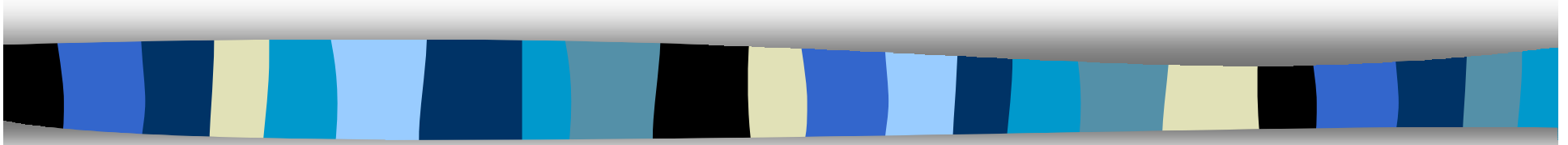


FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction



Dr. Barbara Hartley Grimes
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Onsite Wastewater Section
DEH/ NCDENR
18th Annual Onsite Conference



FIXED FILM WASTEWATER TREATMENT

✍ Wastewater treatment

based on attached microbial growth

✍ Support materials

a wide variety

✍ Two important factors

- flow of wastewater
- size of support material particles



Variety of Fixed Film Filters



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

Fixed media

- sand
- gravel
- plastic
- activated carbon
- peat
- other



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

“Trickle” Filters

- sand
- gravel
- plastic
- activated carbon
- peat
- other



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

Single Pass Filters

- Peat
- Pea gravel
- Crushed glass
- Experimental media
- Sand (the best understood and most predictable)



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

RMF Recirculating Media Filters

- Sand (most widely used)
- Peat
- Textiles



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

✍ Rotating Biological Contactors

Rotating cylinders with attached biofilm
in wastewater flow

✍ Submerged Filters

Downflow and Upflow



Fixed Film Filters Their Biofilms in Relation To Pathogen Reduction



FIXED BIOFILM WASTEWATER TREATMENT

Biofilms

- ✍ Biofilms are highly stratified with microbes and matrix
- ✍ Microorganisms attach to solid materials
- ✍ Microorganisms can reach high concentrations
- ✍ Microbial growth rates depend upon
 - flow rates
 - size and geometric configuration of particles
(more surface area of particles = more growth surface)



FIXED BIOFILM WASTEWATER TREATMENT

Biofilms

Biofilm/Zoogleal (animal gunk) film formation

- bacteria
- fungi
- algae
- protozoa
- nematodes
- rotifers
- annelid worms (mini- aquatic earthworms)
- insect larvae/filter fly larvae

FIXED BIOFILM WASTEWATER TREATMENT

Biofilms/Zoogleal Film Formation & Function in Pathogen Reduction

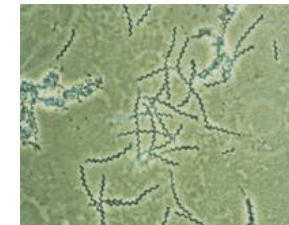
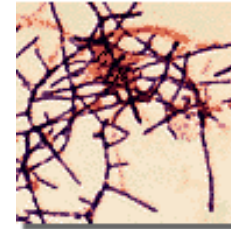
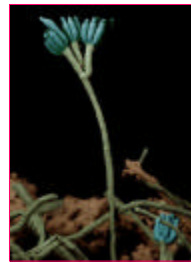
 **Bacteria**

 **Fungi**

 **Algae**

 **Protozoa**

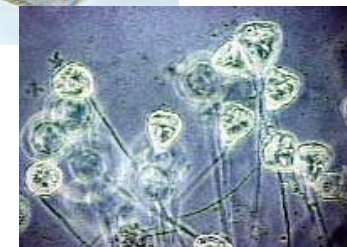
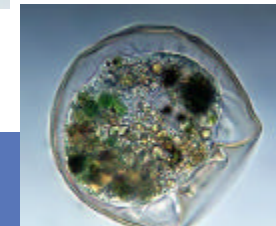
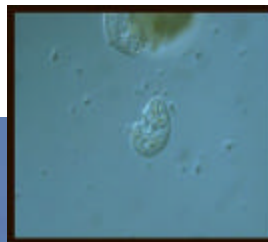
(grazers/predators/absorbers)



cosmic light web



www.microscopy-uk.org.uk



FIXED BIOFILM WASTEWATER TREATMENT

Biofilms/Zoogleal Film Formation & Function in

Pathogen Reduction

Nematodes

- feed on floc

- ingestion
- farm biomat



<http://www.yorkcity.org/>

[cityservices/wwtp/micro.htm](http://www.yorkcity.org/cityservices/wwtp/micro.htm)

