APPENDIX A

Influent Characteristics

A1: The BOD concentration

The BOD concentration in the influent varied considerably over 2 years. Summary statistics are shown in Table A1.

Inlet to Pond	Mean	Median	Range
Blue	553	446	38-2150
Green	658	565	72-1730
Red	594	483	30-1500

Table A1 Summary statistics for inlet BOD (mg/l) to each pond

Plots of the data indicated that the BOD decreased on average after August 2001. However, this decrease was insignificant compared to the overall large fluctuations. The sample autocorrelation function (acf) plot shown as Figure A1, suggests that the BOD concentration could be adequately described as a completely random time-series (nonseasonal and without trend).

The data were skewed (overall skew = 1.773) and did not approximate to a normal distribution (see Figure A2). A useful transformation was found to be log_{10} . The fitted values after this transformation are shown in Figure A3. The data from each pond showed a similar pattern.

After transformation, the data from each pond was tested to ascertain if the overall mean was the same using analysis of variance (p = 0.484). Thus, all inlet BOD data were pooled together; the log-transformed mean was 485 mg/l. All calculations were performed using Genstat 3.2 statistical software.



Figure A1: The sample acf plot of the time series of BOD concentration in the inlet



Figure A2: Plots of BOD data in the inlet as fitted to a normal distribution. (Normal and Half-Normal plots should be straight lines; the histogram should have an even distribution).



Figure A3. Fitted values after a log₁₀ transformation of the BOD data: the fit is improved.

A2: The suspended solids concentration

The SS concentration in the influent had large fluctuations over the 2 years' of operation. Summary statistics are shown in Table A2.

Inlet to Pond	Mean	Median	Range
Blue	1215	877	76-6016
Green	1367	945	183-6081
Red	1181	718	52-4568

Table A2 Summary	^r statistics f	for inlet SS	(mg/l) to	each pond
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The sample autocorrelation function plot (Figure A4) indicates that the SS concentration in the inlets could be adequately described as a completely random time-series (nonseasonal and without trend).

The data were skewed (overall skew = 1.868) and did not approximate to a normal distribution as shown in Figure A5. Again, a useful transformation was found to be log_{10} . The fitted values after this transformation are shown in Figure A6.

After transformation, an ANOVA test for zero mean difference was performed on the data from each pond (p = 0.406). Thus all inlet SS data was pooled together; the grand mean was 1057 mg/l.



Figure A4. The acf plot for the influent SS concentration over 2 years



Figure A5: Normal plot of all inlet SS concentration values; histogram showing skewed data

lgSS



Figure A6. Fitted values after a log₁₀ transformation of the SS data. The fit is improved.

A3: The ammonia concentration

The ammonia concentration in the influent did not appear to be random (Figure A7). Between August 2000 to October 2001 there appeared to be a seasonal effect (i.e. higher in summer than winter), though this was not evident between November 2001 and June 2002. The autocorrelation function plot highlights the possibility of seasonal fluctuations, see Figure A8. A very simple moving average filter was applied to the data to give local averages (Figure A9). These values were used to calculate ammonia removal efficiency. The fitted values range between 18 - 40 mg N/l.



Figure A7. Monthly mean ammonia concentration in the influent: bars show range.



Figure A8: The acf plot of the ammonia concentration time-series: suggests seasonal effects (negative autocorrelations between lags 4-9 and positive between lags 11-17).



Figure A9: Fitted moving average filter for inlet ammonia concentration $Sm(x_t) = 0.25 x_{t-1} + 0.5 x_t + 0.25 x_{t+1}$ $Sm(x_t) =$ smoothed value of x_t

A4. The filtered BOD concentration and the supernatant BOD concentration

The filtered BOD concentration and the BOD concentration in the supernatant both followed a random normal distribution and did not require transforming. An ANOVA test for mean difference gave a significance probability of 0.909 for the filtered BOD and 0.828 for the supernatant BOD. All the data were pooled together and the grand mean was 99 mg/l for filtered BOD and 173 mg/l for the supernatant BOD.

A5 The supernatant SS concentration

The SS concentration in the supernatant followed a random normal distribution and did not require transforming. An ANOVA test for mean difference gave a significance probability of 0.613. All the data were pooled together and the grand mean was 187.5 mg/l.

