Australian Water Quality Centre (AWQC), Adelaide. Similarly, Ecowise, Melbourne, supplied an SOP for manual sample collection and analyses of physico-chemical parameters. Physico-chemical analyses were conducted by Ecowise and La Trobe University. Microbiological analyses were conducted by the AWQC.

Table 4-1. Pilot plant sampling points and parameters measured

Parameter	Influent (stored primary effluent)	Mixed liquor	Effluent
SS	**ü**	**ü**	**ü**
SVI	**û**	**ü**	**û**
NH3-N	**ü**	**û**	**ü**
NO3-N	**ü**	**û**	**ü**
NO2-N	**ü**	**û**	**ü**
TKN	**ü**	**û**	**ü**
COD	**ü**	**û**	**ü**
BOD_5	**ü**	**û**	**ü**
Turbidity	**ü**	**û**	**ü**
Microscopy	**û**	**ü**	**û**
pH	**ü**	**Continuous**	**ü**
Temperature	**ü**	**Continuous**	**ü**
DO	**û**	**Continuous**	**û**
Redox	**û**	**Continuous**	**û**

Note: **ü** - parameter measured; **û** - parameter not measured

4.3. *Cryptosporidium* challenge experiments

The pilot plant was challenged with *Cryptosporidium parvum* (isolated from Swiss calf and passaged in mice, Murdoch University, Perth, Australia). This was achieved by adding 1×10^4 oocysts/L, in addition to native oocyst numbers, to primary effluent freshly drawn from the primary sedimentation tanks at ETP into the influent storage tank.

The removal of *Cryptosporidium* and the selected indicators (see above) through the pilot plant was monitored over a 48 hour period as detailed in Table 4-2. Effluent samples were always removed at the end of the settling stage and compared with those in the feed at 0 h to establish $\log_{10}$ reduction values (LRVs). The data were analysed to identify the most suitable indicators for the removal of *Cryptosporidium* (see Section 5).

The sludge ages investigated were 10 days (1 experiment), 15 days (3 experiments), and 20 days (1 experiment). Spiking of the pilot plant with *Cryptosporidium* and subsequent analyses were only undertaken once 'steady state' had been reached. Steady state was a condition defined as operating the pilot plant for 2 to 3 sludge ages and ensuring all physico-chemical parameters had stabilised and effluent ammonia (as N) was <5 mgL^{-1}.

Table 4-2. *Cryptosporidium*, indicator and physico-chemical sampling times and locations for challenge testing

Sample time (hours)	Influent	Mixed liquor	Effluent
0	**M/S[1] (3)[2] + P/C[3]**	**P**	**P/C**
12	**P/C**	**P**	**M/S (2) + P/C**
16	**no sample**	**P**	**M/S (2) + P/C**
20	**no sample**	**P**	**M/S (2) + P/C**
24	**P/C**	**P**	**M/S (2) + P/C**
36	**P/C**	**P**	**M/S (1) + P/C**
48	**M/S (2) + P/C**	**P**	**M/S (1) + P/C**

Notes:

1. *M/S (Microbiological/Surrogate) - Cryptosporidium, Giardia, C. perfringens, enterococci, coliform, E. coli, F-RNA coliphage, particle profiling.*
2. *Value in parentheses indicate number of samples for M/S. P samples were at least triplicate, C samples were a single sample.*
3. *P/C and P (Physical/Chemical and Physical only) - see Table 4-1.*

4.4. Summary of experimental data

Please refer to Appendix A for comprehensive data tables.

Table 4-3 summarises the experimental data outcomes for the sludge ages trialled. The mean LRVs that can be conservatively attributed to the different pathogen groups are:

- Protozoa 0.5 to 1.0 $\log_{10}$.
- Viral indicators 1.0 to 1.5 $\log_{10}$.
- Bacteria 1.0 to 3.0 $\log_{10}$.

Table 4-3. *Cryptosporidium* and microbiological indicator mean $\log_{10}$ reduction during 48 h sampling regime

***Cryptosporidium* and microbiological surrogates**	**Study (sludge age in days) LRV (mean ± standard deviation) * Estimated at** 19-20°C				
	15 (1 of 3)	**15 (2 of 3)**	**15 (3 of 3)**	**20 (1 of 1)**	**10 (1 of 1)**
Average temperature	19-20°C	19-20°C	Unknown**	19-20°C	26°C*
F-RNA coliphage	0.79 ± 0.04	1.32 ± 0.03	1.68 ± 0.04	2.11 ± 0.12	1.11 ± 0.06
C. perfringens	0.43 ± 0.12	1.27 ± 0.08	1.17 ± 0.08	1.67 ± 0.06	0.84 ±0 .10
Coliforms	0.57 ± 0.10	1.84 ± 0.07	1.39 ± 0.07	3.10 ± 0.12	3.22 ± 0.04
Cryptosporidium	0.51 ± 0.17	1.05 ± 0.06	0.41 ± 0.15	2.06 ± 0.16	1.44 ± 0.14
E. coli	0.78 ± 0.07	2.08 ± 0.06	1.79 ± 0.08	3.20 ± 0.06	3.27 ± 0.06
Enterococci/Faecal Streptococci	0.63 ± 0.13	1.93 ± 0.10	1.62 ± 0.03	2.90 ± 0.09	3.07 ± 0.09
Giardia	1.20 ± 0.21	2.29 ± 0.26	1.82 ± 0.51	2.37 ± 0.17	1.52 ± 0.21
SRC	0.62 ± 0.08	1.17 ± 0.05	1.16 ± 0.08	1.65 ± 0.02	1.02 ± 0.04

* Average temperature was above the recommended temperature range.
** R temperature at 19 – 20°C

5. PREDICTIVE MODEL

5.1. Overview of data analysis

The pilot plant, the experimental trials and the biological and physico-chemical parameters that were monitored during each trial are described in the preceding sections. In this section we focus on the statistical design of the trials and the analysis of data obtained from the study. The data analysis involved three components, namely:

1. Summarising and comparing data between trials using plots and tables.
2. Exploratory data analysis.
3. Development of predictive models for each pathogen group monitored. The predictive model development involved the use of both standard multiple regression techniques and neural networks.

5.2. Comparison of trial data

As has been described above, 5 experiments or 'Trials' were conducted over 2009 at the ETP. The key attribute altered between the trials was sludge age, as follows:

- Trial 1: steady state - 15 day sludge age
- Trial 2: steady state - 15 day sludge age
- Trial 3: steady state - 15 day sludge age
- Trial 4: 20 day sludge age
- Trial 5: 10 day sludge age

As described in Section 4, effluent was sampled for physico-chemical and pathogen variables at the following time periods:

- Pre-spike t = 0 hours
- 12 hours
- 16 hours
- 20 hours
- 24 hours
- 36 hours
- 48 hours.

Physico-chemical variables were generally measured once at each time period, while 2 replicate samples were collected for each pathogen group. These time periods reflect collection of samples at a minimum of 1.5 x the hydraulic residence time (HRT) when it is expected that the spike will have passed through the treatment plant.

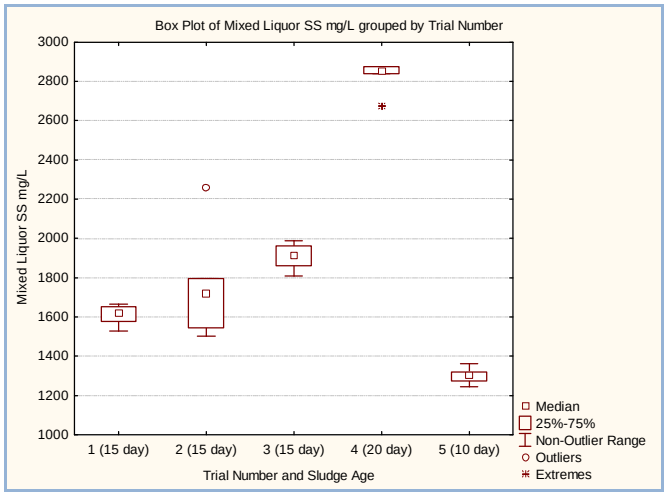
5.2.1. Physico-chemical data

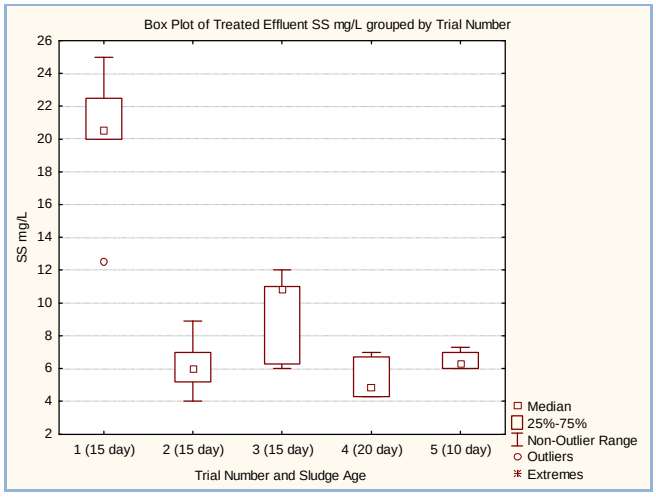
Sludge age has a strong influence on the physico-chemical and biological conditions in the reactor vessel and as a result many of the physico-chemical variables were expected to differ between trials. The differences in physico-chemical variables measured in the effluent between trials are shown as box plots in Figure 5-1. For ease of interpretation, the data have been grouped across sampling times. Visual analysis also showed 'time' had little influence on the range of data values.

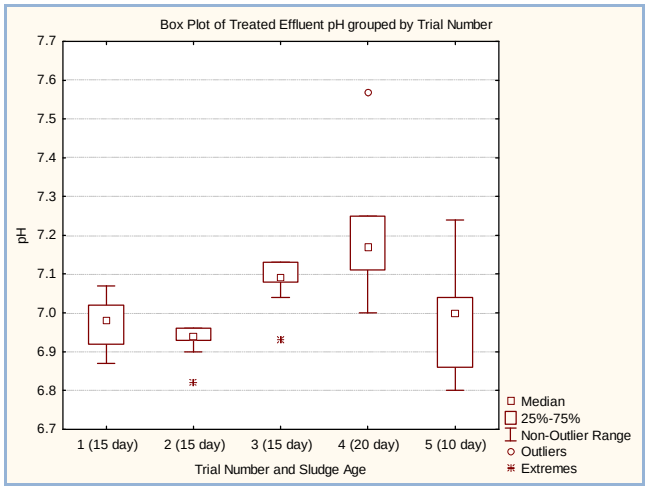
Two of the first three trials were conducted at identical sludge ages and temperatures (19 to 20°C). No temperature data was recorded for the third trial but it was expected to be in the range of the first two trials based on temperatures recorded in Eastern Treatment Plant (ETP) monitoring. For statistical analysis and for the box plot in Figure 5-1, a mean temperature value (19-20°C) of the other 15 day sludge age trials was substituted for the missing values. The temperature range for the 4th trial (20 day sludge age) was similar to Trials 1 and 2, however, Trial 5 (10 day sludge age) was undertaken during a period of very hot weather which raised the reactor temperature from 19-20°C to 26°C. This had the effect of confounding temperature with sludge age across the overall experimental design affecting related physico-chemical parameters (e.g. ammonia), other forms of nitrogen and chemical oxygen demand (COD). The confounding also had implications for the pathogen log reduction rates and subsequent statistical analyses.

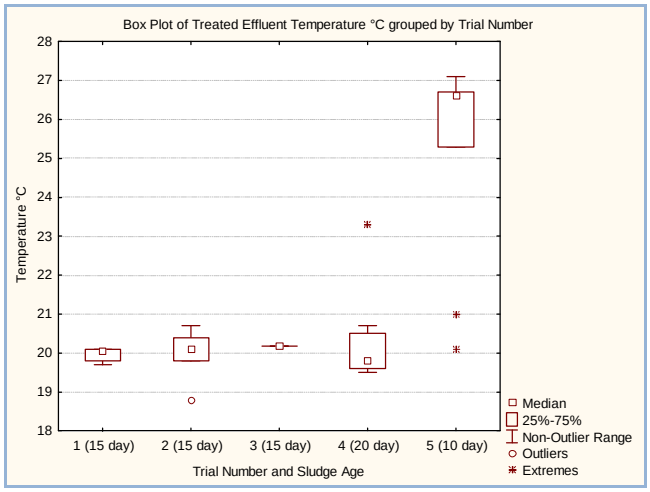
Notable attributes of the physico-chemical data set revealed by the box plots are:

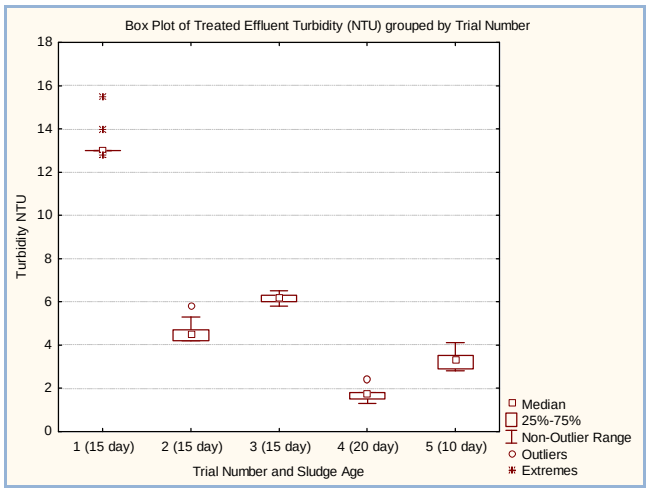
- Moderate to high variability between the three identical 15 day sludge age trials. Trial 1 indicated some possible setup problems in the operation and sampling of the pilot plant with relatively high suspended solids (SS), turbidity and COD.
- The relatively high temperature of Trial 5 (as noted above).
- High Suspended Solids-Mixed Liquor (SS-ML) in the 20 day sludge age trial. This was expected since MLSS is the total amount of organic and mineral suspended solids, including microorganisms, in the mixed liquor and is increased by increasing the sludge age.
- Higher alkalinity and BOD in Trial 3. This effect could be due to temporary variations in the ETP sewage quality. It is not clear if the two factors are related by chemical means or have simply experienced simultaneous higher inputs from the ETP sewer catchment.
- A clear inverse relationship between nitrate (NO_3) and nitrite (NO_2) which may be influenced by sludge age and temperature but also reflects the nature of denitrification conversion.

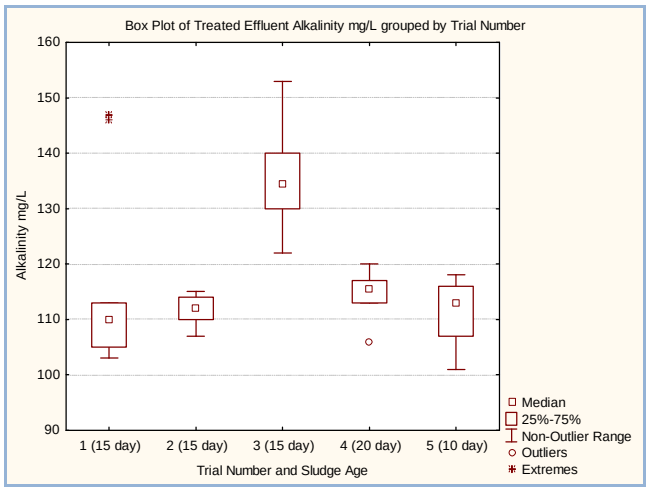


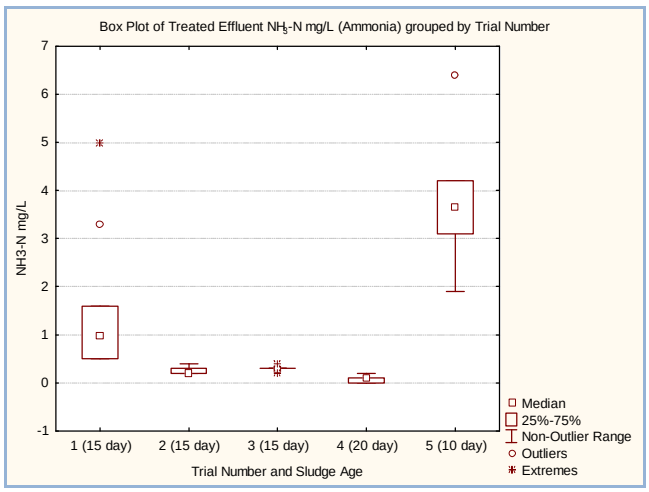


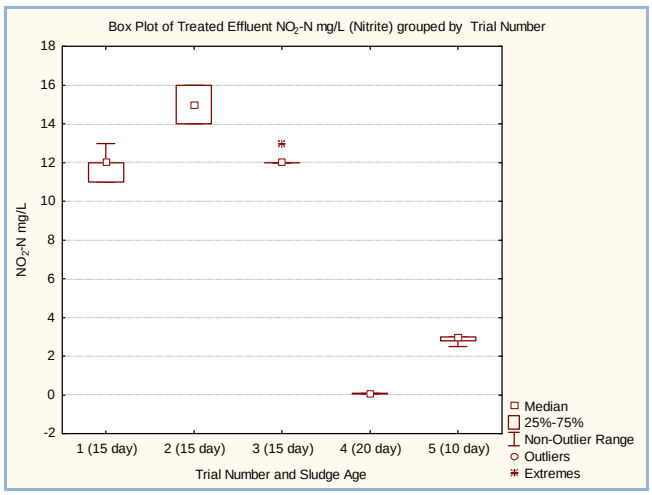

Box Plot of Treated Effluent pH grouped by Trial Number
pH
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
1 (15 day)
2 (15 day)
3 (15 day)
4 (20 day)
5 (10 day)
Trial Number and Sludge Age
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Treated Effluent Temperature °C grouped by Trial Number
Temperature °C
28
27
26
25
24
23
22
21
20
19
18
1 (15 day)
2 (15 day)
3 (15 day)
4 (20 day)
5 (10 day)
Trial Number and Sludge Age
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Treated Effluent Turbidity (NTU) grouped by Trial Number
Turbidity NTU
18
16
14
12
10
8
6
4
2
0
1 (15 day)
2 (15 day)
3 (15 day)
4 (20 day)
5 (10 day)
Trial Number and Sludge Age
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Treated Effluent Alkalinity mg/L grouped by Trial Number
Alkalinity mg/L
160
150
140
130
120
110
100
90
1 (15 day)
2 (15 day)
3 (15 day)
4 (20 day)
5 (10 day)
Trial Number and Sludge Age
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Treated Effluent NH3-N mg/L (Ammonia) grouped by Trial Number
NH3-N mg/L
7
6
5
4
3
2
1
0
-1
1 (15 day)
2 (15 day)
3 (15 day)
4 (20 day)
5 (10 day)
Trial Number and Sludge Age
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Treated Effluent NO2-N mg/L (Nitrite) grouped by Trial Number
NO2-N mg/L
18
16
14
12
10
8
6
4
2
0
-2
1 (15 day)
2 (15 day)
3 (15 day)
4 (20 day)
5 (10 day)
Trial Number and Sludge Age
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