Rotifers

- (+)ingestion/filtering
- (-)protection of pathogens



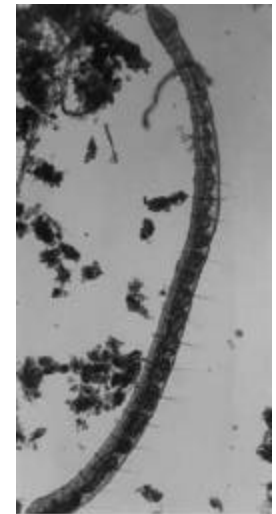
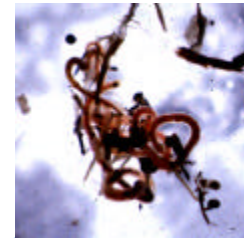
microscopy-uk.org.uk

FIXED BIOFILM WASTEWATER TREATMENT

Biofilms/Zoogleal Film Formation & Function in Pathogen Reduction

Annelid worms (mini-earthworms)

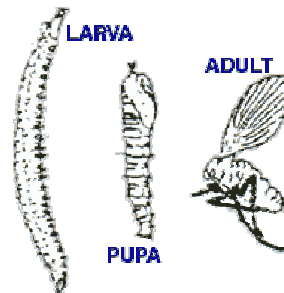
- plow through floc
- ingest floc



<http://www.yorkcity.org/cityservices/wwtp/micro.htm>

Filter fly larvae

- graze biomat
- promote biomat turnover



(&adults)



<http://www.arrowpestcontrol.com/pages/drainf>



FIXED BIOFILM WASTEWATER TREATMENT

Biofilms/Zoogleal Film and Sloughing

- ✍ As biofilms develop organisms in the deepest layers lose access to nutrition and may die -off
- ✍ Then the biofilm may/does slough off
- ✍ The fragments of biofilms carry:
 - the outer attached treatment organisms
 - any attached pathogens with them.



FIXED BIOFILM WASTEWATER TREATMENT

Biofilms/Zoogleal Film Function in Pathogen Reduction

Filtration (packed beds)

Adsorption to biofilm matrix

- (some layers polyanionic)
- pathogens can be stuck in the “gleal goo”

Biofilm organisms

- eat/ingest/digest some pathogens
- however the pathogens can be protected inside body
- can overgrow and clog

Sloughed biofilms

- end up in clarifier
- in systems without clarifier ---on to dispersal



Wastewater Pathogens General Groups



FIXED BIOFILM WASTEWATER TREATMENT

Pathogens : General groupings

- ✍ For this presentation all of the following are called PATHOGENS

- ✍ Sometimes they are divided into two groups:
 - Those called Pathogens
 - Viruses
 - Bacteria
 - Fungi
 - Those called Parasites
 - Protozoa
 - Helminths (Roundworms & Tapeworms)



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Pathogens : General Size ranges

† Virus

particles

particle size 20-100nm

† Bacteria

spores

spore size 1-3-microns

† Fungi

spores

spore size ~5 microns

† Protozoa

cysts

cyst size 10-100's microns

† Helminths

roundworm (ova)eggs 50-100 microns// worm inches - 1 +ft

tapeworms (ova)eggs 50- 100 microns



FIXED BIOFILM WASTEWATER TREATMENT

*Pathogens : Infectious Doses*****

† Virus

Various 1-10 particles

† Bacteria

Shigella 10 - 100 spores

cholera 1,000 - 10,000,000

Campylobacter 100-1,000,000

† Protozoa

Cryptosporidium 1 oocyst

(10 in healthy volunteers)