APPENDIX B Duckweed

The family of duckweeds (*Lemnaceae*) are the smallest flowering plants, and proliferate on still or slow moving water enriched with nutrients. A species of *Lemna (Lemna minor*) (Figure B1) infested all the pilot-ponds; it originated from the humus tank effluent used to fill the ponds at start-up. *Lemna* is a real problem for facultative ponds because the tiny fronds float on the surface, forming a dense blanket which effectively blocks out all the light and prevents air penetration. The micro-algae in the water cannot compete for light and die, thus the water beneath the duckweed becomes anaerobic. The weed must be removed (or harvested), otherwise it will eventually settle to the bottom, greatly augmenting the sludge volume and increasing feedback into the water column.





Figure B1: Duckweed (Lemna minor) on the Red pond

The optimum temperature for duckweed growth is between $20-30^{\circ}$ C, but may tolerate temperatures down to -7° C. The optimum pH is between 4.5 to 7.5; at pH 10 growth is seriously impeded. Wind and waves affect growth by affecting the ability of the fronds to attach to each other and form colonies (Cross, 2002).

The ponds became covered with the duckweed at different times: the Green pond was covered within two weeks of start-up, and the Blue and Red ponds took a month or so to be completely covered. The construction of the outlet meant that the fronds could not flow out. The specific growth rates for duckweed are very high, ranging from 0.1-0.35 g/g.day, and within two months of operation all the ponds were covered with duckweed mats 3 cm thick. Removal was by manually dragging the weed to one side of the pond using a barrier sign from the site (see Figure B2); ropes were attached to each side of the barrier and two people were required to pull the weed, and another person removed it using a special "trawler" net designed for the purpose (Figure B2a). The ponds were cleared of duckweed in November 2000 (Figures B3 and B4). It reappeared in late May 2001 and May 2002; on each occasion it was quickly removed before it could impact on the ponds.



Figure B2. Duckweed removal in November 2000. Two people are dragging the weed to one side using a barrier sign.



Figure B2a "Trawler" net for the removal of duckweed



Figure B3. Duckweed on the surface of the Red pond just before removal



Figure B4. The surface of the Red pond immediately after removal of the duckweed

The design of the pilot-scale ponds increased the problem of duckweed: it was trapped by the steep sides and the outlet design. A pond with flatter sides would be better as the wind could drive the weed onto the banks where it would be easier to remove. Alternatively, a flexible outlet (which could take water from the surface) would be useful: allowing the weed to flow out before it became established. Duckweeds grow best in water with high concentrations of nutrients, which are inevitable in facultative ponds. However, as they are affected by high pH (>10), a high concentration of actively photosynthesising micro-algae may inhibit growth.

Experiences in France for controlling duckweed, given by CEMAGREF (1997), include: manual removal, using domestic waterfowl or chemicals. Waterfowl are not easy control (unless you clip their wings!) and suitable chemicals are difficult to find because microalgae are also usually sensitive to the same chemicals as duckweed (2,6 dichlorobenonitrile was suggested).

Duckweed ponds may be used as a form of wastewater treatment, and recently work has been carried to compare the performance of these systems with algal based ponds ((Awuah *et al.*, 2001; Smith and Moelyowati, 2001; Calcedo *et al.*, 2002; Zimmo *et al.*,

2002). Zimmo *et al.* (2002) found that up to 33% of the total nitrogen was removed by harvesting the duckweed. Harvesting removes the nutrients from the system, so eliminating sedimentation and recycling from the sludge. The harvested duckweed is high in protein and may be used for animal feed.

APPENDIX C

Pond Biology

The identification of the most abundant species in the pond water took place between April 2001 and June 2002. The relative abundance was based on visual or microscopic inspection and the most abundant organism was established by judgement of the relative numbers of individuals. In many cases, more than one species was judged to be dominant. More detailed versions of the figures given in Chapter 5, Section 5.5.4, are given as Figures C1 and C2.

C1. Algae

The most dominant algal genera in the pilot-scale ponds were *Chlamdymonas* (Figure C3) and *Chlorella*. When *Chlamdymonas* was dominant, usually it was the only dominant organism and turned the ponds a uniform green colour. Of all the algae, a very small species of *Chlorella* (about 4μ m in diameter) tended to dominate during the winter. Whilst *Euglena* (Figure C4) and *Phacus* (Figure C5) were usually present, they were rarely dominant. The turbid waters of the facultative ponds favoured the motile algae, and non-motile, heavier algae such as *Scenedesmus* only appeared briefly in the Red pond; diatoms such as *Navicula* and *Fragillaria* were present in early spring and autumn. The non-motile species of *Chlorella* which dominated in the winter appeared to be able to remain buoyant in the water.