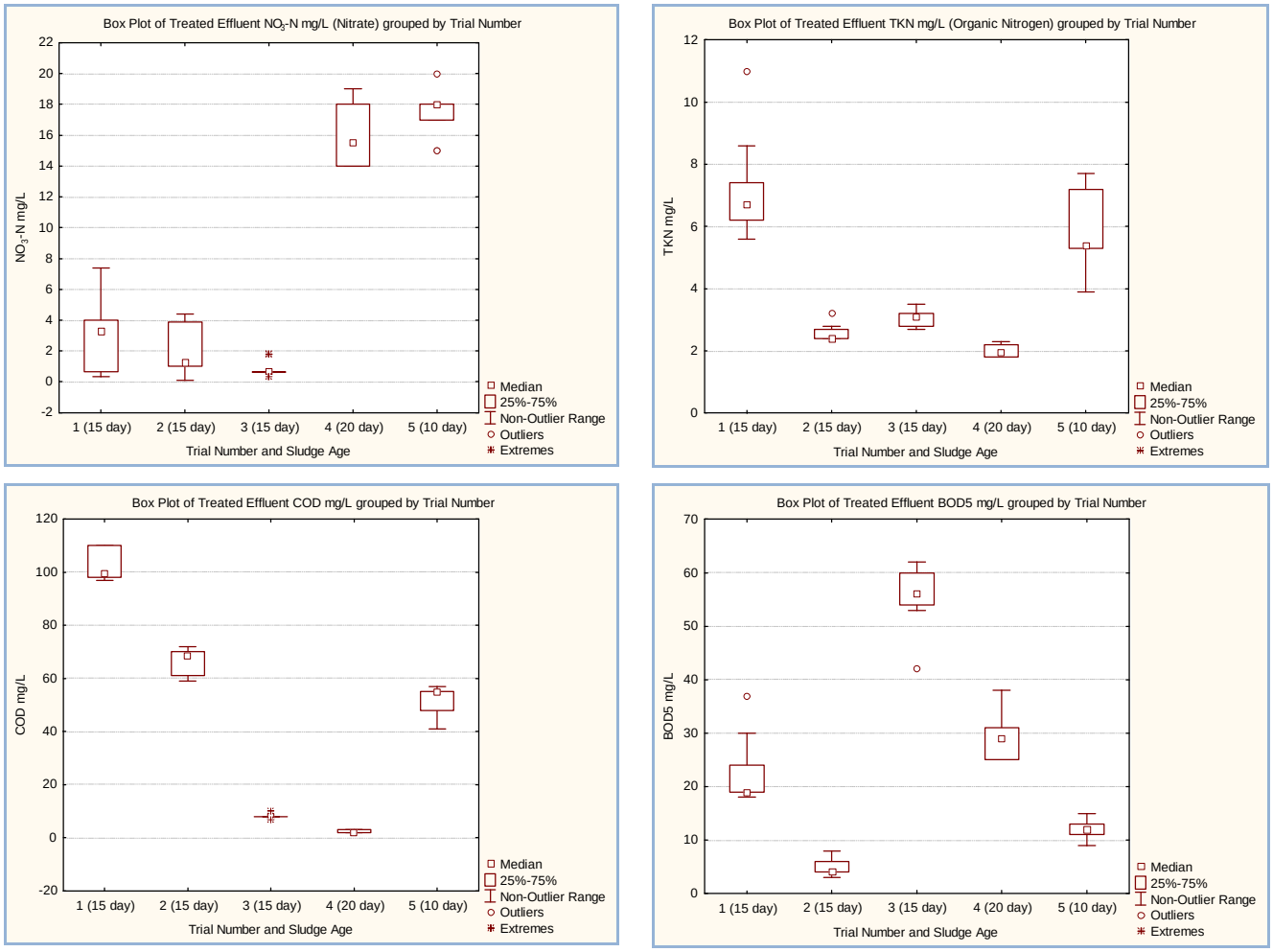


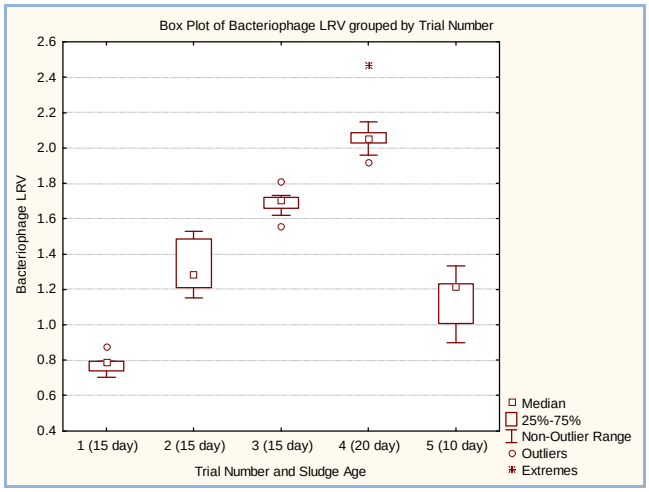
Figure 5-1. Box plots of physico-chemical variables monitored in effluent for the study
Note due to missing temperature data for trial 3 a fixed average temperature of the related 15 day sludge age trials was used. Trial 1, 2 and 3 are 15 day sludge age. Trial 4 is 20 day sludge age. Trial 5 is 10 day sludge age.

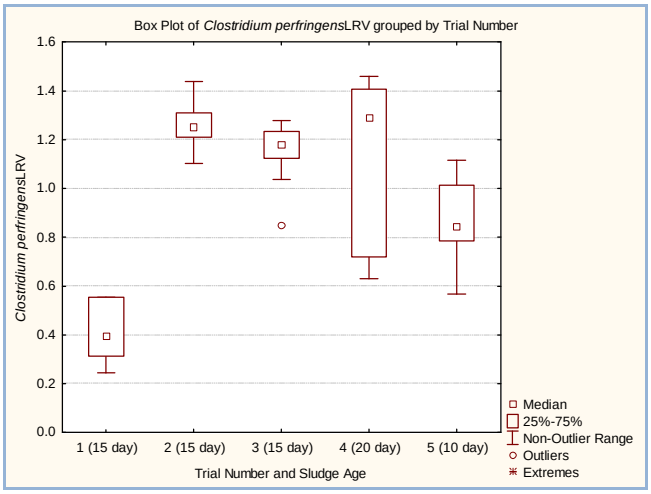
5.2.2. Pathogens and pathogen indicator groups log reduction values

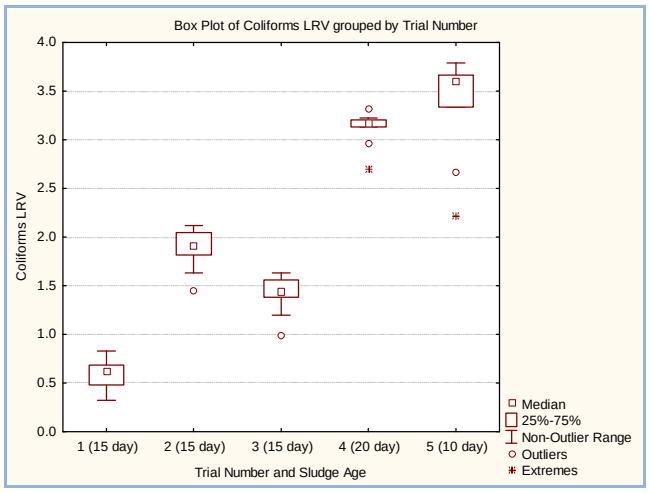
The primary biological variables of interest of the study were the $\log_{10}$ reduction in pathogen and pathogen indicator concentrations between t=0 hour influent to the reactor vessel and the effluent. The differences in pathogen LRVs measured in the effluent between trials are shown as box plots in Figure 5-2. Notable attributes revealed by the box plots are:

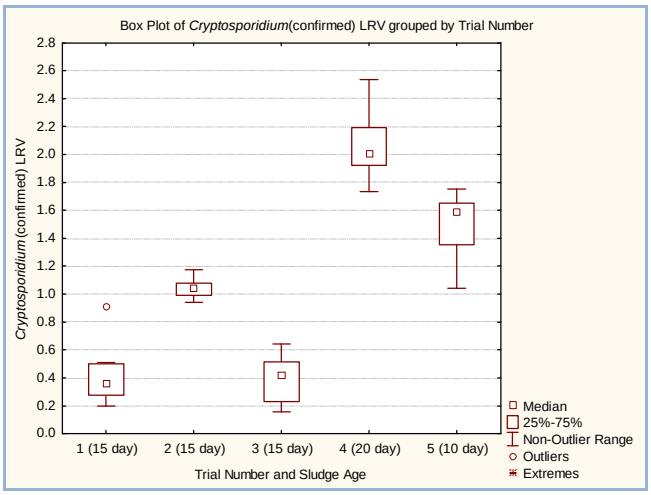
- Low pathogen LRVs in Trial 1. This may be due to setup difficulties associated with sampling and laboratory analysis for this first trial. It is notable that the results of the later trials appeared to be more consistent, particularly Trials 2 and 3.
- Increasing the sludge age to 20 days appeared to increase LRVs for bacteriophage, coliforms, *Cryptosporidium*, *E. coli* and enterococci. Trends in other groups were not so clear.
- Decreasing the sludge age to 10 days and increasing the average reactor temperatures by several degrees (as noted earlier, these effects were confounded) seemed to decrease the bacteriophage LRV, but increased the coliform, *E. coli* and enterococci LRVs. The response of the remaining groups was less clear.

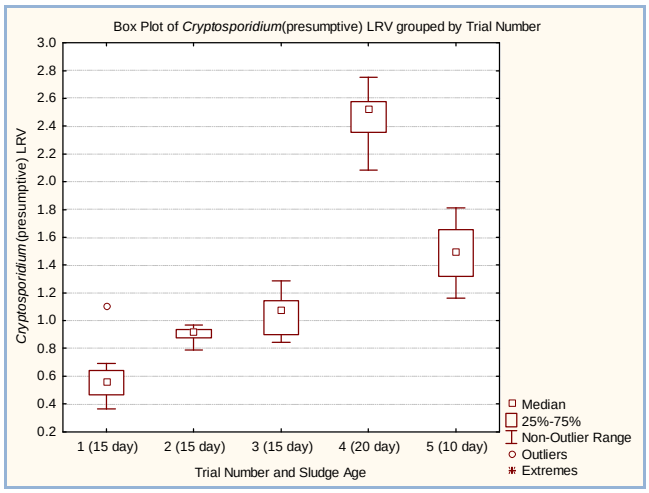
- There is some suggestion in the data of differing sensitivities to temperatures and sludge age (as measured by MLSS). For example the coliform bacteria appear more sensitive to higher temperatures than the MLSS while bacteriophage and *Cryptosporidium* appear more sensitive to sludge age.

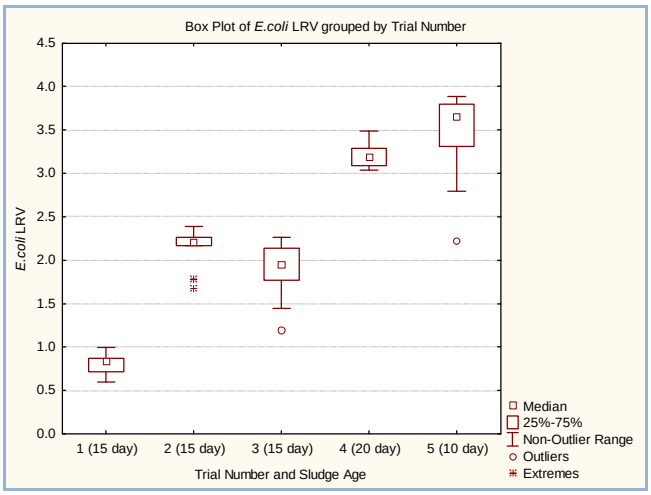












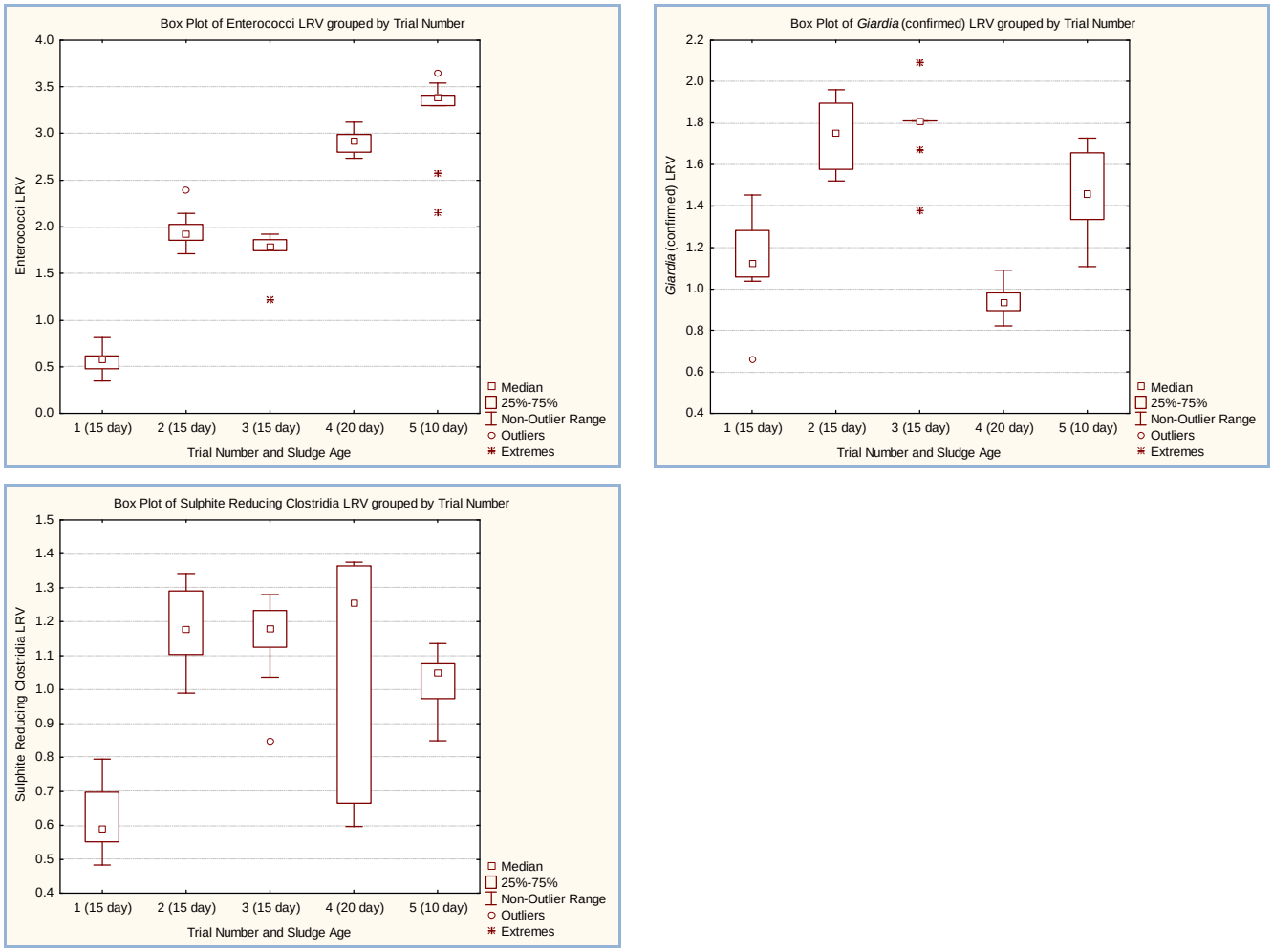


Figure 5-2. Box plots of Log Reduction Values (LRVs) grouped by trial for pathogen groups monitored in the study

Note that *Giardia* data for trial 3 and trial 4 is of lower quality due to the need to substitute average values for multiple missing values.

5.3. Pathogen die-off rates in storage

While every attempt was made to keep pathogen loading rates to the reactor constant over the duration of each trial, some minor changes in pathogen concentration in the effluent storage tank were noted over the 48 hour trial periods (Table 5-1). If such changes are consistent and measurable, then it is possible that a correction factor can be applied. However, despite the fact that the values for changes in influent quality over 48 hours were calculated from the means of 3 samples of 0 hour influent and 2 samples of 48 hour influent, the coefficients of variation (CVs) were very high and not particularly consistent in sign across trials or pathogen species (i.e. sometimes positive, sometimes negative). The causes of such variation are not clear but could be due to:

- variability in sample collection, storage, and analysis;
- variations in sewage quality between trials which affected pathogen die off rates during storage; and
- variations in storage conditions between trials at the site.

Since the pathogens monitored are not expected to proliferate in the storage due to their intrinsic biology, slight decreases in concentrations may be expected over 48 hours. However, the presence of small and sometimes large decreases and also of large increases in concentration means that the behaviour of pathogens in the stored effluent cannot (on the basis of the sampling effort of this study) be predicted with reasonable confidence. Consequently, it was decided that no correction factor would be applied to the trial data to account for changes during storage. If there truly is a small decrease during storage, then the LRVs calculated over the study may be slightly inflated. However, this effect would apply regardless of the type of treatment and values of the independent physico-chemical variables. Thus, a predictive model constructed on the data would consistently over predict LRVs by the same amount and require only a single correction factor to be applied. Future trials should include additional sampling of influent quality over the duration of the trials to provide a higher quality data set from which more accurate correction factors could be calculated.

Table 5-1. Changes in pathogen and indicator LRVs (log_{10}) over each 48 trial duration in the stored sewage which was used as influent for each trial

Trial	Bacteriophage	*Clostridium perfringens*	Coliforms	*Cryptosporidium* - Confirmed	*Cryptosporidium* - Presumptive	E.coli	Enterococci	*Giardia* - Confirmed	Sulphite Reducing Clostridia
LRV changes over 48 hours in stored sewage									
1	-0.07	0.09	0.14	0.14	0.04	0.07	-0.13	-0.72	-0.07
2	0.06	-0.11	-0.04	-0.24	-0.01	-0.19	-0.37	-0.20	-0.11
3	-0.14	-0.28	-0.13	0.34	-0.20	-0.12	-0.12	-0.43	-0.28
4	0.15	0.61	-0.10	-0.79	-0.16	-0.12	-0.22	0.24	0.69
5	0.16	0.15	-0.32	-0.43	-0.46	-0.29	-2.12	-0.61	-0.13
48 hour LRVs in ASP process									
1	0.87	0.28	0.32	0.23	0.51	0.60	0.62	1.45	0.52
2	1.22	1.21	1.45	0.94	0.88	1.68	1.71	1.96	0.99
3	1.73	1.16	0.99	0.16	0.87	1.19	1.22	1.67	1.16
4	2.04	1.22	2.96	1.99	2.51	3.19	2.80	0.89	1.18
5	0.94	0.83	2.67	1.04	1.16	2.80	2.57	1.33	1.12
% change									
1	-8.4	33.4	43.8	60.3	7.6	11.8	-21.2	-49.6	-13.0
2	5.1	-9.2	-2.9	-25.9	-0.6	-11.5	-21.8	-10.4	-11.3
3	-8.0	-23.9	-13.4	216.5	-22.7	-10.2	-9.5	-25.7	-23.9
4	7.5	49.9	-3.5	-39.8	-6.3	-3.7	-8.0	27.1	58.2
5	17.4	18.1	-12.0	-41.2	-39.3	-10.5	-82.6	-45.8	-11.6
Coefficients of Variation									
All Trials	405.3	361.1	-181.3	-226.4	-125.4	-101.7	-145.2	-110.9	1835.4

Note: Large positive LRVs over 48 hours are highlighted in red. These parameters for the particular trials recorded increases over the 48 period. Large negative changes are highlighted in yellow.

5.4. Choosing an appropriate statistical model

Based on the experimental design and the structure of the sampled data set, it was possible to develop a statistical model using either analysis of covariance (ANCOVA) or multiple regression. Both model types use a number of independent variables (IVs) (e.g. physico-chemical data, different sampling times etc.) to predict the value of a single dependent variable (DV) (e.g. E. coli LRV). To predict the values of other dependent variable requires the development of a separate model for each DV. In practice, the same IV data set can be used for each model. The decision as to which type of model to use depends on intrinsic attributes of the data. For example, predicting the values of continuous DVs with categorical IV data is best undertaken using analysis of variance (ANOVA) type statistical models.

When an IV data set consists of a mixture of categorical and continuous variables, an ANCOVA is required. If all the IVs are continuous, then a multiple regression model can be used.

Each of the aforementioned statistical approaches is based on the assumption of a linear relationship between IVs and DVs and it is assumed that the IVs are not correlated with each other. Furthermore for ANCOVA, it is assumed that the slopes of relationships between continuous IVs and DVs are the same for each level of a categorical IV. For example, assuming a positive relationship between suspended solids (SS) and a pathogen LRV and homogeneous slopes, we should expect a plot of LRV versus SS for two levels of a categorical variable (e.g. different trials) to look like the left plot in Figure 5-3. Using the example of different experimental trials as the categorical variable and if the effect of the trial changes the slope, then at some values of the IV there is no difference between the trials and at other values there is a difference (see the right plot in Figure 5-3). The difficulty with ANCOVA is that heterogeneity of slopes is common in biological data more so when there are several IVs.

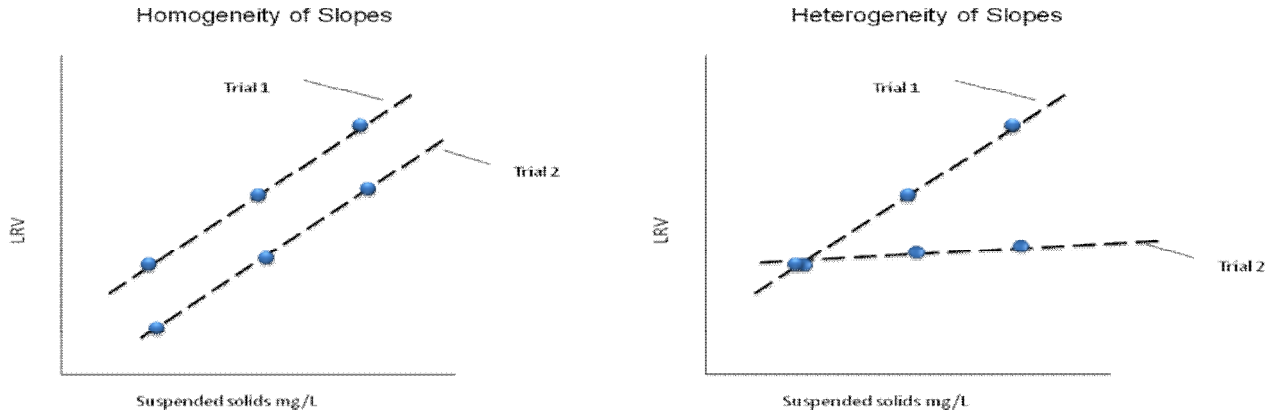


Figure 5-3. Homogeneity-heterogeneity of slopes in Analysis of Covariance (ANOVA)
In this example suspended solids is the independent variable (IV) and LRV is the dependent variable (DV).