Giardia 10-100 oocysts

† Helminths

roundworms 1- 10 eggs

(embryonated)

tapeworms 1-800 eggs



Wastewater Pathogen Indicators



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Criteria for Ideal Microbial Indicator

Wastewater Microbiology: 2nd edition 1999

- ✍ Member of the intestinal microflora of warm-blooded animals
- ✍ Should be present if pathogens are present, and absent in uncontaminated samples
- ✍ Should be present in greater numbers than the pathogens
- ✍ Should be at least equally resistant as the pathogens to environmental insults and to disinfection in water and wastewater treatment(s)
- ✍ Should not multiply in the environment
- ✍ Should be detectable by means of easy, rapid, and inexpensive methods
- ✍ Should be nonpathogenic



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

Microbial Indicators of Fecal Contaminants

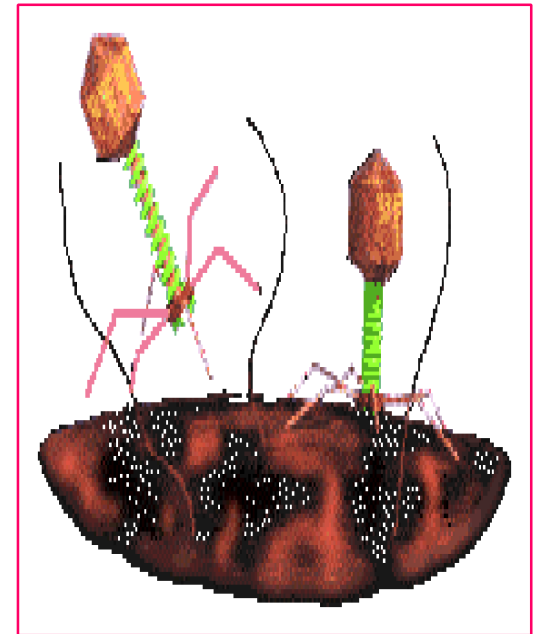
- ✍ **Viral** :Bacteriophage (viruses)
- ✍ **Bacterial** (used for the rest of pathogens):
 - Total Coliforms
 - Fecal Coliform Bacteria
 - Fecal Streptococci
 - Anaerobic Bacteria
 - Clostridium , Bifidobacteria, etc.

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Pathogen (microbial) Indicators

VIRAL INDICATORS:

- environmental detection
- assessing pathogen removal by wastewater treatment
- F-specific bacteriophages
indicator of wastewater
- Bacteriophage of Bacterioides spp.
Chlorine resistant/
some wastewater usefulness



Bacteriophage *from Cells Alive*



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Pathogen (microbial) indicators

Total Coliforms (bacteria)

- Aerobic and facultative anaerobic , gram neg, rod shaped, non-spore formers
- Include Escherichia coli, Enterobacter, Klebsiella etc.
- Less sensitive than viruses or protozoa to env/ disinfection
- Some may regrow (not all detected)

Fecal Coliforms (bacteria)

- All thermotolerant and ferment lactose (44.5C)
- Vertebrate Guts
- E. coli, Klebsiella, etc. :
- Survival pattern similar to path bacteria
- Less resistant than viruses or protozoan cysts to disinfection and environmental conditions



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen (microbial) indicators

Fecal Streptococci

- Streptococcus - 4 species
- subgroup enterococci : S. faecalis and S. faecium
useful for viruses, especially in sludge and seawater

Anaerobic Bacteria

- Clostridium perfringens (viruses & protozoan cysts)
 - useful as a tracer
- Bifidobacteria fecal indicator in the environment
- Bacterioides sp., fecal contamination of water



SOME EFFLUENT NUMBERS



Pathogen Concentration in Raw Wastewater / 100ml

✍	Virus		100-50,000 particles
✍	Bacteria	<u>Shigella</u>	1- 1,000
		<u>Salmonella</u>	400-8,000
✍	Protozoa	<u>Cryptosporidium</u>	1-10,000
		<u>Giardia</u>	50-10,000
✍	Helminths		
		– roundworms	1-1,800
		– tapeworms	not a good number available



Pathogen Concentration in Septic Tank Effluent / 100ml

✍ Virus 0 - 10⁵ e.g. hepatitis, polio, coxsackie, coliphage

✍ Bacteria 10⁶ - 10⁸ e.g. Salmonella, Shigella, etc..