Figure C1. The dominant organisms identified in effluent samples

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Figure C2. The dominant organisms identified in column samples

C2. Purple bacteria

Only large (>1 μ m) bacteria were identified. The most frequently observed was *Chromatium* (Figure C6), a purple sulphur bacterium. This genus dominated the Blue pond for most of the winter. This is a motile, anaerobic bacterium which stores elemental sulphur in intracellular globules. The most abundant purple bacterium observed in the Red pond was *Rhodopseudomonas* (Figure C7), (a purple non-sulphur bacterium) which only really dominated after the algae were wiped out by predation effects. At these times, the surface of the water became clear and the purple colouration below became visible. The Green pond was rarely dominated by purple bacteria.

C3 Other organisms

The other main organisms identified were protozoa (eg. *Vorticella* and various cilliates), rotifers (Figure C8), and mosquito larvae (Figure C9); though nematodes were also present. The most frequently observed algal predator was *Paramecium* (Figure C10). This organism bloomed on the surface giving the pond a grey/brown appearance. Being much greater in size than the average alga, the *Paramecium* could finish off the algae rapidly, before declining themselves.



Figure C3. Chlamdymonas (x 200 BF) Green pond, 19 June 2001



Figure C4 *Euglena* (x 100 BF) Blue pond 19 June 2001



Figure C5 Phacus (x200 BF) Red pond, 9 July 2001



Figure C6 Chromatium (x 200 BF). Green pond, 3 July 2001



Figure C7 Rhodopseudomonas (x200 BF) Red pond, 12 June 2001



Figure C8 Rotifer (x 100 BF). Red pond. 19 June 2001



Figure C9 Mosquito larvae Red pond, July 2002



Figure C10 *Paramecium* (x 200 bright field) Blue pond, 19 June 2001

APPENDIX D

Sludge statistics

D1 Settleable solids concentration in the influent

The concentration of settleable solids in the influent to each pond had a very wide range as shown in Table D1. The very high values correspond to waste sludge from the host works periodically entering the flow. These high values caused the data to be strongly skewed, so a log transformation was required. The ANOVA test for mean difference on the transformed data had a significance probability of p=0.525, thus the data from all the ponds were grouped together. The log transformed (geometric) mean was 32.7 ml/l.

Table D1. Summary statistics of the settleable solid concentration (ml/l) to all ponds

	n	Minimum	Maximum	Mean	Std. Deviation	Skewness
BLUE	54	2	240	43.5	52.191	2.845
GREEN	54	7	225	44.7	41.799	2.186
RED	53	1	180	42.3	42.776	1.701

D2 Estimation of the theoretical sludge volume

The estimated cumulative flow to each pond up to each sludge measurement event was based on the average daily flow from the pumps as shown in Table D2. These values were used together with the mean settleable solids concentration to give the theoretical sludge volume originating from the influent as shown in Table D3.

1st sampling: 3/10/00	Daily flow (m ³ /d)	Days	Total flow (m ³)	Total flow this interval (m ³)	Cumulative flow (m ³)
Blue	0.84	8	6.72		
	0.431	77	33.187		
	1.412	3	4.236	44.143	44.143
Green	0.436	5	2.18		
	0.43	83	35.69	37.87	37.87
Red	0.516	88	45.408	45.408	45.408
2nd sampling: 15/3/01					
Blue	1.412	165	232.98	232.98	277.123
Green	0.43	14	6.02		
	0.801	151	120.951	126.971	164.841
Red	0.516	165	85.14	85.14	130.548
3rd sampling: 2/10/01					
Blue	1.412	8	11.296		
	0.8937	194	173.3778	184.6738	461.7968
Green	0.801	133	106.533		
	0.567	69	39.123	145.656	310.497
Red	0.516	202	104.232	104.232	234.78
4th sampling:4/2/01		<u> </u>			
Blue	0.79697	153	121.9364	121.9364	583.7332
Green	0.59956	153	91.73268	91.73268	402.2297
Red	0.49732	153	76.08996	76.08996	310.87

Table D2. Estimation of the total flow to the ponds up to each sludge sampling event

Table D3. Calculation of the theoretical sludge volume originating from the influent

