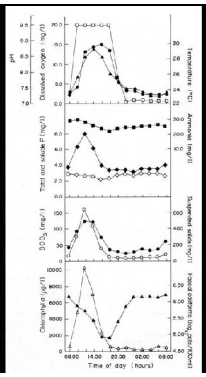
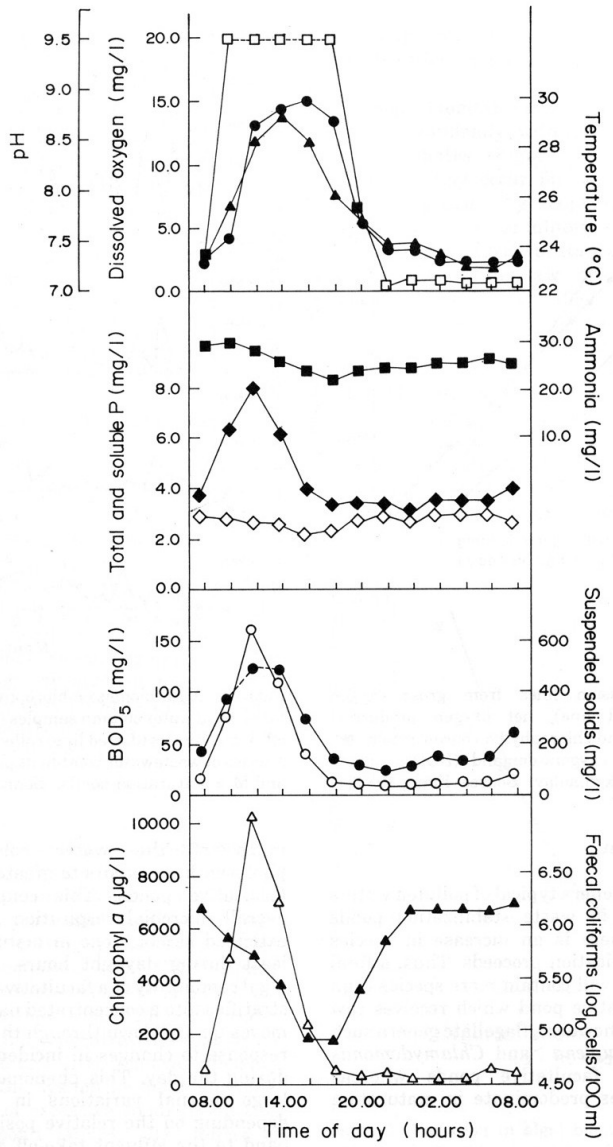
WASTE STABILIZATION PONDS 6a
Excreted pathogen removal mechanisms

1.		This presentation is on the mechanisms of excreted pathogen removal in waste stabilization ponds.
2.	**Removal of excreted pathogens in deep ponds in series in Northeast Brazil, 1984-1985, 25 °C** **Σθ = 21 days** | Raw w'w | A6 | F8 | M4 | M5 | M6 Retention time | – | 1 | 5 | 5 | 5 | 5 F. coliforms | 2E7 | 4E6 | 8E5 | 2E5 | 3E4 | 7E3 Campylobacters | 70 | 20 | 0.2 | 0 | 0 | 0 Salmonellae | 20 | 8 | 0.1 | 0.02 | 0.01 | 0 Enteroviruses** | 1E4 | 6E3 | 1E3 | 400 | 50 | 9 Rotaviruses** | 800 | 200 | 70 | 30 | 10 | 3 Bacterial nos. per 100 ml ** Viral nos. per 10 litres	Normally, when we monitor the performance of a pond system, we just determine the removal of an indicator bacterium, such as *E. coli* or faecal coliforms. The slide shows the results of some work we did quite a few years ago on a series of ponds in northeast Brazil. The pond system comprised a 1-day anaerobic pond followed by a facultative and three maturation ponds, each with a retention time of 5 days. We monitored faecal coliforms, two bacterial pathogens, salmonellae and campylobacters; and two lots of excreted viruses, enteroviruses and rotavirus. The faecal coliforms were reduced from 2×10^7 per 100 ml in the raw wastewater to 7000 per 100 ml in the final effluent, and the campylobacters from 70 per 100 ml to zero (well, at least undetectable) in the effluent of the first maturation pond. The salmonellae were reduced from 20 per 100 ml to zero (undetectable, again) in the effluent of the third maturation pond, although I suspect the removals of the campylobacters and salmonellae were similar; it's just that we're able to detect very small numbers of salmonellae, but not of campylo-bacters.