Sludge age could have been another categorical variable (giving a two-factor ANCOVA) but the experimental design became intractable as trials were nested within sludge age and there were different numbers of trials for each sludge age causing the design to be imbalanced (i.e. three trials within “15 day sludge age” and 1 trial each within “10 day sludge age” and “20 day sludge age”).

Sludge age was also confounded with temperature as Trial 5, 10 day sludge age, was undertaken with average temps of 26°C compared to 19-20°C for the other trials. The effect “Trials” therefore meant a combination of different temperature and different sludge age.

Based on the above considerations, the following decisions were made in relation to the appropriate statistical model for the study:

- Sludge age was ignored as an IV.
- Since the assumption of homogeneity of slopes could not be supported and the Trials IV was confounded between temperature and sludge age it decided to ignore the Trials effect.
- Jettisoning the categorical variables of Sludge Age and Trials meant that the remaining IVs were all continuous variables and so could in principal be analysed by multiple regression.

- Multiple regression requires that the IVs be uncorrelated (i.e. that they are not multicollinear). A check of the correlation matrix of these variables (not presented here for brevity) plus the clear visual evidence of Figure 5-1 indicates that this assumption could not be supported.
- A solution to the multicollinearity problem is to conduct a Principal Components Analyses (PCA) to derive a small subset of new variables which are highly correlated with the original variables but are not correlated with each other. The new principal component axes can then be used as IVs in the multiple regression. This technique is known as Principal Components Regression and is described in Section 5.6.1. The PCA is described in the following section.

5.5. Exploratory data analysis - Principal Components Analysis of physico-chemical variables

PCA was conducted on the 13 IVs in the data set (Table 5-2). Using PCA it is possible to identify subsets of variables that are correlated with each other but largely uncorrelated with other subsets. Groups of correlated variables are combined into "factors" that are thought to represent underlying processes that have created the correlations among the variables (Tabachnick and Fidell 2007). In PCA one eigenvalue is computed for each IV (Figure 5-4) but only larger eigenvalues with a score greater than 1 are retained for further analysis (Table 5-3). The reason for this is that using matrix algebra it can be shown that a correlation matrix of variables, amongst which correlations occur, can be well characterised by a number of eigenvalues which is smaller than the number of variables used to create the matrix. Furthermore, (using the Kaiser criterion) unless an eigenvalue accounts for as much variance at least as much as the equivalent of one original variable, it is discarded (Hill and Lewicki 2006). A detailed account of PCA methodology is described in Tabachnick and Fidell (2007).

Table 5-2. Independent variables (IVs) used in the PCA

Variable	Description	Type
Hours	Time elapsed since beginning of trial in hours	Temporal
SS-ML (MLSS)	Suspended Solids Mixed Liquor. The weight of suspended solids in the mixed liquor	Physico-chemical
SS	Suspended Solids. The weight of suspended solids in the supernatant (effluent from the reactor vessel)	Physico-chemical
pH	Hydrogen ion concentration	Physico-chemical
Temperature	Temperature of effluent in Celsius	Physico-chemical
Turbidity	Turbidity is cloudiness or haziness of a fluid caused by individual particles. It is measured by light scatter with a calibrated nephelometer in Nephelometric Turbidity Units (NTU).	Physico-chemical
Alkalinity	Sum of bases in solution excluding carbonate and bicarbonate	Physico-chemical
NH_3-N	Ammonia nitrogen	Physico-chemical
NO_2-N	Nitrite nitrogen	Physico-chemical
NO_3-N	Nitrate nitrogen	Physico-chemical
TKN	Total Kjeldahl Nitrogen (the sum of organic nitrogen; ammonia (NH_3) and ammonium (NH_4^+))	Physico-chemical
COD	Chemical Oxygen Demand (amount of organic compounds in water)	Physico-chemical
BOD5	Biochemical Oxygen Demand (uptake rate of dissolved oxygen by the biological organisms over 5 days)	Physico-chemical

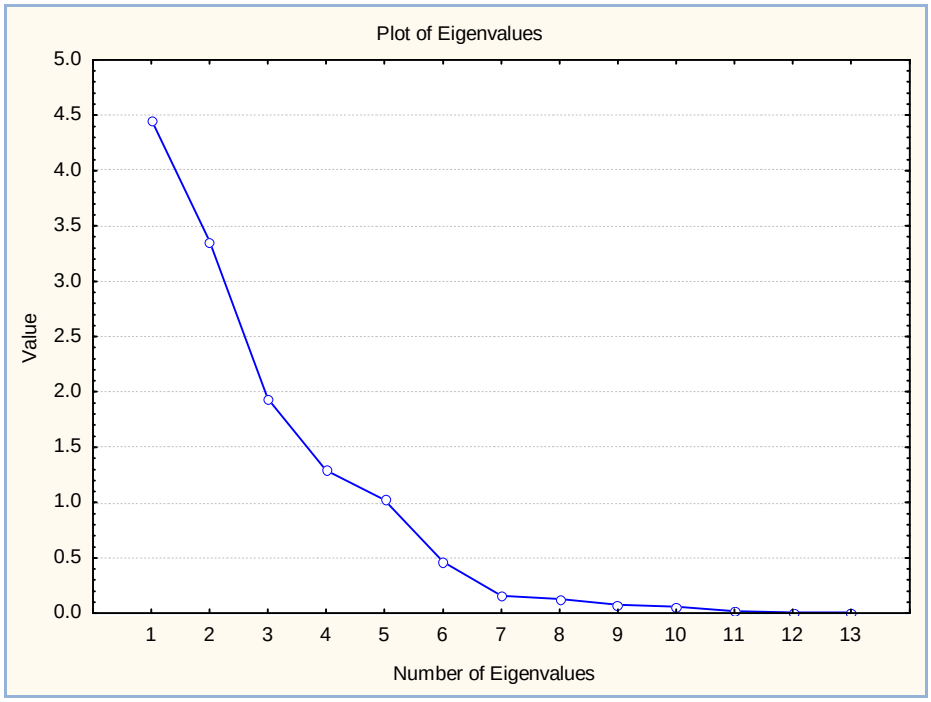


Figure 5-4. Screen plot of eigenvalues of the IVs
The plot indicates that the first 5 eigenvalues are greater than one and account for most the variance in the data set.

Table 5-3. Major eigenvalues and percentage variance explained by each factor

Factor	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	4.46	34.31	4.46	34.31
2	3.36	25.85	7.82	60.16
3	1.94	14.93	9.76	75.09
4	1.29	9.93	11.05	85.02
5	1.02	7.88	12.08	92.89

As noted earlier, PCA creates (or extracts) new variables ("factors") which are correlated with the original variables but are fewer in number.

The extracted factors exist as a score for each case in the data set. Using these scores and the values of the original variables for each case, a correlation matrix of factor scores versus original variables can be constructed (Table 5-4). The correlations between each variable and each factor are referred to as factor loadings. The factor is interpreted from the variables that are highly correlated with it and have high loadings on it (Tabachnick and Fidell 2007). In Table 5-4, the first factor appears to represent the amount of suspended organic matter in the effluent as measured by suspended solids, turbidity, TKN and COD and these variables as a group are inversely correlated with the mixed liquor suspended solids (MLSS). This is useful information, however, interpretation of the factor loadings for all factors would be clearer if only a few variables are highly correlated with a particular factor and the rest are not. This is accomplished by rotating the factor scores (in mathematical space) in such a manner as to maximise the variance of factor loadings by making high loadings higher and low ones lower for each factor.

The particular rotation technique is known as Varimax rotation and is commonly used in conjunction with PCA. The rotated factor loadings are given in Table 5-5. Interpretation of the rotated loadings is given in Table 5-6. The interpretations of the loadings will be helpful in subsequent analyses where factor scores are substituted for the original IVs.

Table 5-4. PCA Factor Loadings (Unrotated)
Marked loadings are >0.650.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Hours	-0.109	-0.069	-0.018	-0.770	-0.594
SS-ML (MLSS)	0.739	-0.405	-0.016	0.291	-0.365
SS	-0.765	-0.219	-0.323	0.342	-0.299
pH	0.576	-0.085	-0.561	0.437	0.052
Temperature	0.044	0.852	-0.203	-0.120	0.328
Turbidity	-0.869	-0.300	-0.167	0.219	-0.202
Alkalinity	-0.011	-0.544	-0.657	-0.357	0.216
NH3-N	-0.327	0.736	-0.502	-0.166	0.085
NO2-N	-0.647	-0.561	0.296	-0.147	0.340
NO3-N	0.533	0.748	-0.090	0.041	-0.322
TKN	-0.754	0.408	-0.485	0.080	-0.121
COD	-0.896	0.233	0.237	0.202	-0.085
BOD5	0.218	-0.623	-0.618	-0.128	0.056
Expl.Var	4.460	3.361	1.941	1.291	1.024
Prp.Totl	34.3%	25.9%	14.9%	9.9%	7.9%

Table 5-5. PCA Factor Loadings (Varimax rotated and normalized)
Marked loadings are >0.650.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Hours	0.022	0.015	0.105	0.975	0.011
SS-ML (MLSS)	-0.228	0.520	0.221	-0.096	0.741
SS	0.956	-0.140	0.093	-0.011	0.008
pH	-0.079	0.591	0.488	-0.486	0.132
Temperature	-0.245	0.257	-0.194	-0.135	-0.842
Turbidity	0.902	-0.379	0.043	0.052	0.023
Alkalinity	0.042	-0.209	0.918	0.107	-0.047
NH3-N	0.219	0.231	0.000	0.074	-0.910
NO2-N	0.244	-0.935	0.048	0.018	0.149
NO3-N	-0.297	0.835	-0.298	0.082	-0.278
TKN	0.751	0.027	-0.014	0.066	-0.649
COD	0.679	-0.384	-0.529	0.022	-0.267
BOD5	0.028	0.021	0.887	0.000	0.224
Expl.Var	3.070	2.666	2.345	1.247	2.749
Prp.Totl	23.6%	20.5%	18.0%	9.6%	21.1%

Table 5-6. Interpretations of rotated factor loadings

Factor	Variable	Interpretation	Code	Comment
Factor 1	SS, Turbidity, TKN, COD	*Concentration of Particulate Organic Matter (POM)*	*POM*	Higher concentrations of fine organic particles.
Factor 2	NO2-N, NO3-N	*Oxidation state of nitrogen*	*NOx*	This axis measures the oxidation state of ionic nitrogen (NO_3 = high, NO_2 = low) in solution.
Factor 3	Alkalinity, BOD5	*Alkalinity and BOD*	*ALKBOD*	The relationship between alkalinity and BOD is likely due to the consumption of alkalinity that occurs to a limiting factor as microbes metabolise BOD.
Factor 4	Hours	*Time*	*TIME*	Time seems quite independent of other factors
Factor 5	SS-ML (MLSS), Temperature, NH3-N	*Temperature, Sludge age and ammonia - probably confounded here*	*TNH3_SL*	Ammonia is linked to temperature across an optimal range as expected. Note the large increase in trial 5 with high temperatures. The correlation with sludge age is also expected given the optimal growth rate required for successful nitrification.

A further measure of how well the factor scores explain the variation in the original variables is given by Communalities. Communalities are the sum of squared loadings for a variable across factors. The communality of variable is the variance accounted for by the loadings/factors (Tabachnick and Fidell 2007). Communalities for the current data set are given in Table 5-7. Variables that have communalities close to zero after five factors are poorly explained and should be excluded from the data set or checked to see if the inclusion of additional factors will help explain their relationship to the rest of the data. In the case of the current data, all communalities are high to very high.

Table 5-7. Communalities (varimax normalized)

	From 1 factor	From 2 factors	From 3 factors	From 4 factors	From 5 factors
Hours	0.000	0.001	0.012	0.963	0.963
SS-ML (MLSS)	0.052	0.322	0.371	0.380	0.928
SS	0.914	0.934	0.943	0.943	0.943
pH	0.006	0.356	0.594	0.830	0.848
Temperature	0.060	0.126	0.164	0.182	0.891
Turbidity	0.813	0.957	0.959	0.962	0.962
Alkalinity	0.002	0.045	0.887	0.899	0.901
NH3-N	0.048	0.101	0.101	0.107	0.935
NO2-N	0.060	0.934	0.936	0.936	0.959
NO3-N	0.088	0.785	0.874	0.880	0.958
TKN	0.564	0.564	0.565	0.569	0.990
COD	0.462	0.609	0.889	0.890	0.961
BOD5	0.001	0.001	0.787	0.787	0.838

5.5.1. Relationships between pathogen groups

The pathogen groups monitored for the study can be divided along taxonomic grounds into standard bacteria, spore-forming bacteria, protozoa and viruses. It is of interest to determine if each group responds in a similar way or if biological differences between members within the groups are more important than differences between the groups. A cluster analysis was undertaken on the pathogen groups to reveal the existence of such associations.

The method used was unweighted pair-group averaging (for determining cluster amalgamation thresholds) on a similarity matrix computed using the City-block (Manhattan) distance measure. Both techniques are described in Hill and Lewicki (2006). The largest distinction was between the non-spore forming bacteria (enterococci, coliforms and *E. coli*) and protozoa and bacteria with encysted or spore-forming life history stages (Figure 5-5). Enterococci are Gram-positive facultative anaerobic organisms while coliform is a name used for members of the Enterobacteriaceae family. The coliform group includes *E. coli* and all members are Gram-negative. Both the coliforms and enterococci are non-spore forming organisms (Brock et al, 1984).

Clostridium perfringens is a Gram-positive, anaerobic, spore-forming bacterium while Sulphite Reducing Clostridia is a group that also includes *Clostridium perfringens* as well as other clostridia. These spore-forming bacterial groups have been suggested as surrogates for *Cryptosporidium*. Based on the cluster analysis, they appear to share some similarities in response to environmental conditions within the activated sludge process. Two measures of *Cryptosporidium* oocysts have been included within the data set; "confirmed" and "presumptive". The "confirmed" data records oocysts in which internal contents are apparent while the "presumptive" data records the total number of oocysts observed. Both measures have been included since the laboratory analysts have noted that freshly sourced oocysts sometimes are not permeable enough to uptake the DAPPI stain to demonstrate the internal contents. Some variation in the *Cryptosporidium* measures was observed and in general it appears from the statistical analyses that the presumptive count provides a more reliable measure for *Cryptosporidium* for this data set. *Giardia* cysts were measured using only the "confirmed" approach as the stain permeability issue was not apparent for this group. In general, the *Giardia* data set was more problematic with high coefficients of variation within cells and several missing values. Nevertheless, the cluster analysis groups *Giardia* with the other cyst or spore-forming groups. Bacteriophage viruses (an indicator virus) also clustered with the cyst and spore-forming groups.

A reasonable conclusion from the cluster analysis results is that the virus indicator, spore and cyst forming groups respond differently (in fact have generally lower LRVs, see Figure 5-2) than non-spore forming bacterial groups. This observation is consistent with the view that the non-spore forming groups are less resistant to environmental extremes and less long-lived than the other groups.

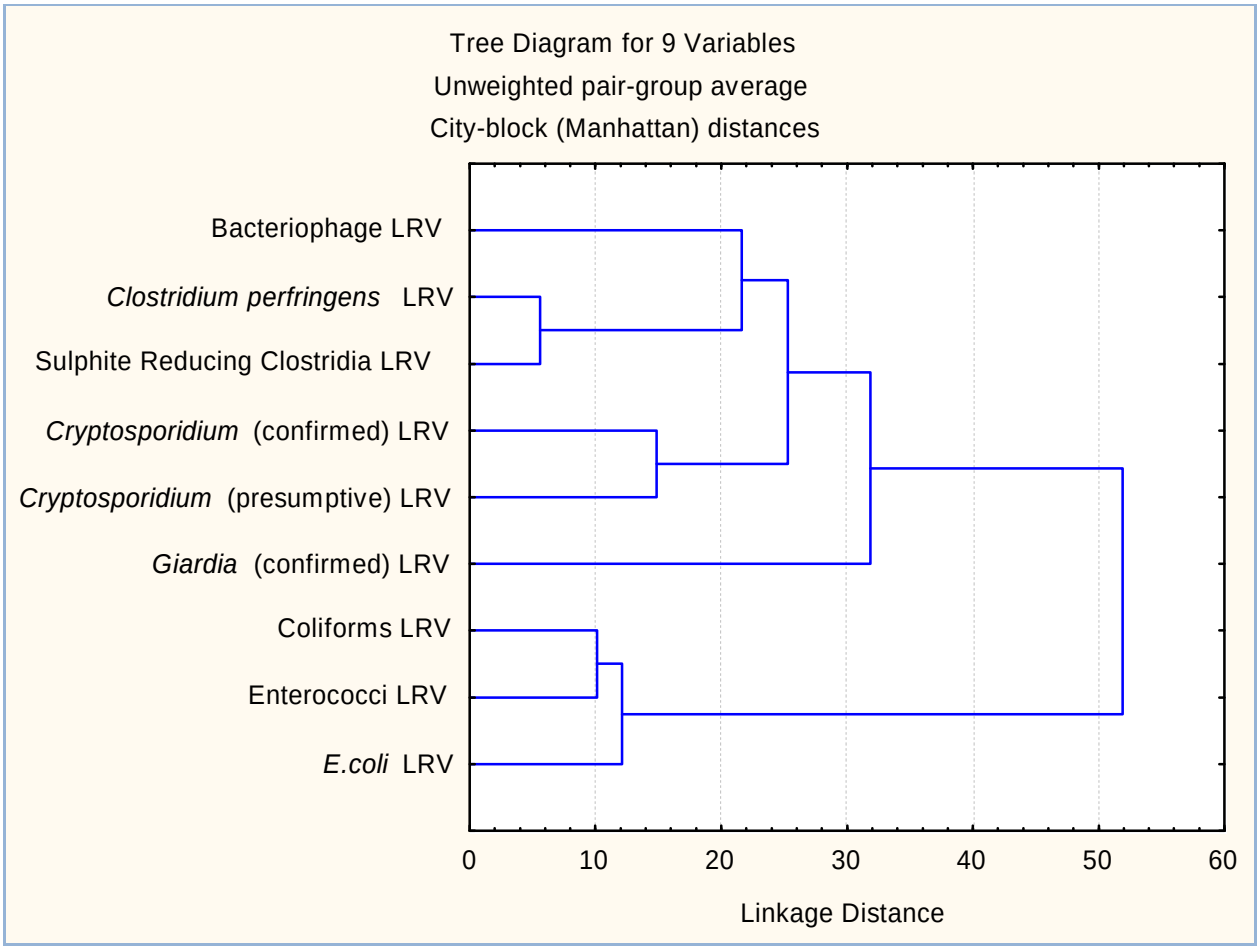


Figure 5-5. Cluster analysis of pathogen groups

5.6. Predictive model development

5.6.1. Principal Components Regression

Multiple regression analyses were conducted using the 5 PCA factor scores as IVs and pathogen LRVs as the DVs. Separate analyses were undertaken for each pathogen group and the results are given in Table 5-8 The Multiple R^2 values indicate the proportion of variance explained by the model while the ANOVA table shows details of the F test of the hypothesis that the multiple regression is zero (i.e. that all correlations between DVs and IVs and all regression coefficients are zero). The results in Table 5-8 clearly show that the hypothesis of zero multiple regression is rejected for all pathogen groups and furthermore that:

- The models for bacteriophage (91%), coliforms (96%), *Cryptosporidium* (presumptive) (92.6%), *E. coli* (94.3%) and enterococci (92.9%) all explain over 90% of the variance in the modelled parameter. This is generally an excellent result and implies that a model built on a larger data set could be very reliable.
- The model for *Cryptosporidium* (confirmed) explains 87.4% of the variance in this parameter which provides some support to the view that this parameter is a little less reliable than *Cryptosporidium* (presumptive).
- The models for *Clostridium perfringens* and Sulphite Reducing Clostridia were less successful, explaining 65.6% and 57.5% of the variance respectively and indicating that other, unmeasured parameters were important in determining the LRVs of these groups.