	Total flow to	Total flow to	Total settleable	Total settleable
	pond	pond	solids	solids
	(m^3)	(1)	(ml)	(m ³)
to 2nd October 2000				
Blue pond	44.14	44143	1444800	1.44
Green pond	37.87	37870	1239485	1.24
Red pond	45.41	45408	1486203	1.49
to 15th March 2001				
Blue pond	277.12	277123	9070235	9.07
Green pond	164.84	164841	5395245	5.40
Red pond	130.55	130548	4272836	4.27
to 3rd October 2001				
Blue pond	461.80	461796	15114609	15.11
Green pond	310.50	310497	10162566	10.16
Red pond	234.78	234780	7684349	7.68
to 4th March 2002				
Blue pond	583.73	583733	19105587	19.11
Green pond	402.23	402229	13164977	13.16
Red pond	310.87	310869	10174773	10.17

D3 Calculation of actual sludge accumulation volume

The base areas were 16.4, 15.6 and 15.4 m^2 for the Red, Green and Blue ponds respectively. The bases were roughly rectangular in shape with 90° sides as shown in Figure 4.3 (Chapter 4) and for sludge measurement were divided into 16 square metre areas as shown in Figure D1. The average sludge volume for each square metre of base was estimated to be numerically equal to the average height of sludge (m) measured at its four corner points. The total sludge volume in the pond was taken to be the sum of the 16 volumes.



Figure D1. The pond base showing the sludge height measuring locations (circles). Each square represents 1 m².

D4 Calculation of the contribution per person per year

The number of people served by each pond was based on BOD load, assuming each person contributes 50 g BOD per day, as shown in Table D4. These figures were used to calculate the contribution per person per year of sludge volume accumulated.

	BOD load (g/d)	(A)	(B)	A x B	average
		number of people	number of days		no. of people
BLUE					
Phase 1	208.8	4.2	81	338.256	
Phase 2	684.8	13.7	168	2300.928	
Phase 3	473.2	9.5	113	1069.432	
Phase 4	434.6	8.7	244	2120.848	
total			606	5829.464	9.6
GREEN					
Phase 1	208.7	4.2	81	338.094	
Phase 2	388.7	7.8	168	1306.032	
Phase 3	388.7	7.8	113	878.462	
Phase 4	274.7	5.5	244	1340.536	
total			606	3863.124	6.4
RED					
Phase 1	250.5	5.0	81	405.81	
Phase 2	250.5	5.0	168	841.68	
Phase 3	250.5	5.0	113	566.13	
Phase 4	250.5	5.0	244	1222.44	
total			606	3036.06	5.0

Table D4. Calculation of the average number of people served by each pondover 20 months

D5. Percentage of volatile solids to total solids in the influent settleable solids.

The concentration of volatile solids (VS) and total solids (TS) was measured regularly on the influent settleable solids. Although the percentage of VS in TS varied widely, between 40-80%, overall there appeared to be an upward trend over the 20 months of operation as shown in Figure D2. This phenomenon remains unaccounted for, though is it most likely due to changes in influent composition over time, or possibly, a change in the performance of the muffle furnace over time. Either way, the change is important as it will have affected either the composition or measurement of the volatile solids in the sludge samples.



Figure D2. The percentage of volatile solids to total solids in the influent

APPENDIX E POND TEMPERATURE

Between 19 June 2001 and 20 July 2002, temperatures were logged in each pond every hour at six depth intervals from the surface to 1.25 m depth. The measurements were taken using *Thermochron iButton* (made by Dallas Instruments) as detailed in Section 4.5.3. In total, approximately 170,600 readings were taken. This data has not been included in the main thesis, though the readings from four days with distinct profiles are shown in Figures E1-4. Perhaps the data may be used together with the data from the weather station to create a thermal balance model.



Figure E1 Diurnal temperature profiles for 5 July 2001 High temperatures, stratification. Air temperature: 13.2-26.4°C Wind speed : 0.4-39. m/s Solar intensity: 242.7 W/m² Rainfall = 0.0 mm



Figure E2 Diurnal temperature profiles for 25 October 2001 Medium temperatures; mixing below surface. Air temperature: 7.1-11.4°C Wind speed : 0.1-5.6. m/s Solar intensity: 31.4 W/m² Rainfall = 1.2 mm







Figure E3 Diurnal temperature profiles for 8 January 2002 Frozen surface, temperature rises with depth, steady all day. Air temperature: 3.9-5.2°C Wind speed : 0.1-2.0. m/s. Solar intensity: 4.3 W/m². Rainfall = 0.0 mm







Figure E4 Diurnal temperature profiles for 12 March 2002 Spring mixing: layers not stable relative to each other. Air temperature: -1.4-10.1°C. Wind speed : 0.0-3.8. m/s Solar intensity: 85.1 W/m² . Rainfall = 0.0 mm