	Removal of excreted pathogens in deep ponds in series in Northeast Brazil, 1984-1985, 25 °C Σθ = 21 days | Raw w'w | A6 | F8 | M4 | M5 | M6 Retention time | – | 1 | 5 | 5 | 5 | 5 F. coliforms | 2E7 | 4E6 | 8E5 | 2E5 | 3E4 | 7E3 Campylobacters | 70 | 20 | 0.2 | 0 | 0 | 0 Salmonellae | 20 | 8 | 0.1 | 0.02 | 0.01 | 0 Enteroviruses** | 1E4 | 6E3 | 1E3 | 400 | 50 | 9 Rotaviruses** | 800 | 200 | 70 | 30 | 10 | 3 Bacterial nos. per 100 ml ** Viral nos. per 10 litres ***Slide repeated for clarity***	The enteroviruses were reduced from 10,000 per 10 litres to just under 10 per 10 litres in the final effluent, and the rotaviruses from 800 per 10 litres to around 3 per 10 litres. These results show that when the effluent contained ~7000 faecal coliforms per 100 ml, there were no campylobacters, no salmonellae and only very small numbers of viruses.
3.	**Removal of *Vibrio cholerae* O1 in WSP** Northeast Brazil, 25–27°C Series of 10 ponds (1-day anaerobic pond followed by nine 2-day ponds) Sample | *V. cholerae* (per litre) | FC (/100 ml) | BOD (mg/l) | Chl.a (µg/l) Wastewater | 485 | 2E7 | 215 | -- An. pond | 28 | 8E6 | 79 | -- Fac. pond | 8 | 3E8 | 63 | 253 1st mat. pond | 3 | 1E6 | 53 | 113 2nd | 8 | 5E5 | 43 | 70 3rd | 3 | 2E5 | 36 | 115 4th 11 days | 0 | 6E4 | 31 | 113 It's clear from these results that most *V. cholerae* are removed in the anaerobic pond, but why is this? To answer this, we did some lab. work in Brazil and found that *V. cholerae* is very sensitive to quite low sulphide concentrations, ~3 mg/l. In anaerobic ponds the sulphide concentration is ~10−12 mg/l, so there's more than enough to kill off most of the cholera vibrios.	We were also able to study the removal of *Vibrio cholerae*, the causative agent of cholera, soon after the current cholera pandemic showed up in northeast Brazil in the 1980s. The pond system we had was a little odd: a 1-day anaerobic pond followed by several 2-day ponds. The number of *V. cholerae* was just under 500 per litre in the raw wastewater, and most were removed in the anaerobic pond, and thereafter a little in each of the next five ponds, becoming undetectable in the effluent of the fourth 2-day pond, after a total retention time of 11 days. At this point the faecal coliform count was 60,000 per 100 ml. **[Continued opposite]**
4.	**Removal of intestinal nematode eggs in a series of five ponds, Northeast Brazil, 25 °C** | Retention time (days) | Eggs per litre Raw w'w | – | 804 A1 | 6.8 | 29 F1 | 5.5 | 1 M1 | 5.5 | 0 M2 | 5.5 | 0 M3 | 5.8 | 0 Raw w'w | – | 1489 A1 | 2.0 | 45 F1 | 1.6 | 18 M1 | 1.6 | 7 M2 | 1.6 | 5 M3 | 1.6 | 2	We also monitored the removal of human intestinal nematode eggs, the eggs of *Ascaris*, *Trichuris* and the human hookworms; these are the geohelminths which comprise Category C of the unitary environmental classification of water- and excreta-related communicable diseases. Most of these eggs were removed in the anaerobic pond, with the remaining small numbers being gradually reduced in the subsequent ponds.