Table 5-8 Principal Components Regression model summaries

Pathogen LRV	Multiple R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
Bacteriophage	0.910	9.732	5	1.946	0.968	44.000	0.022	88.475	0.000
Clostridium perfringens	0.656	4.198	5	0.840	2.204	44.000	0.050	16.766	0.000
Coliforms	0.960	55.672	5	11.134	2.329	44.000	0.053	210.383	0.000
Cryptosporidium (confirmed)	0.874	19.383	5	3.877	2.803	44.000	0.064	60.861	0.000
Cryptosporidium (presumptive)	0.926	20.767	5	4.153	1.656	44.000	0.038	110.369	0.000
E. coli	0.943	47.043	5	9.409	2.833	44.000	0.064	146.122	0.000
Enterococci	0.929	44.118	5	8.824	3.390	44.000	0.077	114.510	0.000
Giardia (confirmed)	0.780	5.577	5	1.115	1.577	44.000	0.036	31.117	0.000
Sulphite Reducing Clostridia	0.575	2.085	5	0.417	1.539	44.000	0.035	11.919	0.000

Table 5-9 presents the regression coefficients and related statistics for each pathogen LRV. To help visualise the pattern of response, a summary of the regression signs (i.e. positive or negative) is presented in Table 5-10. The following observations can be drawn from the analysis (note for consistency, only the *Cryptosporidium* (presumptive) variable is discussed):

- For all pathogens, the amount of particulate organic matter (POM) present was negatively correlated with the pathogen LRV.
- High scores for the NO_x factor occurred for Coliforms, *E. coli,* enterococci, bacteriophage and *Cryptosporidium*; i.e. LRVs for these groups were greater when NO_3 was higher. *Giardia* had an opposite response.
- High alkalinities and BOD were associated with lower LRVs for Coliforms, *E. coli*, enterococci, and *Cryptosporidium.* In contrast Bacteriophage, *Giardia* and Sulphite Reducing Clostridia had higher LRVs in response to increases in these variables.
- The longer the trial duration, the lower the LRV for Coliforms, *E. coli*, Enterococci, and *Cryptosporidium* but otherwise, time did not affect pathogen LRVs.
- Higher temperatures, ammonia and shorter sludge ages increased LRVs for Coliforms, *E. coli*, Enterococci and *Giardia* but decreased them for the remaining groups. It seems likely that temperature was the dominant effect here and that the shorter sludge age finding is an anomaly due to the lack of trials with (i) high temperature and long sludge age and (ii) low temperature and short sludge age.

Table 5-9. Regression coefficients for each pathogen LRV

The t-test tests the significance of the hypothesis that the slope of the relationship between each factor and the respective pathogen LRV is zero. Standardized (Beta) and non-standardized (B) regression coefficients (weights), their standard error and statistical significance are shown. Significant relationships ($p \leq 0.05$) are shown in red font.

Factor	B-coefficient	Std.Err	t	p	Beta (ß)	St.Err.ß
Bacteriophage						
Intercept	1.406	0.021	67.038	0.000	-	-
POM	-0.293	0.021	-13.849	0.000	-0.628	0.045
NOx	0.184	0.021	8.699	0.000	0.394	0.045
ALKBOD	0.190	0.021	8.953	0.000	0.406	0.045
TIME	-0.040	0.021	-1.899	0.064	-0.086	0.045
TNH3_SL	0.202	0.021	9.546	0.000	0.433	0.045
Clostridium perfringens						
Intercept	0.959	0.032	30.312	0.000	-	-
POM	-0.276	0.032	-8.640	0.000	-0.764	0.088
NOx	-0.048	0.032	-1.506	0.139	-0.133	0.088
ALKBOD	0.042	0.032	1.319	0.194	0.117	0.088
TIME	0.028	0.032	0.877	0.385	0.078	0.088
TNH3_SL	0.067	0.032	2.099	0.042	0.186	0.088
Coliforms						
Intercept	2.076	0.033	63.801	0.000	-	-
POM	-0.710	0.033	-21.597	0.000	-0.652	0.030
NOx	0.673	0.033	20.482	0.000	0.619	0.030
ALKBOD	-0.202	0.033	-6.161	0.000	-0.186	0.030
TIME	-0.163	0.033	-4.947	0.000	-0.149	0.030
TNH3_SL	-0.334	0.033	-10.175	0.000	-0.307	0.030
Cryptosporidium (confirmed)						
Intercept	1.082	0.036	30.309	0.000	-	-
POM	-0.339	0.036	-9.409	0.000	-0.504	0.054
NOx	0.482	0.036	13.379	0.000	0.717	0.054
ALKBOD	-0.212	0.036	-5.876	0.000	-0.315	0.054
TIME	-0.052	0.036	-1.452	0.154	-0.078	0.054
TNH3_SL	0.013	0.036	0.374	0.710	0.020	0.054
Cryptosporidium (presumptive)						
Intercept	1.299	0.027	47.337	0.000	-	-
POM	-0.338	0.028	-12.185	0.000	-0.499	0.041
NOx	0.542	0.028	19.559	0.000	0.801	0.041
ALKBOD	0.023	0.028	0.847	0.402	0.035	0.041
TIME	-0.059	0.028	-2.121	0.040	-0.087	0.041
TNH3_SL	0.109	0.028	3.950	0.000	0.162	0.041
E. coli						
Intercept	2.297	0.036	64.014	0.000	-	-
POM	-0.694	0.036	-19.158	0.000	-0.688	0.036
NOx	0.576	0.036	15.879	0.000	0.571	0.036

Factor	B-coefficient	Std.Err	t	p	Beta (ß)	St.Err.ß
ALKBOD	-0.093	0.036	-2.573	0.014	-0.092	0.036
TIME	-0.214	0.036	-5.896	0.000	-0.212	0.036
TNH3_SL	-0.303	0.036	-8.370	0.000	-0.301	0.036
Enterococci						
Intercept	2.070	0.039	52.725	0.000	-	-
POM	-0.699	0.040	-17.638	0.000	-0.710	0.040
NOx	0.537	0.040	13.536	0.000	0.545	0.040
ALKBOD	-0.082	0.040	-2.062	0.045	-0.083	0.040
TIME	-0.134	0.040	-3.377	0.002	-0.136	0.040
TNH3_SL	-0.314	0.040	-7.911	0.000	-0.319	0.040
Giardia (confirmed)						
Intercept	1.420	0.027	53.019	0.000	-	-
POM	-0.137	0.027	-5.048	0.000	-0.357	0.071
NOx	-0.279	0.027	-10.301	0.000	-0.729	0.071
ALKBOD	0.082	0.027	3.035	0.004	0.215	0.071
TIME	-0.010	0.027	-0.369	0.714	-0.026	0.071
TNH3_SL	-0.104	0.027	-3.827	0.000	-0.271	0.071
Sulphite Reducing Clostridia						
Intercept	1.004	0.026	37.939	0.000	-	-
POM	-0.195	0.027	-7.289	0.000	-0.716	0.098
NOx	-0.034	0.027	-1.260	0.214	-0.124	0.098
ALKBOD	0.019	0.027	0.701	0.487	0.069	0.098
TIME	0.054	0.027	2.011	0.050	0.198	0.098
TNH3_SL	0.015	0.027	0.578	0.566	0.057	0.098

Table 5-10. Pattern of correlations of regression coefficients with dependent variables
Key: + positive, - negative, ++ strong positive, -- strong negative

	Coliforms LRV	***E. coli* LRV**	**Enterococci LRV**	**Bacteriophage LRV**	***Cryptosporidium* (presumptive) LRV**	***Giardia* (confirmed) LRV**	***Cryptosporidium* (confirmed) LRV**	**Sulphite Reducing Clostridia LRV**	***Clostridium perfringens* LRV**
Intercept	++	++	++	++	++	++	++	++	++
POM	--	--	--	--	--	-	-	-	-
NOx	++	++	++	+	++	--	++		
ALKBOD	-	-	-	+		+	-	+	
TIME	-	-	-		-				
TNH3_SL	--	-	-	+	+	-		+	+

Predictive models for each pathogen or indicator are shown in Table 5-11 along with an assessment of overall model performance. In general, model performance was good although it should be noted that no attempt was made to validate the models by partitioning and quarantining a subset of the data (say 20%) to use as a testing data set. Given the limited size of the current data set, loss of 20% of the cases from the training data set would have reduced our confidence in the predictive capacity of the models. It was felt that for the Principal Components Regression approach, it was better to strengthen the data set by conducting more trials at different temperatures and sludge ages before continuing with a validation exercise. Nevertheless, the results from the training data set look promising and it is likely that accurate predictive models for pathogen LRVs can be developed using the Principal Components Regression approach.

Table 5-11. Predictive model formulas for Principal Components Regression, summary variance explained by multiple regression model and assessment of model performance
Note: the assessment is based on the training data only and the models are not validated due to insufficient data at this stage.

Pathogen/indicator	**Predictive model**	**Multiple R^2**	**Assessment of model performance**
Bacteriophage	"Bacteriophage LRV"=10.406-0.2930*"POM"+0.1843*"NOx"+0.1897*"ALKBOD" -0.0400*"TIME"+0.2023*"TNH3_SL"	**91.0%**	Very Good to Excellent
Clostridium perfringens	"*Clostridium perfringens* LRV"=0.9593-0.276*"POM"-0.048*"NOx"+0.0422*"ALKBOD"+ 0.0280*"TIME"+0.0671*"TNH3_SL"	65.6%	Moderate
Coliforms	"Coliforms LRV"=20.0760-0.7100*"POM"+0.6731*"NOx"-0.2020*"ALKBOD"-0.1630*"TIME"-0.3340*"TNH3_SL"	**96.0%**	Excellent
Cryptosporidium (confirmed)	"*Cryptosporidium* (confirmed) LRV"=10.0818-0.3392*"POM"+0.4824*"NOx"-0.2119*"ALKBOD"-0.0524*"TIME"+0.0135*"TNH3_SL"	**87.4%**	Very Good
Cryptosporidium (presumptive)	"*Cryptosporidium* (presumptive) LRV"=10.2990-0.3380*"POM"+0.5420*"NOx"+0.0235*"ALKBOD"-0.059*"TIME"+0.1095*"TNH3_SL"	**92.6%**	Very Good to Excellent
E. coli	"*E. coli* LRV"=20.298-0.6944*"POM"+0.5756*"NOx"-0.0933*"ALKBOD"-0.2137*"TIME"-0.3034*"TNH3_SL"	**94.3%**	Excellent
Enterococci	"Enterococci LRV"=20.0700-0.6990*"POM"+0.5368*"NOx"-0.0820*"ALKBOD"-0.1340*"TIME"-0.3140*"TNH3_SL"	**92.9%**	Very Good to Excellent
Giardia (confirmed)	"*Giardia* (confirmed) LRV"=10.4200-0.1370*"POM"-0.2790*"NOx"+0.0821*"ALKBOD"-0.0100*"TIME"-0.1040*"TNH3_SL"	78.0%	Good
Sulphite Reducing Clostridia	"Sulphite Reducing Clostridia LRV"=10.004-0.1950*"POM"-0.0340*"NOx"+0.0187*"ALKBOD"+0.0537*"TIME"+0.0154*"TNH3_SL"	57.5%	Poor to Moderate

Key:

Factor 1	SS, Turbidity, TKN, COD	*Concentration of Particulate Organic Matter (POM)*	*POM*	Higher concentrations of fine organic particles.
Factor 2	NO2-N, NO3-N	*Oxidation state of nitrogen*	*NOx*	This axis measures the oxidation state of ionic nitrogen (NO_3 = high, NO_2 = low) in solution.
Factor 3	Alkalinity, BOD5	*Alkalinity and BOD*	*ALKBOD*	The relationship between alkalinity and BOD is likely due to the consumption of alkalinity that occurs to a limiting factor as microbes metabolise BOD.
Factor 4	Hours	*Time*	*TIME*	Time seems quite independent of other factors.
Factor 5	SS-ML (MLSS), Temperature, NH3-N	*Temperature, Sludge age and ammonia - probably confounded here*	*TNH3_SL*	Ammonia is linked to temperature across an optimal range as expected. Note the large increase in trial 5 with high temperatures. The correlation with sludge age is also expected given the optimal growth rate required for successful nitrification.

5.7. Neural Network Model

In addition to the Principal Components Regression approach described above, an alternative method based on a Generalized Regression Neural Network (GRNN), was undertaken. Neural networks or 'nets' derive their name from their computational structure which is similar to a brain. Neural nets provide an alternative to more traditional statistical methods and have the advantage that they are capable of modelling extremely complex functions (Palisade Corp. 2009). The process of training the nets on a data set fine tunes the net so that the net outputs (or predicts) approximately correct values for given inputs. While there are many kinds of neural network algorithms, GRNN is used in this study because it is designed for the prediction of continuous numeric variables (i.e. variables that can any have value within a select range, in contrast to categorical variables which are limited to discrete values). A key feature of GRNNs is that the closer a known case is to the unknown one, the more weight it is given when estimating the unknown dependent value. Every training case is represented in the net. When presented with a case, the net computes the predicted dependent value using the dependent values of every training case, with closer training cases contributing more significantly to the value of the output (Palisade Corp. 2009).

5.7.1. GRNN results

For the current project, a separate net was developed for each dependent variable but the independent variables were the same for each net. Unlike the situation with multiple regression, neural nets make no assumptions about the nature of the relationships between variables. Consequently, there is no need to deal with the issues of multicollinearity or heterogeneity of slopes which posed challenges for the earlier analyses described in Sections 5.4 to 5.6 above. GRNN results for each pathogen/indicator are described in Table 5-12. Each net involved 50 input cases of which 40 were used as a training data set and 10 were quarantined for subsequent testing.

Overall the nets performed well with the training data set with the exception of *Cryptosporidium* (confirmed) and Giardia (confirmed). With the exception of the clostridia and *Cryptosporidium* (confirmed) the testing data was predicted with 100% accuracy. As was the case with the Principal Components Regression above, the *Cryptosporidium* (confirmed) variable tended to have higher error rates than the presumptive version, consequently, it is not discussed further here. The clostridia groups (*C. perfringens* and Sulphite Reducing Clostridia) were predicted with less accuracy (20% bad predictions). The reasons for this are not clear but are likely to be due to intrinsic biological attributes of the clostridia which are not influenced by the measured IVs to such an extent as the other pathogen/indicator groups.

Table 5-12. Generalized Regression Neural Network (GRNN) performance statistics for the pathogen/indicator data set

Statistics explanation: % Bad Predictions; a prediction counts as "bad" if it falls outside a defined margin around the actual value (here arbitrarily set at 30%). Root Mean Square Error is a measure of deviation of predictions from actual value (calculated as square root of average square deviation). The Mean Absolute Error is the average deviation of predictions from actual values. Higher error statistics are shown in red font.

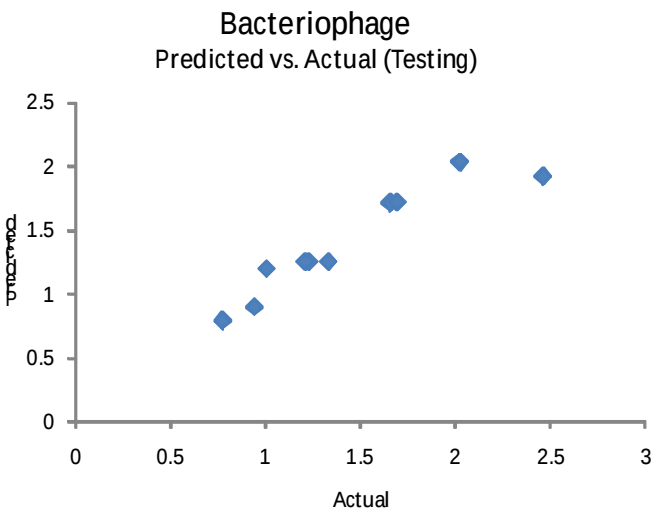
	Dependent Variables (Pathogen/indicator) LRVs								
Item	**Bacteriophage**	***Clostridium perfringens***	**Coliforms**	***Cryptosporidium* (confirmed)**	***Cryptosporidium* (presumptive)**	***E. coli***	**Enterococci**	***Giardia* (confirmed)**	**Sulphite Reducing Clostridia**
Independent Variables (numeric)	Hours, SS-ML (MLSS), SS, pH, Temperature, Turbidity, Alkalinity, NH3-N, NO2-N, NO3-N, TKN, COD, BOD5								
Training									
Number of Cases	40	40	40	40	40	40	40	40	40
Number of Trials	53	69	61	104	82	68	59	92	170
% Bad Predictions (30% Tolerance)	0.0%	5.0%	0.0%	15.0%	5.0%	0.0%	2.5%	7.5%	0.0%
Root Mean Square Error	0.024	0.067	0.064	0.130	0.100	0.061	0.072	0.177	0.035
Mean Absolute Error	0.016	0.043	0.033	0.099	0.078	0.042	0.052	0.128	0.023
Std. Deviation of Abs. Error	0.018	0.052	0.055	0.084	0.061	0.044	0.049	0.122	0.026
Testing									
Number of Cases	10	10	10	10	10	10	10	10	10
% Bad Predictions (30% Tolerance)	0.0%	20.0%	10.0%	20.0%	0.0%	0.0%	0.0%	0.0%	20.0%
Root Mean Square Error	0.187	0.159	0.188	0.245	0.178	0.103	0.179	0.124	0.130
Mean Absolute Error	0.103	0.131	0.137	0.185	0.151	0.080	0.140	0.094	0.085
Std. Deviation of Abs. Error	0.156	0.090	0.129	0.161	0.094	0.065	0.112	0.081	0.098

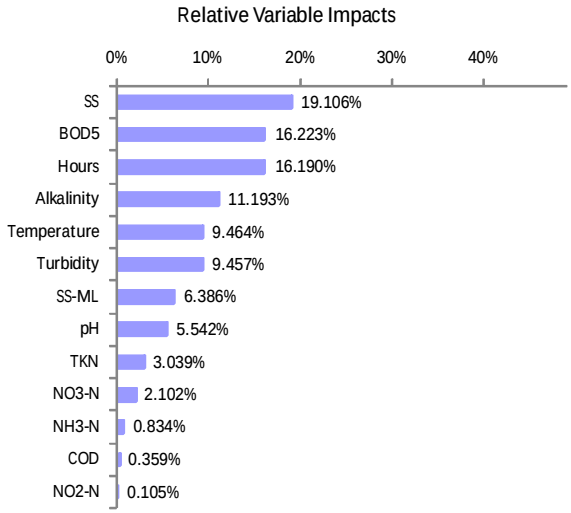
The Palisade Neural Net software produces some comparative statistics to contrast the performance of the neural net to a multiple regression. As described above, the IV data set is multicollinear, so that the multiple regression R-square statistics are inflated compared to those of the Principal Components Regression data set. Nevertheless, the comparison is informative as it shows the superior performance of the neural net (Table 5-13). Although testing was not performed on the Principal Components Regression data set, it was clear from the general improvements in the root mean square error statistics for the training and testing data sets that the Neural Network approach had superior predictive powers. Furthermore, it can be performed without the need to conduct Principal Components Analysis on the IVs.

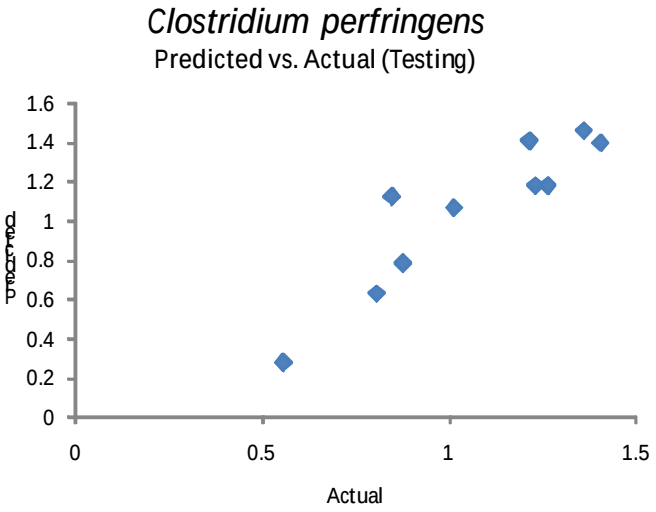
Table 5-13. Comparison of GRNN predictive performance to multiple regression

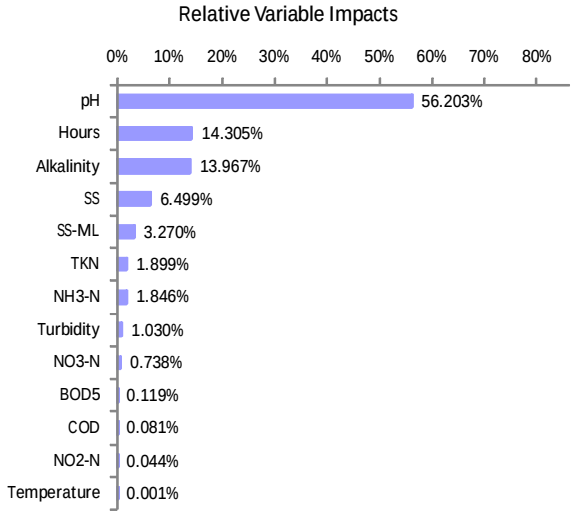
DV and statistics	Linear Predictor (multiple regression)	Neural Net	% improvement of Neural Net Error over Linear predictor
Bacteriophage			
R-Square (Training)	0.974	--	--
Root Mean Sq. Error (Training)	0.074	0.024	67%
Root Mean Sq. Error (Testing)	0.191	0.187	3%
Clostridium perfringens			
R-Square (Training)	0.848	--	--
Root Mean Sq. Error (Training)	0.145	0.067	54%
Root Mean Sq. Error (Testing)	0.214	0.159	26%
Coliforms			
R-Square (Training)	0.984	--	--
Root Mean Sq. Error (Training)	0.136	0.064	53%
Root Mean Sq. Error (Testing)	0.222	0.188	15%
***Cryptosporidium* (confirmed)**			
R-Square (Training)	0.927	--	--
Root Mean Sq. Error (Training)	0.174	0.130	25%
Root Mean Sq. Error (Testing)	0.255	0.245	4%
***Cryptosporidium* (presumptive)**			
R-Square (Training)	0.960	--	--
Root Mean Sq. Error (Training)	0.130	0.100	23%
Root Mean Sq. Error (Testing)	0.236	0.178	25%
E. coli			
R-Square (Training)	0.978	--	--
Root Mean Sq. Error (Training)	0.144	0.061	58%
Root Mean Sq. Error (Testing)	0.242	0.103	57%
Enterococci			
R-Square (Training)	0.967	--	--
Root Mean Sq. Error (Training)	0.175	0.072	59%
Root Mean Sq. Error (Testing)	0.272	0.179	34%
***Giardia* (confirmed)**			
R-Square (Training)	0.838	--	--
Root Mean Sq. Error (Training)	0.158	0.177	-11%
Root Mean Sq. Error (Testing)	0.248	0.124	50%
Sulphite Reducing Clostridia			
R-Square (Training)	0.785	--	
Root Mean Sq. Error (Training)	0.129	0.035	73%
Root Mean Sq. Error (Testing)	0.192	0.130	32%

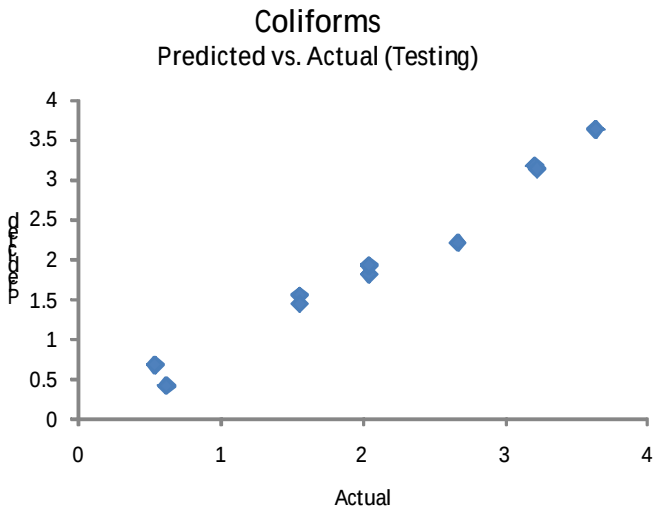
Plots of predicted versus actual LRVs for each net are shown in Figure 5-6 along with a "Relative Variable Impact" value for each IV. These are percent values and add up to 100%. The lower the percent value for a given variable, the less that variable affects the predictions (Palisade Corp. 2009). Despite the limited data set, the plots show generally excellent predictive powers (with the aforementioned exceptions of the clostridia bacteria and *Cryptosporidium* (confirmed)). The identity of the IVs making the greatest contribution to model performance varied radically between models. With neural nets, it is important to recognise that the results are particular to each individual net. While similar nets are likely to “learn” to disregard similar variables; different nets are also likely to "discover" how the variable can make a significant contribution to accurate “predictions” (Palisade Corp. 2009). With data sets that have fewer cases and/or greater numbers of variables, the differences in the relative impact variables between trained nets are likely to be more marked. It is also important to note that variables with low relative variable impact percentages may nevertheless contribute significantly to predictive accuracy. For the current data set, when IVs that contributed less than 5 % to the relative variable impact statistic were removed and the analysis rerun, model performance deteriorated greatly. It is likely that augmenting the training data set with a greater number of cases across a broader range of IV values would improve model consistency with respect to the more important IVs and may assist in identifying key subsets of IVs that could be monitored at less expense.

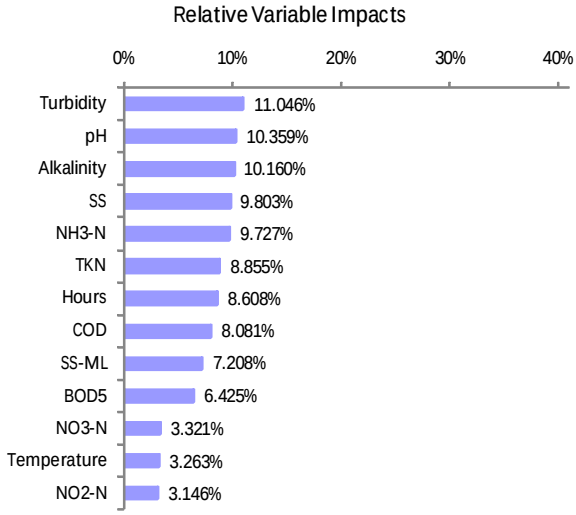


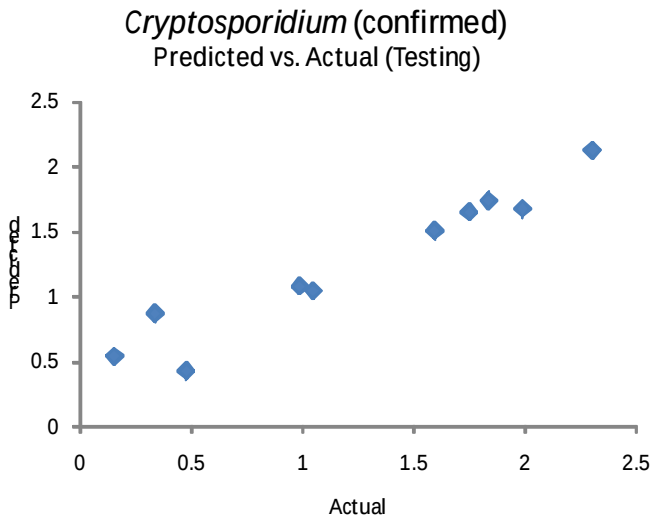


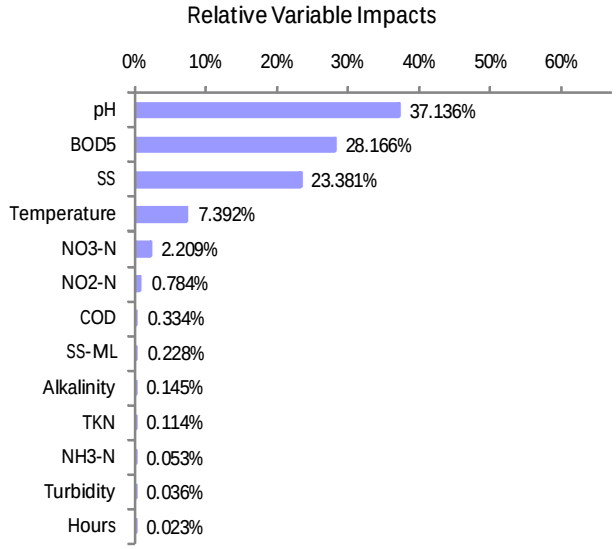


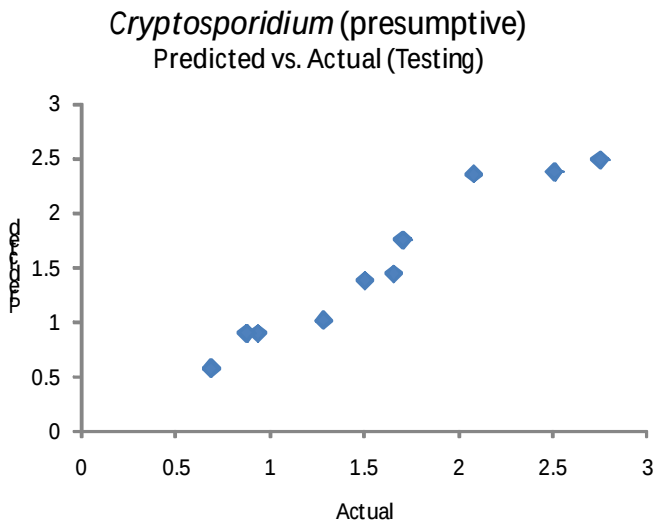


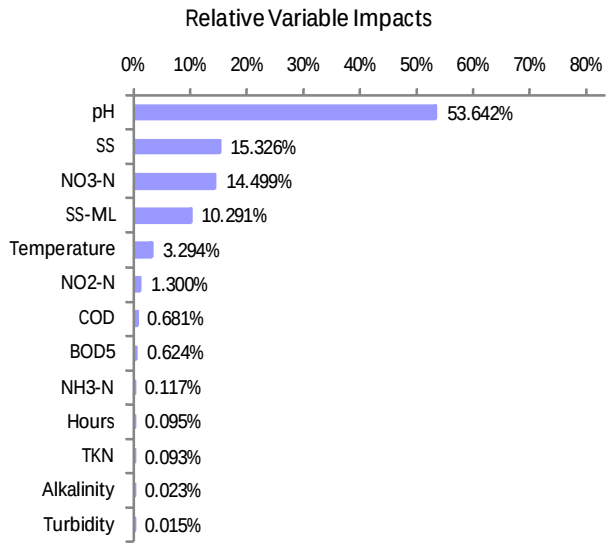

Coliforms
Predicted vs. Actual (Testing)
Predicted
Actual
0
0.5
1
1.5
2
2.5
3
3.5
4


Relative Variable Impacts
0%
10%
20%
30%
40%
Turbidity 11.046%
pH 10.359%
Alkalinity 10.160%
SS 9.803%
NH3-N 9.727%
TKN 8.855%
Hours 8.608%
COD 8.081%
SS-ML 7.208%
BOD5 6.425%
NO3-N 3.321%
Temperature 3.263%
NO2-N 3.146%


Cryptosporidium (confirmed)
Predicted vs. Actual (Testing)
Predicted
Actual
0
0.5
1
1.5
2
2.5


Relative Variable Impacts
0%
10%
20%
30%
40%
50%
60%
pH 37.136%
BOD5 28.166%
SS 23.381%
Temperature 7.392%
NO3-N 2.209%
NO2-N 0.784%
COD 0.334%
SS-ML 0.228%
Alkalinity 0.145%
TKN 0.114%
NH3-N 0.053%
Turbidity 0.036%
Hours 0.023%


Cryptosporidium (presumptive)
Predicted vs. Actual (Testing)
Predicted
Actual
0
0.5
1
1.5
2
2.5
3


Relative Variable Impacts
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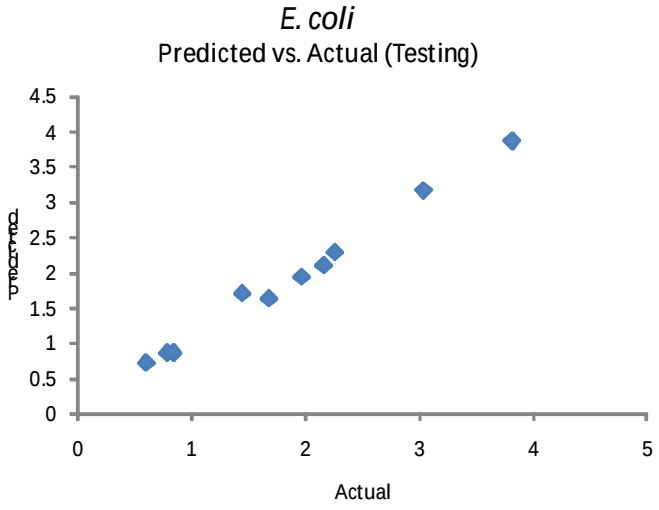
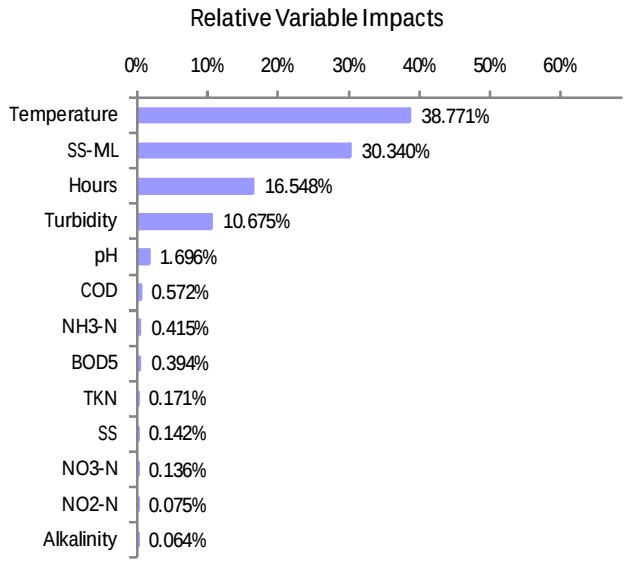
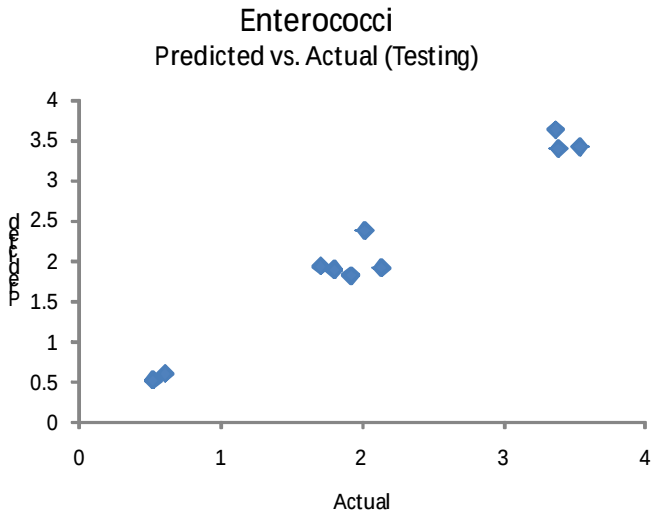
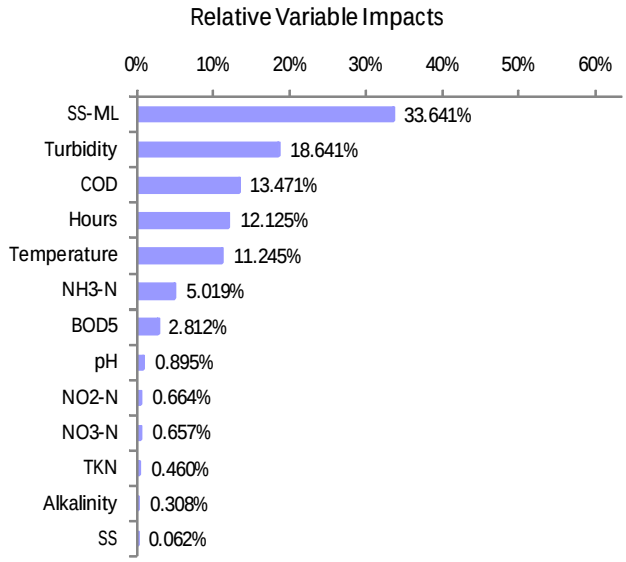
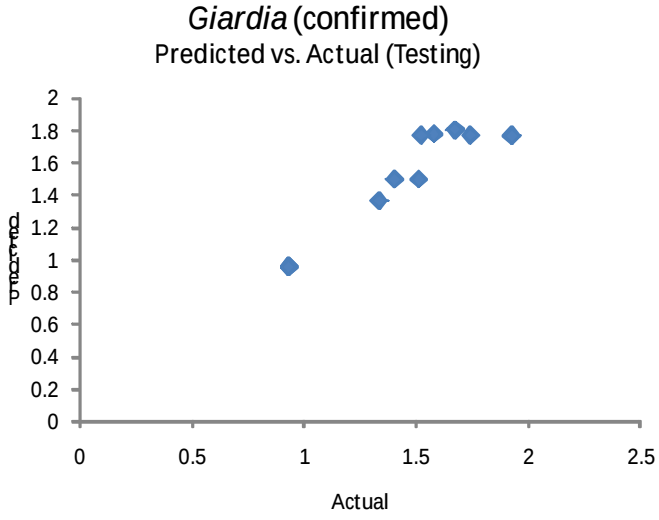
20%
30%
40%
50%
60%
70%
80%
pH 53.642%
SS 15.326%
NO3-N 14.499%
SS-ML 10.291%
Temperature 3.294%
NO2-N 1.300%
COD 0.681%
BOD5 0.624%
NH3-N 0.117%
Hours 0.095%
TKN 0.093%
Alkalinity 0.023%
Turbidity 0.015%

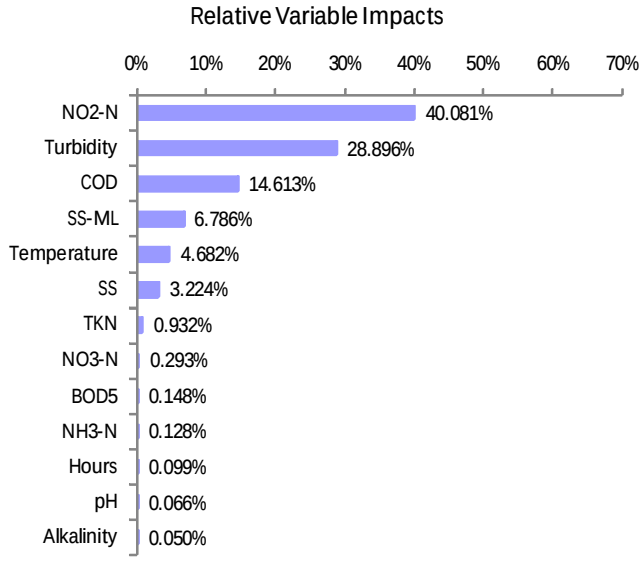

E. coli
Predicted vs. Actual (Testing)
Actual


Relative Variable Impacts
0%
10%
20%
30%
40%
50%
60%
Temperature 38.771%
SS-ML 30.340%
Hours 16.548%
Turbidity 10.675%
pH 1.696%
COD 0.572%
NH3-N 0.415%
BOD5 0.394%
TKN 0.171%
SS 0.142%
NO3-N 0.136%
NO2-N 0.075%
Alkalinity 0.064%


Enterococci
Predicted vs. Actual (Testing)
Actual


Relative Variable Impacts
0%
10%
20%
30%
40%
50%
60%
SS-ML 33.641%
Turbidity 18.641%
COD 13.471%
Hours 12.125%
Temperature 11.245%
NH3-N 5.019%
BOD5 2.812%
pH 0.895%
NO2-N 0.664%
NO3-N 0.657%
TKN 0.460%
Alkalinity 0.308%
SS 0.062%


Giardia (confirmed)
Predicted vs. Actual (Testing)
Actual


Relative Variable Impacts
0%
10%
20%
30%
40%
50%
60%
70%
NO2-N 40.081%
Turbidity 28.896%
COD 14.613%
SS-ML 6.786%
Temperature 4.682%
SS 3.224%
TKN 0.932%
NO3-N 0.293%
BOD5 0.148%
NH3-N 0.128%
Hours 0.099%
pH 0.066%
Alkalinity 0.050%

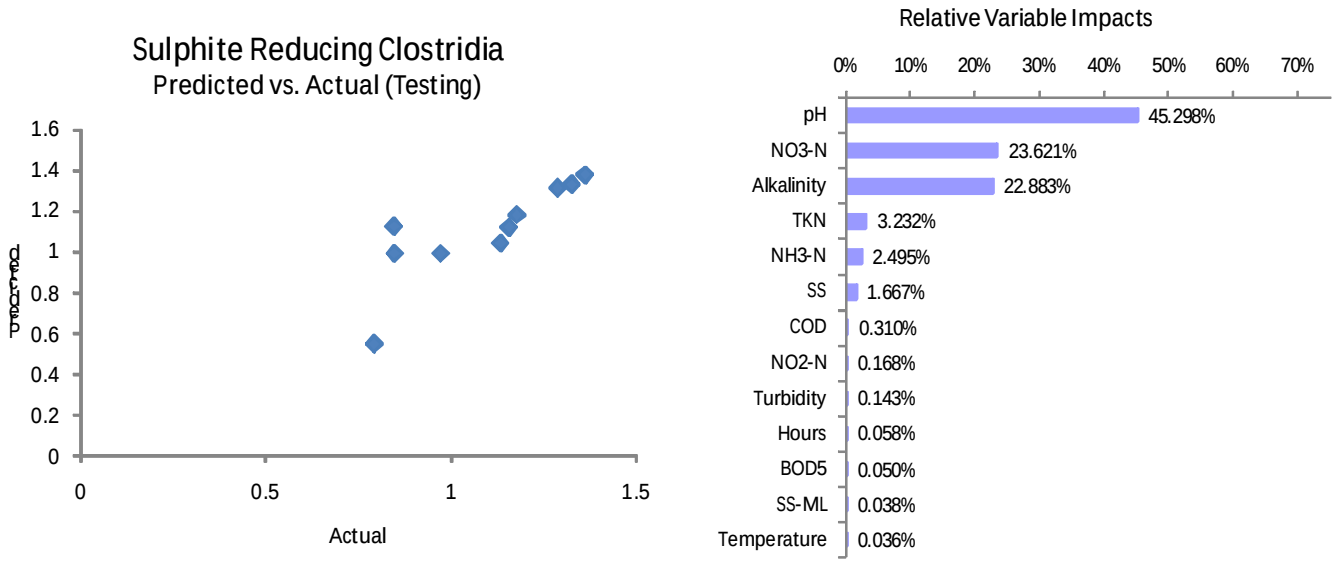


Figure 5-6. Neural net model performance (predicted versus actual LRV on test data set) and relative contribution of IVs to the model

5.8. Predictive model outcomes

While predictive model performance was generally good, meeting the assumptions required for multiple regression required some complex preliminary analyses (i.e. Principal Components Analysis) and the results indicated that more data is necessary to improve model performance and provide sufficient data for a testing data set.