5.	**Mechanisms of Pathogen Removal in WSP** **Helminth eggs & protozoan cysts:** sedimentation **Viruses:** adsorption & sedimentation (probably) **BACTERIA:** Several possible responsible factors − eg: * **Time & Temperature** * Algal toxins * **High DO** * Low CO_2 * **High pH** ———————————————————— With the **bacteria** there are several candidate removal mechanisms. Firstly, there's time and temperature: whatever bacteria are going to do, grow or die, they do it more quickly at higher temperatures, and they require more time to grow or die at lower temperatures. Secondly, some algae excrete toxins which can kill faecal bacteria. Thirdly, ponds are low in CO_2 and high in dissolved oxygen [DO], and this is the exact opposite of what occurs in their natural habitat − the gut. And then there's high pH and we know that high pH values occur in ponds as a result of algal activity; the in-pond pH can be above 9, even above 10.	Now we come to the actual mechanisms of excreted pathogen removal in ponds. Well, it's pretty clear that **helminth eggs** are simply removed by sedimentation, and the same is true for **protozoan cysts** although, because these are much smaller than helminth eggs, their sedimentation rate, and hence their removal, is much lower. With the **viruses** we are less certain, because there are very few data on viral removal in ponds; but it seems that they are mostly removed by adsorption on to settleable solids and thus by sedimentation when these solids settle. Virus have quite a high surface charge, so at least this is a reasonable hypothesis. They also adsorb on to algae, we know this from scanning-electron-micrographs, and so they will settle with the algae as these algae die. **[Continued opposite]**
6.	**Removal of *E. coli*** 1. Marais (1974) **Time and temperature only:** $N_e = N_i / [1 + (2.6(1.19)^{T-20}\theta)]$ 2. Northeast Brazil **Time, temperature & organic loading**	Marais considered time and temperature only, and his design equation for *E. coli* removal is given on the slide. And we know from our work on facultative ponds in northeast Brazil that organic loading, BOD loading, is also important in this type of pond.
7.	□ DO ▲ pH ● Temp. ********** ■ NH_3-N ♦ Total P ◇ Sol. P ********** ● BOD ○ SS ********** △ Chl. *a* ▲ FC  **Diurnal variation in fac. pond effluent quality − northeast Brazil**	***Note:*** *the transcript for this slide is given on the next page together with a larger version of the central diagram.*

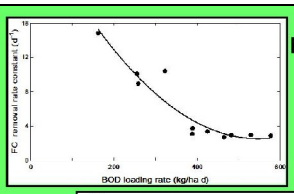
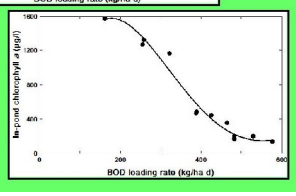
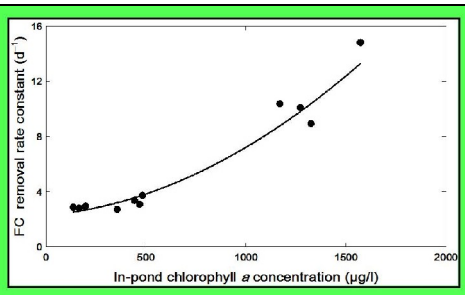
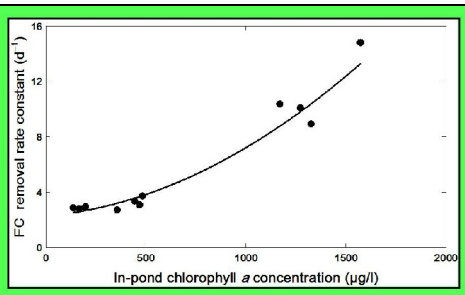


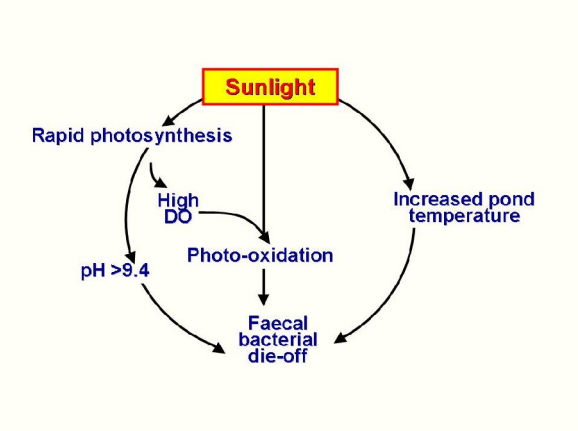
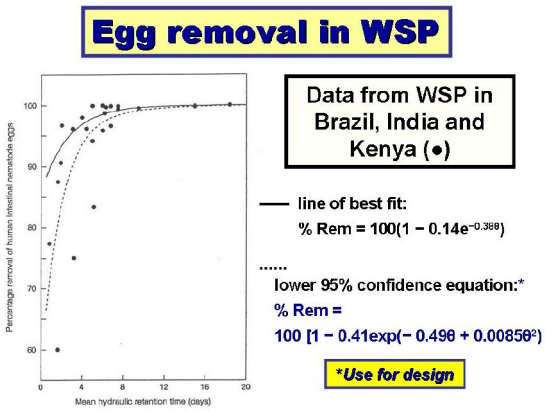
This somewhat complicated slide tells us how the values of several parameters in the effluent of a primary facultative pond, one of the ones we studied in northeast Brazil, varied over a 24-hour period. The *x*-axis is <u>time</u>, starting at 8 am one day and finishing at 8 am the following day.