Overall, the Principal Components Regression approach showed promise as a predictive tool but the superior performance of the neural network approach, the lack of any need to conduct complex (and to some, opaque) preliminary analyses and data transformations and the ease of its application recommend the use of neural nets as the better predictive tool. Using Palisade's Neural Tools software (Palisade Corp. 2009), live predictions can be made within a Microsoft Excel spreadsheet. Such software makes it easy to envisage a situation in which the results of online measurements of independent physico-chemical variables are deposited automatically into a spreadsheet and the spreadsheet plotting and predictive tools automatically plot a continuous LRV statistic for each pathogen and indicator.

While extremely encouraging, the use of Generalised Regression Neural Nets, Principal Components Regression, (both conducted here) or other statistical methods such as Bayesian networks or other linear approaches such as multilevel linear modelling requires a larger data set with significantly more cases measured across a wider range of values of the independent variables. In addition, future trials should include additional sampling of influent quality over the duration of the trials. This should provide a higher quality data set from which more accurate correction factors to account for pathogen die-off in the effluent storage can be calculated.

6. CONCLUSIONS

Tables 6.1 summarises the pathogen and indicator LRVs noted for the various stages of the project. These could be considered as conservative but scientifically justified LRV to apply for the ASP for the given pathogen groups.

Table 6-1. Summary of observed LRVs for pathogens and indicators

Study	Log_{10} reduction value		
	Protozoa	**Viruses and viral surrogates**	**Bacteria**
International literature review	0.5 – 1.5 *Cryptosporidium* 1 – 1.5 *Giardia*	1 - 2	1 - 2
Australian literature review	1 *Cryptosporidium* 1.9 *Giardia*	1	1.8
Data review survey	1 *Cryptosporidium* 1.5 *Giardia*	1.5 – 2.9	2.8
Detailed data review	2.2	2.8	2.5
Experimental pilot plant	0.5 – 2 *Cryptosporidium* 1.2 – 2.3 *Giardia*	1 – 2	1 – 3

Overall, the Principal Components Regression approach showed promise as a predictive tool; however, the superior performance of the neural network approach, the lack of any need to conduct complex (and to some, opaque) preliminary analyses and data transformations and the ease of its application highlighted the use of neural nets as the better predictive tool. Using Palisade's Neural Tools software (Palisade Corporation 2009), live predictions can be made within a Microsoft Excel spreadsheet. Such software makes it easy to envisage a situation in which the results of online measurements of independent physico-chemical variables are deposited automatically into a spreadsheet and the spreadsheet plotting and predictive tools automatically plot a continuous LRV statistic for each pathogen and indicator.

While extremely encouraging, the use of Generalised Regression Neural Nets, Principal Components Regression, (both conducted here) or other statistical methods such as Bayesian networks or other linear approaches such as multilevel linear modelling requires a larger data set with significantly more cases measured across a wider range of values of the independent variables. In particular, the data set should be augmented with trials incorporating (i) high temperature and 20 day sludge age and (ii) low temperature and 10 day sludge age. Consideration should also be given to conducting the following trials:

- 15 day sludge age trials at high/er temperatures;
- 10, 15 and 20 day sludge age trials at temperatures intermediate between those of trials 1 to 4 and trial 5; and
- Maximum sludge age experienced by the ETP which may be higher than the maximum sludge age trialled here.

In addition, future trials should include additional sampling of influent quality over the duration of the trials. This should provide a higher quality data set from which more accurate correction factors to account for pathogen die-off in the effluent storage can be calculated. The predictive model approach may offer the best 'surrogate' for attributing LRV as they can be modelled to online operating data and perform as critical control points in recycled water quality management plans.

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Appendix A – Data Tables from Pilot Plant Experiments

Table A1.1a: Statistical summary of microbiological data for 15 days sludge age replicate 1 (of 3)

Microbiological parameter, time (hours) and source	Actual value summary		Log_{10} reduction value (LRV) summary					
	Mean	Standard deviation	Mean	Standard deviation	95th %ile	50th %ile	Min	Max
Bacteriophage								
0 (influent)	73333	12097						
12 (effluent)	13250	1626	0.74	0.05	0.78	0.74	0.71	0.78
16 (effluent)	12900	1556	0.75	0.05	0.79	0.76	0.72	0.79
20 (effluent)	11950	354	0.79	0.01	0.80	0.79	0.78	0.80
24 (effluent)	12450	1061	0.77	0.04	0.79	0.77	0.74	0.80
36 (effluent)	11800	n/a	0.79	n/a	n/a	n/a	n/a	n/a
48 (effluent)	9700	n/a	0.88	n/a	n/a	n/a	n/a	n/a
48 (influent)	61500	6364	0.08	0.05	0.11	0.08	0.05	0.11
Clostridium Presumptive								
0 (influent)	126667	28868						
12 (effluent)	37000	5657	0.53	0.07	0.58	0.54	0.49	0.58
16 (effluent)	33000	2828	0.58	0.04	0.61	0.58	0.56	0.61
20 (effluent)	25500	2121	0.70	0.04	0.72	0.70	0.67	0.72
24 (effluent)	27500	10607	0.66	0.17	0.79	0.68	0.56	0.80
36 (effluent)	25000	n/a	0.70	n/a	n/a	n/a	n/a	n/a
48 (effluent)	38000	n/a	0.52	n/a	n/a	n/a	n/a	n/a
48 (influent)	107500	17678	0.07	0.07	0.12	0.07	0.02	0.12
Clostridium perfringens								
0 (influent)	76000	28000						
12 (effluent)	30500	14849	0.40	0.22	0.56	0.42	0.27	0.58
16 (effluent)	33000	2828	0.36	0.04	0.39	0.36	0.34	0.39
20 (effluent)	25500	2121	0.47	0.04	0.50	0.48	0.45	0.50
24 (effluent)	27500	10607	0.44	0.17	0.57	0.46	0.34	0.58
36 (effluent)	20000	n/a	0.58	n/a	n/a	n/a	n/a	n/a
48 (effluent)	38000	n/a	0.30	n/a	n/a	n/a	n/a	n/a
48 (influent)	89000	4243	-0.07	0.02	-0.06	-0.07	-0.08	-0.05
Coliforms								
0 (influent)	65333333	21501938						
12 (effluent)	11150000	2616295	0.77	0.10	0.84	0.77	0.70	0.85
16 (effluent)	14000000	1414214	0.67	0.04	0.70	0.67	0.64	0.70
20 (effluent)	15500000	3535534	0.62	0.10	0.69	0.63	0.56	0.70
24 (effluent)	19500000	6363961	0.53	0.14	0.63	0.54	0.43	0.64
36 (effluent)	21000000	n/a	0.49	n/a	n/a	n/a	n/a	n/a
48 (effluent)	30000000	n/a	0.34	n/a	n/a	n/a	n/a	n/a
48 (influent)	87000000	0	-0.12	0.00	-0.12	-0.12	-0.12	-0.12
Coliforms - Presumptive								
0 (influent)	60333333	20599353						
12 (effluent)	6500000	7778175	0.97	0.76	1.73	1.24	0.70	1.78
16 (effluent)	12000000	1414214	0.70	0.05	0.74	0.70	0.67	0.74

20 (effluent)	14000000	4242641	0.63	0.13	0.73	0.64	0.55	0.74
24 (effluent)	18500000	4949747	0.51	0.12	0.60	0.52	0.44	0.60
36 (effluent)	20000000	n/a	0.48	n/a	n/a	n/a	n/a	n/a
48 (effluent)	26000000	n/a	0.37	n/a	n/a	n/a	n/a	n/a
48 (influent)	73000000	0	-0.08	0.00	-0.08	-0.08	-0.08	-0.08
Cryptosporidium **- Confirmed**								
0 (influent)	7966	6880						
12 (effluent)	1701	1394	0.67	0.41	1.02	0.76	0.47	1.05
16 (effluent)	1985	202	0.60	0.04	0.63	0.60	0.57	0.64
20 (effluent)	2752	1328	0.46	0.22	0.63	0.49	0.33	0.64
24 (effluent)	2972	168	0.43	0.02	0.44	0.43	0.41	0.45
36 (effluent)	2396	n/a	0.52	n/a	n/a	n/a	n/a	n/a
48 (effluent)	3465	n/a	0.36	n/a	n/a	n/a	n/a	n/a
48 (influent)	8709	4799	-0.04	0.25	0.16	0.00	-0.18	0.18
Cryptosporidium **- Presumptive**								
0 (influent)	16573	12561						
12 (effluent)	2229	1597	0.87	0.34	1.15	0.94	0.69	1.18
16 (effluent)	3054	310	0.73	0.04	0.76	0.74	0.70	0.77
20 (effluent)	4759	588	0.54	0.05	0.58	0.54	0.51	0.58
24 (effluent)	3963	1120	0.62	0.12	0.71	0.63	0.54	0.72
36 (effluent)	5989	n/a	0.44	n/a	n/a	n/a	n/a	n/a
48 (effluent)	4331	n/a	0.58	n/a	n/a	n/a	n/a	n/a
48 (influent)	15287	2827	0.04	0.08	0.09	0.04	-0.02	0.10
E.coli								
0 (influent)	6866667	1357694						
12 (effluent)	1045000	502046	0.82	0.22	0.98	0.84	0.69	1.00
16 (effluent)	1040000	84853	0.82	0.04	0.84	0.82	0.80	0.85
20 (effluent)	895000	35355	0.88	0.02	0.90	0.89	0.87	0.90
24 (effluent)	990000	14142	0.84	0.01	0.85	0.84	0.84	0.85
36 (effluent)	1300000	n/a	0.72	n/a	n/a	n/a	n/a	n/a
48 (effluent)	1700000	n/a	0.61	n/a	n/a	n/a	n/a	n/a
48 (influent)	8050000	1484924	-0.07	0.08	-0.01	-0.07	-0.12	-0.01
E.coli **- Presumptive**								
0 (influent)	6166667	1301281						
12 (effluent)	1045000	502046	0.77	0.22	0.94	0.80	0.64	0.95
16 (effluent)	980000	0	0.80	0.00	0.80	0.80	0.80	0.80
20 (effluent)	845000	35355	0.86	0.02	0.87	0.86	0.85	0.88
24 (effluent)	950000	42426	0.81	0.02	0.82	0.81	0.80	0.83
36 (effluent)	1300000	n/a	0.68	n/a	n/a	n/a	n/a	n/a
48 (effluent)	1200000	n/a	0.71	n/a	n/a	n/a	n/a	n/a
48 (influent)	6450000	777817	-0.02	0.05	0.02	-0.02	-0.06	0.02
Ent/F.Strep - Presumptive								
0 (influent)	1316667	950912						
12 (effluent)	380000	169706	0.54	0.20	0.69	0.56	0.42	0.70
16 (effluent)	350000	28284	0.58	0.04	0.60	0.58	0.55	0.60
20 (effluent)	335000	77782	0.59	0.10	0.67	0.60	0.53	0.67
24 (effluent)	240000	98995	0.74	0.18	0.88	0.76	0.63	0.89
36 (effluent)	280000	n/a	0.67	n/a	n/a	n/a	n/a	n/a
48 (effluent)	270000	n/a	0.69	n/a	n/a	n/a	n/a	n/a
48 (influent)	825000	7071	0.20	0.00	0.21	0.20	0.20	0.21

***Giardia* - Confirmed**								
0 (influent)	1589	838						
12 (effluent)	93	53	1.23	0.26	1.44	1.27	1.09	1.46
16 (effluent)	99	35	1.21	0.16	1.32	1.22	1.11	1.33
20 (effluent)	92	21	1.24	0.10	1.31	1.25	1.18	1.31
24 (effluent)	209	144	0.88	0.33	1.15	0.94	0.71	1.17
36 (effluent)	123	n/a	1.11	n/a	n/a	n/a	n/a	n/a
48 (effluent)	50	n/a	1.50	n/a	n/a	n/a	n/a	n/a
48 (influent)	277	91	0.76	0.14	0.86	0.77	0.67	0.87
***Giardia* - Presumptive**								
0 (influent)	5546	1325						
12 (effluent)	186	105	1.48	0.26	1.68	1.51	1.33	1.70
16 (effluent)	221	123	1.40	0.26	1.60	1.44	1.26	1.62
20 (effluent)	244	29	1.36	0.05	1.39	1.36	1.32	1.40
24 (effluent)	398	313	1.14	0.38	1.47	1.22	0.95	1.50
36 (effluent)	413	n/a	1.13	n/a	n/a	n/a	n/a	n/a
48 (effluent)	192	n/a	1.46	n/a	n/a	n/a	n/a	n/a
48 (influent)	1574	779	0.55	0.22	0.72	0.58	0.42	0.73
Sulphite Reducing Clostridia								
0 (influent)	126667	28868						
12 (effluent)	37000	5657	0.53	0.07	0.58	0.54	0.49	0.58
16 (effluent)	33000	2828	0.58	0.04	0.61	0.58	0.56	0.61
20 (effluent)	25500	2121	0.70	0.04	0.72	0.70	0.67	0.72
24 (effluent)	27500	10607	0.66	0.17	0.79	0.68	0.56	0.80
36 (effluent)	25000	n/a	0.70	n/a	n/a	n/a	n/a	n/a
48 (effluent)	38000	n/a	0.52	n/a	n/a	n/a	n/a	n/a
48 (influent)	107500	17678	0.07	0.07	0.12	0.07	0.02	0.12

Note: n/a (not applicable)

Table A1.2b: Summary of physico-chemical data for 15 days sludge age replicate 1 (of 3)

Phys-Chem parameter and source	Time (hours) 0	12	16	20	24	36	48
SS (mgL^{-1})							
Mixed liquor	1637	nd	1665	1577	1603	1527	1637
Influent	95	95	nd	nd	113	93	112
Effluent	17.5	12.5	22.5	20.5	20	21.5	25
Turbidity (NTU)							
Influent	113	125	nd	nd	116	95	100
Effluent	15	13	12.8	13	13	14	15.5
pH							
Influent	7.55	7.68	nd	nd	7.77	7.69	7.7
Effluent	7.18	6.87	6.92	6.98	7.02	7.05	7.07
Alkalinity (mgL^{-1})							
Influent	225	257	nd	nd	233	247	247
Effluent	129	103	105	110	113	147	146
Temperature (°C)							
Influent	17.5	16	nd	nd	15	13	13
Effluent	19.8	20.1	20	19.8	20.1	20.1	19.7
NH_3 ($mgN\ L^{-1}$)							
Influent	42	42	nd	nd	41	41	40
Effluent	0.6	0.5	0.5	1	1.6	3.3	5
NO_2^- ($mgN\ L^{-1}$)							
Influent	0	0.05	nd	nd	0	0	0
Effluent	11	11	12	11	12	13	12
NO_3^- ($mgN\ L^{-1}$)							
Influent	0	0.19	nd	nd	0	0.03	0
Effluent	0.19	4	7.4	3.3	0.65	0.46	0.35
TKN ($mgN\ L^{-1}$)							
Influent	57	58	nd	nd	55	57	60
Effluent	6.7	5.6	6.2	6.7	7.4	8.6	11
BOD_5 (mgL^{-1})							
Influent	180	140	nd	nd	120	94	160
Effluent	18	19	19	18	24	30	37
COD (mgL^{-1-1})							
Influent	440	320	nd	nd	320	270	260
Effluent	100	98	100	97	110	110	99
SVI ($mL\ g^{-1}$)							
Mixed liquor	67.2	nd	nd	nd	nd	nd	67.2

Note:
Data is presented as mean values
nd = not determined

Table A1.2a: Statistical summary of microbiological data for 15 days sludge age replicate 2 (of 3)

Microbiological parameter, time (hours) and source	Actual value summary		Log_{10} reduction value (LRV) summary					
	Mean	Standard deviation	Mean	Standard deviation	95th %ile	50th %ile	Min	Max
Bacteriophage								
0 (influent)	38033	3253						
12 (effluent)	2425	346	1.20	0.06	1.24	1.20	1.15	1.24
16 (effluent)	2415	120	1.20	0.02	1.21	1.20	1.18	1.21
20 (effluent)	1180	85	1.51	0.03	1.53	1.51	1.49	1.53
24 (effluent)	1230	14	1.49	0.00	1.49	1.49	1.49	1.49
36 (effluent)	1800	n/a	1.32	n/a	n/a	n/a	1.32	1.32
48 (effluent)	2280	n/a	1.22	n/a	n/a	n/a	1.22	1.22
48 (influent)	43900	4101	-0.06	0.04	-0.04	-0.06	-0.09	-0.03
***Clostridium* - Presumptive**								
0 (influent)	126667	5774						
12 (effluent)	11100	2687	1.06	0.11	1.13	1.06	0.99	1.14
16 (effluent)	9200	1131	1.14	0.05	1.17	1.14	1.10	1.18
20 (effluent)	7950	636	1.20	0.03	1.23	1.20	1.18	1.23
24 (effluent)	6350	212	1.30	0.01	1.31	1.30	1.29	1.31
36 (effluent)	5800	n/a	1.34	n/a	n/a	n/a	1.34	1.34
48 (effluent)	13000	n/a	0.99	n/a	n/a	n/a	0.99	0.99
48 (influent)	100000	28284	0.10	0.12	0.19	0.11	0.02	0.20
Clostridium perfringens								
0 (influent)	126667	5774						
12 (effluent)	8400	1131	1.18	0.06	1.22	1.18	1.14	1.22
16 (effluent)	8350	2333	1.18	0.12	1.27	1.19	1.10	1.28
20 (effluent)	7100	566	1.25	0.03	1.27	1.25	1.23	1.28
24 (effluent)	5400	1131	1.37	0.09	1.43	1.38	1.31	1.44
36 (effluent)	4600	n/a	1.44	n/a	n/a	n/a	1.44	1.44
48 (effluent)	7800	n/a	1.21	n/a	n/a	n/a	1.21	1.21
48 (influent)	100000	28284	0.10	0.12	0.19	0.11	0.02	0.20
Coliforms								
0 (influent)	87333333	22501852						
12 (effluent)	1035000	374767	1.93	0.16	2.04	1.94	1.83	2.05
16 (effluent)	885000	162635	1.99	0.08	2.05	2.00	1.94	2.05
20 (effluent)	1150000	70711	1.88	0.03	1.90	1.88	1.86	1.90
24 (effluent)	670000	28284	2.12	0.02	2.13	2.12	2.10	2.13
36 (effluent)	2000000	n/a	1.64	n/a	n/a	n/a	1.64	1.64
48 (effluent)	3000000	n/a	1.46	n/a	n/a	n/a	1.46	1.46
48 (influent)	77500000	6363961	0.05	0.04	0.08	0.05	0.03	0.08
Coliforms - Presumptive								
0 (influent)	87333333	22501852						
12 (effluent)	1035000	374767	1.93	0.16	2.04	1.94	1.83	2.05
16 (effluent)	885000	162635	1.99	0.08	2.05	2.00	1.94	2.05
20 (effluent)	960000	56569	1.96	0.03	1.98	1.96	1.94	1.98
24 (effluent)	650000	56569	2.13	0.04	2.15	2.13	2.10	2.16
36 (effluent)	2000000	n/a	1.64	n/a	n/a	n/a	1.64	1.64
48 (effluent)	2900000	n/a	1.48	n/a	n/a	n/a	1.48	1.48
48 (influent)	63000000	2828427	0.14	0.02	0.15	0.14	0.13	0.16

***Cryptosporidium* - Confirmed**								
0 (influent)	7622	321						
12 (effluent)	605	135	1.10	0.10	1.17	1.11	1.04	1.18
16 (effluent)	710	100	1.03	0.06	1.07	1.03	0.99	1.08
20 (effluent)	696	47	1.04	0.03	1.06	1.04	1.02	1.06
24 (effluent)	761	112	1.00	0.06	1.04	1.00	0.96	1.05
36 (effluent)	510	n/a	1.17	n/a	n/a	n/a	1.17	1.17
48 (effluent)	875	n/a	0.94	n/a	n/a	n/a	0.94	0.94
48 (influent)	4437	1237	0.24	0.12	0.32	0.24	0.16	0.33
***Cryptosporidium* - Presumptive**								
0 (influent)	8805	597						
12 (effluent)	1092	103	0.91	0.04	0.93	0.91	0.88	0.94
16 (effluent)	1246	256	0.85	0.09	0.91	0.85	0.79	0.92
20 (effluent)	992	71	0.95	0.03	0.97	0.95	0.93	0.97
24 (effluent)	1206	224	0.86	0.08	0.92	0.87	0.81	0.92
36 (effluent)	1020	n/a	0.94	n/a	n/a	n/a	0.94	0.94
48 (effluent)	1170	n/a	0.88	n/a	n/a	n/a	0.88	0.88
48 (influent)	8730	1238	0.00	0.06	0.05	0.01	-0.04	0.05
E.coli								
0 (influent)	9133333	152753						
12 (effluent)	59000	4243	2.19	0.03	2.21	2.19	2.17	2.21
16 (effluent)	43500	9192	2.32	0.09	2.39	2.33	2.26	2.39
20 (effluent)	59000	4243	2.19	0.03	2.21	2.19	2.17	2.21
24 (effluent)	45000	9899	2.31	0.10	2.37	2.31	2.24	2.38
36 (effluent)	150000	n/a	1.78	n/a	n/a	n/a	1.78	1.78
48 (effluent)	190000	n/a	1.68	n/a	n/a	n/a	1.68	1.68
48 (influent)	5850000	494975	0.19	0.04	0.22	0.19	0.17	0.22
***E.coli* - Presumptive**								
0 (influent)	9133333	152753						
12 (effluent)	59000	4243	2.19	0.03	2.21	2.19	2.17	2.21
16 (effluent)	43500	9192	2.32	0.09	2.39	2.33	2.26	2.39
20 (effluent)	55000	5657	2.22	0.04	2.25	2.22	2.19	2.25
24 (effluent)	45000	9899	2.31	0.10	2.37	2.31	2.24	2.38
36 (effluent)	120000	n/a	1.88	n/a	n/a	n/a	1.88	1.88
48 (effluent)	170000	n/a	1.73	n/a	n/a	n/a	1.73	1.73
48 (influent)	5500000	989949	0.22	0.08	0.27	0.22	0.17	0.28
Ent/F.Strep - Presumptive								
0 (influent)	1800000	173205						
12 (effluent)	21500	4950	1.92	0.10	1.99	1.93	1.86	2.00
16 (effluent)	17500	6364	2.01	0.16	2.13	2.03	1.91	2.14
20 (effluent)	26500	2121	1.83	0.03	1.85	1.83	1.81	1.86
24 (effluent)	12100	6930	2.17	0.26	2.38	2.21	2.02	2.40
36 (effluent)	21000	n/a	1.93	n/a	n/a	n/a	1.93	1.93
48 (effluent)	35000	n/a	1.71	n/a	n/a	n/a	1.71	1.71
48 (influent)	760000	42426	0.37	0.02	0.39	0.37	0.36	0.39
***Giardia* - Confirmed**								
0 (influent)	1897	2165						
12 (effluent)	13		2.16					
16 (effluent)								
20 (effluent)	32		1.77					

24 (effluent)	19		2.00					
36 (effluent)	33	n/a	1.76	n/a	n/a	n/a	1.76	1.76
48 (effluent)								
48 (influent)	684	8	0.44	0.01	0.45	0.44	0.44	0.45
***Giardia* - Presumptive**								
0 (influent)	6258	1180						
12 (effluent)	27	18	2.37	0.31	2.63	2.43	2.21	2.65
16 (effluent)	19		2.52	n/a				
20 (effluent)	65		1.98	n/a				
24 (effluent)	44	21	2.16	0.21	2.32	2.18	2.03	2.33
36 (effluent)	67	n/a	1.97	n/a	n/a	n/a	1.97	1.97
48 (effluent)	12	n/a	2.72	n/a	n/a	n/a	2.72	2.72
48 (influent)	1823	139	0.54	0.03	0.56	0.54	0.51	0.56
Sulphite Reducing Clostridia								
0 (influent)	126667	5774						
12 (effluent)	11100	2687	1.06	0.11	1.13	1.06	0.99	1.14
16 (effluent)	9200	1131	1.14	0.05	1.17	1.14	1.10	1.18
20 (effluent)	7950	636	1.20	0.03	1.23	1.20	1.18	1.23
24 (effluent)	6350	212	1.30	0.01	1.31	1.30	1.29	1.31
36 (effluent)	5800	n/a	1.34	n/a	n/a	n/a	1.34	1.34
48 (effluent)	13000	n/a	0.99	n/a	n/a	n/a	0.99	0.99
48 (influent)	100000	28284	0.10	0.12	0.19	0.11	0.02	0.20

Note:
n/a (not applicable)
Blank space: no data

Table A1.2b: Summary of physico-chemical data for 15 days sludge age replicate 2 (of 3)

Phys-Chem parameter and source	Time (hours) 0	12	16	20	24	36	48
SS (mgL^{-1})							
Mixed liquor	1872		1718	1545	2261	1565	1503
Influent	96.5	94.4			94.4	76	70.7
Effluent	8.1	5.2	4	7	8.9	6.7	5.3
Turbidity (NTU)							
Influent	138	126			122	107	95
Effluent	6.5	4.7	4.2	4.2	4.5	5.8	5.3
pH							
Influent	7.23	7.48			7.63	7.78	7.74
Effluent	6.99	6.93	6.96	6.96	6.94	6.9	6.82
Alkalinity (mgL^{-1})							
Influent	271	266			257	261	264
Effluent	116	115	113	110	111	107	114
Temperature (°C)							
Influent	18	16.5			15	13	11.7
Effluent	20.9	20.7	20.4	20.1	19.8	19.8	18.8
NH_3 ($mgN\ L^{-1}$)							
Influent	44	43			43	43	44
Effluent	0.2	0.2	0.2	0.2	0.3	0.2	0.4
NO_2^- ($mgN\ L^{-1}$)							
Influent	0	0			0	0	0.09
Effluent	14	15	14	14	16	16	16
NO_3^- ($mgN\ L^{-1}$)							
Influent	0	0			0	0.21	0.69
Effluent	1.2	1	1.4	1.1	4.4	3.9	0.1
TKN ($mgN\ L^{-1}$)							
Influent	57	47			52	54	54
Effluent	3.2	2.4	2.4	2.7	2.4	2.8	3.2
BOD_5 (mgL^{-1})							
Influent	200	200			180	190	200
Effluent	6	4	4	3	6	7	8
COD (mgL^{-1-1})							
Influent	400	380			360	320	310
Effluent	68	72	70	68	61	59	69
SVI ($mL\ g^{-1}$)							
Mixed liquor	69.4						79.8

Note:
1. Data is presented as mean values
2. blank space (not determined)

Table A1.3a: Statistical summary of microbiological data for 15 days sludge age replicate 3 (of 3)

Microbiological parameter, time (hours) and source	Actual value summary		Log reduction value (LRV) summary					
	Mean	Standard deviation	Mean	Standard deviation	95th %ile	50th %ile	Min	Max
Bacteriophage								
0 (influent)	38933	4046						
12 (effluent)	870	85	1.65	0.04	1.68	1.65	1.62	1.68
16 (effluent)	800	71	1.69	0.04	1.71	1.69	1.66	1.72
20 (effluent)	760	28	1.71	0.02	1.72	1.71	1.70	1.72
24 (effluent)	670	99	1.76	0.06	1.81	1.77	1.72	1.81
36 (effluent)	1080	n/a	1.56	n/a	n/a	n/a	1.56	1.56
48 (effluent)	720	n/a	1.73	n/a	n/a	n/a	1.73	1.73
48 (influent)	28250	2333	0.14	0.04	0.16	0.14	0.11	0.17
***Clostridium* - Presumptive**								
0 (influent)	120000	10000						
12 (effluent)	7900	0	1.18	0.00	1.18	1.18	1.18	1.18
16 (effluent)	13000	5657	0.97	0.20	1.11	0.99	0.85	1.12
20 (effluent)	6750	354	1.25	0.02	1.26	1.25	1.23	1.27
24 (effluent)	9300	2404	1.11	0.11	1.19	1.12	1.04	1.20
36 (effluent)	6300	n/a	1.28	n/a	n/a	n/a	1.28	1.28
48 (effluent)	8300	n/a	1.16	n/a	n/a	n/a	1.16	1.16
48 (influent)	63500	7778	0.28	0.05	0.31	0.28	0.24	0.32
Clostridium perfringens								
0 (influent)	120000	10000						
12 (effluent)	7900	0	1.18	0.00	1.18	1.18	1.18	1.18
16 (effluent)	13000	5657	0.97	0.20	1.11	0.99	0.85	1.12
20 (effluent)	6750	354	1.25	0.02	1.26	1.25	1.23	1.27
24 (effluent)	9300	2404	1.11	0.11	1.19	1.12	1.04	1.20
36 (effluent)	6300	n/a	1.28	n/a	n/a	n/a	1.28	1.28
48 (effluent)	6600	n/a	1.26	n/a	n/a	n/a	1.26	1.26
48 (influent)	63500	7778	0.28	0.05	0.31	0.28	0.24	0.32
Coliforms								
0 (influent)	77333333	32501282						
12 (effluent)	2000000	0	1.59	0.00	1.59	1.59	1.59	1.59
16 (effluent)	2350000	919239	1.52	0.17	1.65	1.53	1.41	1.66
20 (effluent)	2750000	70711	1.45	0.01	1.46	1.45	1.44	1.46
24 (effluent)	2300000	424264	1.53	0.08	1.58	1.53	1.47	1.59
36 (effluent)	4600000	n/a	1.23	n/a	n/a	n/a	1.23	1.23
48 (effluent)	7500000	n/a	1.01	n/a	n/a	n/a	1.01	1.01
48 (influent)	54500000	14849242	0.15	0.12	0.24	0.16	0.08	0.24
Coliforms - Presumptive								
0 (influent)	77333333	32501282						
12 (effluent)	2000000	0	1.59	0.00	1.59	1.59	1.59	1.59
16 (effluent)	2350000	919239	1.52	0.17	1.65	1.53	1.41	1.66
20 (effluent)	2200000	282843	1.55	0.06	1.58	1.55	1.51	1.59
24 (effluent)	2050000	494975	1.58	0.11	1.65	1.58	1.51	1.66
36 (effluent)	3100000	n/a	1.40	n/a	n/a	n/a	1.40	1.40
48 (effluent)	7500000	n/a	1.01	n/a	n/a	n/a	1.01	1.01
48 (influent)	54500000	14849242	0.15	0.12	0.24	0.16	0.08	0.24
***Cryptosporidium* - Confirmed**								
0 (influent)	2145	1248						

12 (effluent)	631	286	0.53	0.20	0.68	0.55	0.41	0.70
16 (effluent)	938	442	0.36	0.21	0.52	0.39	0.23	0.54
20 (effluent)	711	188	0.48	0.12	0.56	0.49	0.41	0.57
24 (effluent)	551	96	0.59	0.08	0.64	0.59	0.54	0.65
36 (effluent)	1113	n/a	0.28	n/a	n/a	n/a	0.28	0.28
48 (effluent)	1316	n/a	0.21	n/a	n/a	n/a	0.21	0.21
48 (influent)	4088	427	-0.28	0.05	-0.25	-0.28	-0.31	-0.25
***Cryptosporidium* - Presumptive**								
0 (influent)	10362	998						
12 (effluent)	730	274	1.15	0.17	1.27	1.17	1.05	1.29
16 (effluent)	1066	340	0.99	0.14	1.09	1.00	0.90	1.10
20 (effluent)	851	118	1.09	0.06	1.13	1.09	1.05	1.13
24 (effluent)	737	8	1.15	0.00	1.15	1.15	1.15	1.15
36 (effluent)	1488	n/a	0.84	n/a	n/a	n/a	0.84	0.84
48 (effluent)	1382	n/a	0.87	n/a	n/a	n/a	0.87	0.87
48 (influent)	6543	291	0.20	0.02	0.21	0.20	0.19	0.21
E.coli								
0 (influent)	7600000	519615						
12 (effluent)	51500	14849	2.17	0.13	2.26	2.18	2.09	2.27
16 (effluent)	53500	2121	2.15	0.02	2.16	2.15	2.14	2.16
20 (effluent)	90500	13435	1.92	0.06	1.97	1.93	1.88	1.97
24 (effluent)	109500	28991	1.84	0.12	1.92	1.85	1.77	1.93
36 (effluent)	270000	n/a	1.45	n/a	n/a	n/a	1.45	1.45
48 (effluent)	490000	n/a	1.19	n/a	n/a	n/a	1.19	1.19
48 (influent)	5750000	636396	0.12	0.05	0.15	0.12	0.09	0.16
***E.coli* - Presumptive**								
0 (influent)	7600000	519615						
12 (effluent)	51500	14849	2.17	0.13	2.26	2.18	2.09	2.27
16 (effluent)	53500	2121	2.15	0.02	2.16	2.15	2.14	2.16
20 (effluent)	84000	9899	1.96	0.05	1.99	1.96	1.92	1.99
24 (effluent)	87500	17678	1.94	0.09	2.00	1.94	1.88	2.01
36 (effluent)	270000	n/a	1.45	n/a	n/a	n/a	1.45	1.45
48 (effluent)	490000	n/a	1.19	n/a	n/a	n/a	1.19	1.19
48 (influent)	5550000	919239	0.14	0.07	0.19	0.14	0.09	0.19
Ent/F.Strep - Presumptive								
0 (influent)	613333	30551						
12 (effluent)	8250	212	1.87	0.01	1.88	1.87	1.86	1.88
16 (effluent)	8200	1273	1.87	0.07	1.92	1.88	1.83	1.92
20 (effluent)	10050	1344	1.79	0.06	1.82	1.79	1.75	1.83
24 (effluent)	11000	0	1.75	0.00	1.75	1.75	1.75	1.75
36 (effluent)	37000	n/a	1.22	n/a	n/a	n/a	1.22	1.22
48 (effluent)	37000	n/a	1.22	n/a	n/a	n/a	1.22	1.22
48 (influent)	470000	14142	0.12	0.01	0.12	0.12	0.11	0.12
***Giardia* - Confirmed**								
0 (influent)	2147	1073						
12 (effluent)	no data							
16 (effluent)	no data							
20 (effluent)	50	47	1.64	0.51	2.09	1.77	1.41	2.13
24 (effluent)	16		2.13					
36 (effluent)	42	n/a	1.71	n/a	n/a	n/a	1.71	1.71
48 (effluent)	no data							
48 (influent)	754	235	0.45	0.14	0.55	0.47	0.37	0.56
***Giardia* - Presumptive**								

0 (influent)	3924	1478						
12 (effluent)	no data							
16 (effluent)	no data							
20 (effluent)	71	77	1.75	0.63	2.35	1.94	1.50	2.39
24 (effluent)	48	n/a	1.91	n/a	n/a	n/a	n/a	n/a
36 (effluent)	42	n/a	1.97	n/a	n/a	n/a	1.97	1.97
48 (effluent)	no data							
48 (influent)	1736	180	0.35	0.05	0.38	0.36	0.32	0.39
Sulphite Reducing Clostridia								
0 (influent)	120000	10000						
12 (effluent)	7900	0	1.18	0.00	1.18	1.18	1.18	1.18
16 (effluent)	13000	5657	0.97	0.20	1.11	0.99	0.85	1.12
20 (effluent)	6750	354	1.25	0.02	1.26	1.25	1.23	1.27
24 (effluent)	9300	2404	1.11	0.11	1.19	1.12	1.04	1.20
36 (effluent)	6300	n/a	1.28	n/a	n/a	n/a	1.28	1.28
48 (effluent)	8300	n/a	1.16	n/a	n/a	n/a	1.16	1.16
48 (influent)	63500	7778	0.28	0.05	0.31	0.28	0.24	0.32

Note: n/a (not applicable)

Table A1.3b: Summary of physico-chemical data for 15 days sludge age replicate 3 (of 3)

Phys-Chem parameter and source	Time (hours) 0	12	16	20	24	36	48
SS (mgL^{-1})							
Mixed liquor	1938		1962	1898	1862	1880	1808
Influent	103	89			76.5	70	58
Effluent	15	12	11	10.8	6.3	6	6
Turbidity (NTU)							
Influent	118	109			107	96	86
Effluent	9.5	6.5	6.2	6.3	6	5.8	6
pH							
Influent	7.26	7.46			7.58	7.71	7.78
Effluent	7.08	7.09	7.13	7.13	7.08	7.04	6.93
Alkalinity (mgL^{-1})							
Influent	264	255			277	264	279
Effluent	137	132	153	122	137	130	140
Temperature (°C)							
Influent	18.2	17.5			16.9	15.5	15
Effluent	21.1	21.3	21.1	20.9	21.1	18.8	18.8
NH_3 ($mgN\ L^{-1}$)							
Influent	41	35			43	42	41
Effluent	0.4	0.3	0.3	0.4	0.3	0.2	0.3
NO_2^- ($mgN\ L^{-1}$)							
Influent	0	0			0	0	0
Effluent	11	12	12	12	12	13	13
NO_3^- ($mgN\ L^{-1}$)							
Influent	0	0			0	0	0.05
Effluent	1.7	0.38	0.66	0.64			1.8
TKN ($mgN\ L^{-1}$)							
Influent	54	52			51	51	51
Effluent	4.1	3.2	3.5	3.1	2.8	2.9	2.7
BOD_5 (mgL^{-1})							
Influent	200	230			210	180	180
Effluent	15	8	8	8	7	8	10
COD (mgL^{-1-1})							
Influent	380	350			330	300	260
Effluent	64	60	56	62	54	53	42
SVI ($mL\ g^{-1}$)							
Mixed liquor	119						144