In the box at the bottom of the central diagram we're plotting algal biomass as chlorophyll *a*, the open triangles, and faecal coliform numbers, the solid triangles. You can see that when the chlorophyll is low (at night), faecal coliform numbers are high; and *vice versa*, albeit with a slight lag in the decrease of faecal coliforms. So high chlorophyll = high algae and = low faecal coliforms. So are the algae doing something to kill off the bacteria?

The box immediately above the bottom box gives the data for BOD and suspended solids. Both these peak at the same time as the algal chlorophyll peaks; and this is what we would expect as most of the effluent BOD and suspended solids is due to the algae in the effluent. In the box second from the top we see the same peak with total phosphorus, the solid diamonds, but not of course for the other two parameters in this box, ammonia and soluble phosphorus, as these are not associated with the algae.

Now in the top box we have the data for temperature, the solid circles; for pH, the solid triangles; and for dissolved oxygen, the open triangles − our DO meter had a maximum reading of 20 mg/l, which is why there's no peak shown for DO, although clearly in reality there was one. All three of these parameters peaked at the same time as the chlorophyll, and this tells us that algal activity increases with increasing temperature, and that both pH and DO increase with increasing algal activity; and, as we've seen already, increasing algal activity means a fall in faecal coliform numbers. We can't be sure from the data here, but it's likely that either the high pH or the high DO, or both, are responsible for the decrease in faecal coliform numbers.

8.	  **Primary fac. ponds Northeast Brazil ~25 ℃** **So….**	Here we have two plots of results from that facultative pond in northeast Brazil: at the top the plot of the first-order rate constant for faecal coliform removal, k_B in day^{-1}, against the BOD loading in kg/ha day. And at the bottom the plot of the **in-pond**, not effluent, chlorophyll *a* concentration in µg/l against the loading rate. So we can …
9.		simply plot the first-order rate constant against the **in-pond** chlorophyll concentration and we get, not quite but almost, a straight line.
10.	 **Thus the pond algae are largely responsible for FC (and other excreted bacterial) removal**	This tells us for sure that the algae are doing something which kills off the faecal coliforms and presumably other faecal bacteria as well.
11.	**4. Laboratory work in UK and fieldwork in Portugal and Brazil:** ➢ **High pH, >9.4** ➢ **High light intensity with high DO** ❑ **Conceptual model:**	In lab. studies here in Leeds and in fieldwork in Portugal and Brazil we found that there were two main factors responsible for faecal bacterial die-off in ponds: firstly, high pH, and pH values >9.4 are rapidly lethal to all faecal bacteria (with the exception of *Vibrio cholerae* which tolerates high pH quite well, so it's fortunate that it's killed off quickly by the sulphides in anaerobic ponds). And secondly, the combination of high DO and high visible light intensity.

12.	 _____ *T. P. Curtis, D. D. Mara and S. A. Silva (1992). Influence of pH, oxygen & humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water. *Applied and Environmental Microbiology* **58** (4), 1335−1345.	This is the conceptual model we developed for faecal bacterial removal in facultative and maturation ponds, but remember it doesn't apply to *Vibrio cholerae*. The sun clearly has the most important role: solar energy heats up the pond, so the faecal bacteria die more quickly; and it's the raw energy for photosynthesis. Rapid photosynthesis raises the pH to above 9.4, which leads to rapid faecal bacterial die-off. And finally, the combination of high visible light intensity and high dissolved oxygen leads to rapid photo-oxidative death of the faecal bacteria.[*]
13.		Finally, we looked at helminth egg removal in ponds in Brazil, India and Kenya. The slide shows the plot of egg numbers, on the *y* axis, against pond retention time, on the *x* axis. The upper solid line is the line of best fit, and the lower dotted line is the boundary of the lower 95% confidence interval, and this is what we use for design. This equation allows us to design a pond system specifically for helminth egg removal and we need to be able to do this if the effluent is going to be used for crop irrigation.