Note:
Data is presented as mean values
Blank space = not determined

Table A1.4a: Statistical summary of microbiological data for 20 days sludge age

Microbiological parameter, time (hours) and source	Actual value summary		Log reduction value (LRV) summary					
	Mean	Standard deviation	Mean	Standard deviation	95th %ile	50th %ile	Min	Max
Bacteriophage								
0 (influent)	81533	36892						
12 (effluent)	735	120	2.05	0.07	2.09	2.05	2.00	2.10
16 (effluent)	578	456	2.15	0.39	2.48	2.23	1.96	2.50
20 (effluent)	625	21	2.12	0.01	2.12	2.12	2.11	2.13
24 (effluent)	695	7	2.07	0.00	2.07	2.07	2.07	2.07
36 (effluent)	529	n/a	2.19	n/a	n/a	n/a	2.19	2.19
48 (effluent)	680	n/a	2.08	n/a	n/a	n/a	2.08	2.08
48 (influent)	106500	16263	-0.12	0.07	-0.07	-0.11	-0.16	-0.07
***Clostridium* - Presumptive**								
0 (influent)	38820	33758						
12 (effluent)	2500	283	1.19	0.05	1.22	1.19	1.16	1.23
16 (effluent)	2350	212	1.22	0.04	1.24	1.22	1.19	1.25
20 (effluent)	455	7	1.93	0.01	1.94	1.93	1.93	1.94
24 (effluent)	500	0	1.89	0.00	1.89	1.89	1.89	1.89
36 (effluent)	450	n/a	1.94	n/a	n/a	n/a	1.94	1.94
48 (effluent)	700	n/a	1.74	n/a	n/a	n/a	1.74	1.74
48 (influent)	52000	1414	-0.13	0.01	-0.12	-0.13	-0.14	-0.12
Clostridium perfringens								
0 (influent)	38820	33758						
12 (effluent)	2250	636	1.24	0.12	1.32	1.25	1.16	1.33
16 (effluent)	2350	212	1.22	0.04	1.24	1.22	1.19	1.25
20 (effluent)	455	7	1.93	0.01	1.94	1.93	1.93	1.94
24 (effluent)	450	71	1.94	0.07	1.98	1.94	1.89	1.99
36 (effluent)	450	n/a	1.94	n/a	n/a	n/a	1.94	1.94
48 (effluent)	700	n/a	1.74	n/a	n/a	n/a	1.74	1.74
48 (influent)	47000	8485	-0.08	0.08	-0.03	-0.08	-0.14	-0.02
Coliforms								
0 (influent)	81333333	14364308						
12 (effluent)	46000	9899	3.25	0.09	3.31	3.25	3.19	3.32
16 (effluent)	108000	73539	2.88	0.32	3.14	2.93	2.71	3.16
20 (effluent)	51500	2121	3.20	0.02	3.21	3.20	3.19	3.21
24 (effluent)	53500	7778	3.18	0.06	3.22	3.18	3.14	3.23
36 (effluent)	58000	n/a	3.15	n/a	n/a	n/a	3.15	3.15
48 (effluent)	88000	n/a	2.97	n/a	n/a	n/a	2.97	2.97
48 (influent)	64500000	17677670	0.10	0.12	0.19	0.11	0.02	0.19
Coliforms - Presumptive								
0 (influent)	70333333	16072751						
12 (effluent)	37500	7778	3.27	0.09	3.34	3.28	3.21	3.34
16 (effluent)	105000	77782	2.83	0.36	3.12	2.90	2.64	3.15
20 (effluent)	51500	2121	3.14	0.02	3.15	3.14	3.12	3.15
24 (effluent)	53500	7778	3.12	0.06	3.16	3.12	3.08	3.17
36 (effluent)	58000	n/a	3.08	n/a	n/a	n/a	3.08	3.08
48 (effluent)	88000	n/a	2.90	n/a	n/a	n/a	2.90	2.90

48 (influent)	64500000	17677670	0.04	0.12	0.12	0.05	-0.04	0.13
***Cryptosporidium* - Confirmed**								
0 (influent)	3504	903						
12 (effluent)	57	9	1.79	0.07	1.84	1.80	1.75	1.85
16 (effluent)	25	11	2.15	0.20	2.30	2.17	2.03	2.31
20 (effluent)	19	12	2.28	0.31	2.52	2.33	2.11	2.54
24 (effluent)	38	4	1.96	0.05	2.00	1.97	1.93	2.00
36 (effluent)	22	n/a	2.20	n/a	n/a	n/a	2.20	2.20
48 (effluent)	35	n/a	2.00	n/a	n/a	n/a	2.00	2.00
48 (influent)	625	413	0.75	0.31	1.00	0.80	0.58	1.02
***Cryptosporidium* - Presumptive**								
0 (influent)	11421	1649						
12 (effluent)	72	31	2.20	0.19	2.35	2.22	2.08	2.36
16 (effluent)	33	0	2.54	0.00	2.54	2.54	2.54	2.54
20 (effluent)	24	5	2.69	0.09	2.75	2.69	2.63	2.76
24 (effluent)	53	6	2.33	0.05	2.36	2.33	2.30	2.37
36 (effluent)	30	n/a	2.58	n/a	n/a	n/a	2.58	2.58
48 (effluent)	35	n/a	2.51	n/a	n/a	n/a	2.51	2.51
48 (influent)	7959	1356	0.16	0.07	0.21	0.16	0.11	0.21
E.coli								
0 (influent)	10200000	1708801						
12 (effluent)	3300	0	3.49	0.00	3.49	3.49	3.49	3.49
16 (effluent)	5850	919	3.24	0.07	3.29	3.24	3.20	3.29
20 (effluent)	7150	1485	3.15	0.09	3.22	3.16	3.09	3.22
24 (effluent)	8050	1626	3.10	0.09	3.16	3.11	3.04	3.17
36 (effluent)	9200	n/a	3.04	n/a	n/a	n/a	3.04	3.04
48 (effluent)	6500	n/a	3.20	n/a	n/a	n/a	3.20	3.20
48 (influent)	7700000	282843	0.12	0.02	0.13	0.12	0.11	0.13
***E.coli* - Presumptive**								
0 (influent)	9166667	907377						
12 (effluent)	2750	212	3.52	0.03	3.54	3.52	3.50	3.55
16 (effluent)	5700	1131	3.21	0.09	3.27	3.21	3.15	3.27
20 (effluent)	7150	1485	3.11	0.09	3.17	3.11	3.05	3.18
24 (effluent)	8050	1626	3.06	0.09	3.12	3.06	3.00	3.12
36 (effluent)	9200	n/a	3.00	n/a	n/a	n/a	3.00	3.00
48 (effluent)	6500	n/a	3.15	n/a	n/a	n/a	3.15	3.15
48 (influent)	7700000	282843	0.08	0.02	0.09	0.08	0.06	0.09
Ent/F.Strep - Presumptive								
0 (influent)	1500000	435890						
12 (effluent)	1300	283	3.06	0.10	3.13	3.07	3.00	3.13
16 (effluent)	1750	354	2.93	0.09	2.99	2.94	2.88	3.00
20 (effluent)	1950	495	2.89	0.11	2.96	2.89	2.81	2.97
24 (effluent)	1700	283	2.95	0.07	2.99	2.95	2.90	3.00
36 (effluent)	2700	n/a	2.74	n/a	n/a	n/a	2.74	2.74
48 (effluent)	2300	n/a	2.81	n/a	n/a	n/a	2.81	2.81
48 (influent)	865000	7071	0.24	0.00	0.24	0.24	0.24	0.24
***Giardia* - Confirmed**								
0 (influent)								
12 (effluent)								
16 (effluent)								

20 (effluent)	13	0					1.41	2.13
24 (effluent)	8	1						
36 (effluent)	9	n/a	n/a	n/a	n/a	n/a	1.71	1.71
48 (effluent)	11	n/a	n/a	n/a	n/a	n/a		
48 (influent)	162	81					0.37	0.56
***Giardia* - Presumptive**								
0 (influent)	4597	704						
12 (effluent)								
16 (effluent)								
20 (effluent)	32	9	2.16	0.13	2.26	2.17	2.08	2.26
24 (effluent)	12	4	2.60	0.14	2.70	2.61	2.52	2.71
36 (effluent)	19	n/a	2.38	n/a	n/a	n/a	2.38	2.38
48 (effluent)	21	n/a	2.34	n/a	n/a	n/a	2.34	2.34
48 (influent)	1234	419	0.57	0.15	0.68	0.58	0.48	0.69
Sulphite Reducing Clostridia								
0 (influent)	38820	33758						
12 (effluent)	2500	283	1.19	0.05	1.22	1.19	1.16	1.23
16 (effluent)	2350	212	1.22	0.04	1.24	1.22	1.19	1.25
20 (effluent)	455	7	1.93	0.01	1.94	1.93	1.93	1.94
24 (effluent)	500	0	1.89	0.00	1.89	1.89	1.89	1.89
36 (effluent)	450	n/a	1.94	n/a	n/a	n/a	1.94	1.94
48 (effluent)	700	n/a	1.74	n/a	n/a	n/a	1.74	1.74
48 (influent)	52000	1414	-0.13	0.01	-0.12	-0.13	-0.14	-0.12

Note:
n/a (not applicable)
Blank space (no data)

Table A1.4b: Summary of physico-chemical data for 20 days sludge age

Phys-Chem parameter and source	Time (hours) 0	12	16	20	24	36	48
SS (mgL^{-1})							
Mixed liquor	2875		2855	2875	2875	2675	2838
Influent	70	85.3			77.3	72.7	72.7
Effluent	6.3	6.7	4.3	7	4.3	4.7	5
Turbidity (NTU)							
Influent	96	90			92	84	80
Effluent	3	2.4	1.5	1.8	1.7	1.3	1.8
pH							
Influent	7.46	7.2			7.69	7.77	7.8
Effluent	7	7.57	7.25	7.17	7.11	7.12	7
Alkalinity (mgL^{-1})							
Influent	239	231			235	245	247
Effluent	111	115	117	113	116	120	106
Temperature (°C)							
Influent						19	20
Effluent	20	19.8	19.5	19.6	20.5	20.7	23.3
NH_3 ($mgN\ L^{-1}$)							
Influent	38	35			34	36	36
Effluent	0.7	0.2	0.1	0.1	0	0	0
NO_2^- ($mgN\ L^{-1}$)							
Influent	0	0			0	0	0
Effluent	0.07	0.08	0.07	0.07	0.07	0.08	0.08
NO_3^- ($mgN\ L^{-1}$)							
Influent	0	0			0.02	0.06	0
Effluent	18	14	14	16	18	15	19
TKN ($mgN\ L^{-1}$)							
Influent	65	48			52	46	51
Effluent	3.6	2.2	1.8	2.3	1.8	1.9	2
BOD_5 (mgL^{-1})							
Influent	260				140	130	120
Effluent	5	2	2	3	2	3	2
COD (mgL^{-1-1})							
Influent	340	310			300	260	240
Effluent	42	25	31	27	31	25	38
SVI ($mL\ g^{-1}$)							
Mixed liquor	278						233

Note:
Data is presented as mean values
Blank space (not determined)

Table A1.5a: Statistical summary of microbiological data for 10 days sludge age

Microbiological parameter, time (hours) and source	Actual value summary		Log reduction value (LRV) summary					
	Mean	Standard deviation	Mean	Standard deviation	95th %ile	50th %ile	Min	Max
Bacteriophage								
0 (influent)	103000	10536						
12 (effluent)	6175	92	1.22	0.01	1.23	1.22	1.22	1.23
16 (effluent)	5885	205	1.24	0.02	1.25	1.24	1.23	1.25
20 (effluent)	5525	1096	1.27	0.09	1.33	1.27	1.21	1.34
24 (effluent)	8300	2546	1.09	0.14	1.19	1.10	1.01	1.20
36 (effluent)	13000	n/a	0.90	n/a	n/a	n/a	0.90	0.90
48 (effluent)	11700	n/a	0.94	n/a	n/a	n/a	0.94	0.94
48 (influent)	150000	7071	-0.16	0.02	-0.15	-0.16	-0.18	-0.15
***Clostridium* - Presumptive**								
0 (influent)	85000	8544						
12 (effluent)	8350	1768	1.01	0.09	1.07	1.01	0.95	1.08
16 (effluent)	8800	283	0.98	0.01	0.99	0.99	0.98	0.99
20 (effluent)	6950	1061	1.09	0.07	1.13	1.09	1.04	1.14
24 (effluent)	7350	71	1.06	0.00	1.07	1.06	1.06	1.07
36 (effluent)	12000	n/a	0.85	n/a	n/a	n/a	0.85	0.85
48 (effluent)	6500	n/a	1.12	n/a	n/a	n/a	1.12	1.12
48 (influent)	63000	4243	0.13	0.03	0.15	0.13	0.11	0.15
Clostridium perfringens								
0 (influent)	45333	11015						
12 (effluent)	4050	354	1.05	0.04	1.07	1.05	1.02	1.08
16 (effluent)	5300	2687	0.93	0.23	1.11	0.96	0.80	1.12
20 (effluent)	6950	1061	0.81	0.07	0.86	0.82	0.77	0.86
24 (effluent)	6600	990	0.84	0.07	0.88	0.84	0.79	0.89
36 (effluent)	12000	n/a	0.58	n/a	n/a	n/a	0.58	0.58
48 (effluent)	6500	n/a	0.84	n/a	n/a	n/a	0.84	0.84
48 (influent)	63000	4243	-0.14	0.03	-0.12	-0.14	-0.16	-0.12
Coliforms								
0 (influent)	61000000	10816654						
12 (effluent)	10400	849	3.77	0.04	3.79	3.77	3.74	3.79
16 (effluent)	14500	2121	3.62	0.06	3.67	3.63	3.58	3.67
20 (effluent)	14000	0	3.64	0.00	3.64	3.64	3.64	3.64
24 (effluent)	26000	2828	3.37	0.05	3.40	3.37	3.34	3.41
36 (effluent)	370000	n/a	2.22	n/a	n/a	n/a	2.22	2.22
48 (effluent)	130000	n/a	2.67	n/a	n/a	n/a	2.67	2.67
48 (influent)	31500000	17677670	0.29	0.26	0.49	0.32	0.14	0.51
Coliforms - Presumptive								
0 (influent)	49666667	9712535						
12 (effluent)	9100	1273	3.74	0.06	3.78	3.74	3.70	3.78
16 (effluent)	13500	3536	3.57	0.12	3.65	3.57	3.49	3.65
20 (effluent)	14000	0	3.55	0.00	3.55	3.55	3.55	3.55
24 (effluent)	22000	2828	3.35	0.06	3.39	3.36	3.32	3.40
36 (effluent)	290000	n/a	2.23	n/a	n/a	n/a	2.23	2.23
48 (effluent)	110000	n/a	2.65	n/a	n/a	n/a	2.65	2.65

48 (influent)	30000000	19798990	0.22	0.31	0.47	0.27	0.05	0.49
***Cryptosporidium* - Confirmed**								
0 (influent)	6815	1418						
12 (effluent)	134	21	1.71	0.07	1.75	1.71	1.66	1.76
16 (effluent)	282	149	1.38	0.24	1.57	1.42	1.25	1.59
20 (effluent)	150	30	1.66	0.09	1.72	1.66	1.60	1.73
24 (effluent)	225	87	1.48	0.17	1.61	1.50	1.38	1.62
36 (effluent)	298	n/a	1.36	n/a	n/a	n/a	1.36	1.36
48 (effluent)	611	n/a	1.05	n/a	n/a	n/a	1.05	1.05
48 (influent)	2505	230	0.43	0.04	0.46	0.44	0.41	0.46
***Cryptosporidium* - Presumptive**								
0 (influent)	9766	1684						
12 (effluent)	170	29	1.76	0.07	1.81	1.76	1.71	1.82
16 (effluent)	377	151	1.41	0.18	1.55	1.43	1.30	1.56
20 (effluent)	264	71	1.57	0.12	1.65	1.58	1.49	1.66
24 (effluent)	354	73	1.44	0.09	1.50	1.45	1.38	1.51
36 (effluent)	464	n/a	1.32	n/a	n/a	n/a	1.32	1.32
48 (effluent)	667	n/a	1.17	n/a	n/a	n/a	1.17	1.17
48 (influent)	3398	474	0.46	0.06	0.50	0.46	0.42	0.50
E.coli								
0 (influent)	10666667	577350						
12 (effluent)	1500	141	3.85	0.04	3.88	3.85	3.82	3.88
16 (effluent)	1900	141	3.75	0.03	3.77	3.75	3.73	3.77
20 (effluent)	2250	778	3.68	0.15	3.79	3.69	3.58	3.80
24 (effluent)	5050	212	3.32	0.02	3.34	3.32	3.31	3.34
36 (effluent)	64000	n/a	2.22	n/a	n/a	n/a	2.22	2.22
48 (effluent)	17000	n/a	2.80	n/a	n/a	n/a	2.80	2.80
48 (influent)	5900000	3252691	0.26	0.25	0.45	0.29	0.11	0.47
***E.coli* - Presumptive**								
0 (influent)	10100000	854400						
12 (effluent)	1350	71	3.87	0.02	3.89	3.87	3.86	3.89
16 (effluent)	1750	71	3.76	0.02	3.77	3.76	3.75	3.77
20 (effluent)	2200	849	3.66	0.17	3.79	3.68	3.56	3.80
24 (effluent)	4500	141	3.35	0.01	3.36	3.35	3.34	3.36
36 (effluent)	53000	n/a	2.28	n/a	n/a	n/a	2.28	2.28
48 (effluent)	16000	n/a	2.80	n/a	n/a	n/a	2.80	2.80
48 (influent)	5700000	3535534	0.25	0.29	0.48	0.29	0.09	0.50
Ent/F.Strep - Presumptive								
0 (influent)	1006667	90185						
12 (effluent)	330	141	3.48	0.19	3.63	3.51	3.37	3.64
16 (effluent)	345	78	3.47	0.10	3.53	3.47	3.40	3.54
20 (effluent)	400	14	3.40	0.02	3.41	3.40	3.39	3.41
24 (effluent)	475	49	3.33	0.05	3.36	3.33	3.30	3.36
36 (effluent)	7100	n/a	2.15	n/a	n/a	n/a	2.15	2.15
48 (effluent)	2700	n/a	2.57	n/a	n/a	n/a	2.57	2.57
48 (influent)	7550	495	2.12	0.03	2.14	2.13	2.11	2.15
***Giardia* - Confirmed**								
0 (influent)	960	364						
12 (effluent)								
16 (effluent)	20	1	1.69	0.02	1.70	1.69	1.68	1.70

20 (effluent)								
24 (effluent)	44	38	1.34	0.44	1.72	1.44	1.13	1.75
36 (effluent)								
48 (effluent)								
48 (influent)								
***Giardia* - Presumptive**								
0 (influent)	3218	512						
12 (effluent)	108	24	1.47	0.10	1.54	1.48	1.41	1.55
16 (effluent)	69	45	1.67	0.31	1.92	1.72	1.51	1.94
20 (effluent)	28		2.06	n/a				
24 (effluent)	159	78	1.31	0.22	1.48	1.34	1.18	1.49
36 (effluent)	208	n/a	1.19	n/a	n/a	n/a	1.19	1.19
48 (effluent)	125	n/a	1.41	n/a	n/a	n/a	1.41	1.41
48 (influent)	1141	482	0.45	0.19	0.59	0.47	0.34	0.60
Sulphite Reducing Clostridia								
0 (influent)	85000	8544						
12 (effluent)	8350	1768	1.01	0.09	1.07	1.01	0.95	1.08
16 (effluent)	8800	283	0.98	0.01	0.99	0.99	0.98	0.99
20 (effluent)	6950	1061	1.09	0.07	1.13	1.09	1.04	1.14
24 (effluent)	7350	71	1.06	0.00	1.07	1.06	1.06	1.07
36 (effluent)	12000	n/a	0.85	n/a	n/a	n/a	0.85	0.85
48 (effluent)	6500	n/a	1.12	n/a	n/a	n/a	1.12	1.12
48 (influent)	63000	4243	0.13	0.03	0.15	0.13	0.11	0.15

Note:
n/a (not applicable)
Blank space (no data)

Table A1.5b: Summary of physico-chemical data for 10 days sludge age

Phys-Chem parameter and source	Time (hours) 0	12	16	20	24	36	48
SS (mgL^{-1})							
Mixed liquor	1268		1315	1245	1320	1360	1273
Influent	75	88			82	81.3	67
Effluent	3	6	7.3	7	6.3	6	6.3
Turbidity (NTU)							
Influent	87.7	92			89	85	70
Effluent	2.7	2.8	3.5	2.9	3.3	4.1	4
pH							
Influent	7.32	7.52			7.73	7.79	7.85
Effluent	6.99	7.24	7.04	7	6.86	6.99	6.8
Alkalinity (mgL^{-1})							
Influent	245	253			250	259	257
Effluent	129	118	113	107	101	115	116
Temperature (°C)							
Influent	22	23		23.5		20.5	18
Effluent	26	27.1	26.6	26.7	25.3	21	20.1
NH_3 ($mgN\ L^{-1}$)							
Influent	40	40			37	37	38
Effluent	8	6.4	4.2	3.1	1.9	3.8	3.5
NO_2^- ($mgN\ L^{-1}$)							
Influent	0	0			0	0	0
Effluent	3	2.8	3	3	3	2.5	2.6
NO_3^- ($mgN\ L^{-1}$)							
Influent	0	0			0	0	0
Effluent	16	15	18	18	18	20	17
TKN ($mgN\ L^{-1}$)							
Influent	49	48			44	49	50
Effluent	9.4	7.7	7.2	5.3	3.9	5.3	5.5
BOD_5 (mgL^{-1})							
Influent	190	160			140	200	100
Effluent	13	12	13	11	9	15	14
COD (mgL^{-1-1})							
Influent	320				290	260	240
Effluent	53	55	57	55	41	48	50
SVI ($mL\ g^{-1}$)							
Mixed liquor	63.1						70.7

Note:
Data is presented as mean values
Blank space (not determined)