✍ Protozoa

✍ Helminths

– roundworms

– tapeworms



Pathogen Indicators

Concentration in Septic Tank Effluent / 100ml

Viruses

specific 0 - 10^7 pfu (episodic high levels)

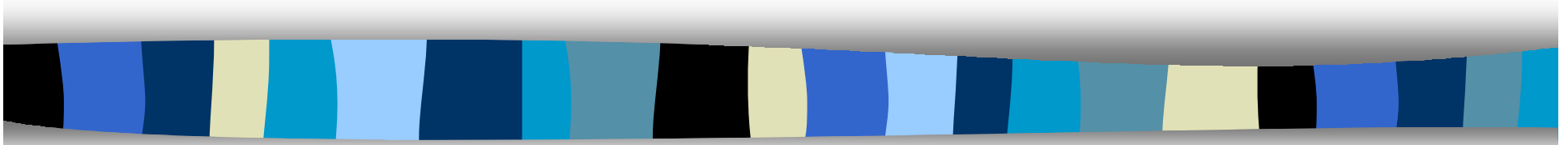
Fecal Coliforms

10^5 - 10^8 (EPA 600/2-78)

Fecal Streptococcus

10^4 - 10^5 (EPA 600/2-78)

FIXED FILMS FILTERS



*PATHOGEN REDUCTION
DATA*



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

TRICKLING FILTER SYSTEMS

- septic tanks
- fixed film reactor
- clarifier (excess biomass)
- optional recirculation of effluent



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

Trickling filter

- EPA manual -
 - 1 - 2 log reduction fecal coliforms
 - says require minimum effluent disinfection for surface effluent requirements
- Bitton summarizes
 - that removal rate generally lower than activated sludge



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction TRICKLING FILTERS Viruses/ viral indicators

- Viruses
 - generally low and erratic removal
 - e.g. 59 - 91%
 - eg 0 - 20 % removal . Yet high coliform >90%
 - e.g. Efficiency of viral removal lower than coliforms
- Bacteriophage
 - erratic also
 - 40 - 90%
 - depends upon season



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

TRICKLING FILTERS : **Pathogen removal erratic**

Virus : 59 - 95% ; phage 40 - 90%

† fecal coliform indicators -e.g. (0-20% viruses ; >90%fecal)

† Lewis, Austin, Loutit, Sharples (1986) no significant red. In fecal coliforms or viruses.

Bacteria

- vary from 20 - >90%, depending upon the operation
- Salmonella 73 - 95% (Feacham et al 1983)
- gen. 20-90% - some pathogenic species removal lower

Protozoa

- Giardia, Entamoeba 74-91%



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

✍️ TRICKLING FILTERS - Parasites

- Protozoan Entamoeba histolytica
 - 71 - 91% in India
- Protozoan Giardia lamblia
 - similar removal rates as E. coli
 - 4- 44 cysts/L in the effluent



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

TRICKLING FILTERS

(General according to Gabriel and Bitton 1999)

- Low and erratic removal of pathogens and parasite
- filtration rate great affects the removal rate, lower rate = greater removal



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

Rotating Biological Contactors

- not much is known about pathogen removal
- one study by Sagy and Kott 1990 - one log removal of fecal coliforms and Salmonella
- blue green algae helped? Reduce #'s

Sagy and Kott 1990. Efficiency of rotating biological contactors in removing pathogenic bacteria from domestic sewage. Water Res. 24:1125 - 1128.



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

- ✍ Rotating Biological Contactors (called rotating trickling filter)
 - Clarke and Chang 1975 *Applied Microbiology* 30:223 - 228
 - partially removed three types of viruses
 - low flow rates - 85-94% removal
 - hi flow rates - 59-81% removal
 - in this system fecal coliform and fecal streptococci are appropriate for estimating some viral reductions.



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

SAND FILTERS - single pass

- viruses from 0- 10^7 to 0 - 10^7 pfu / 100 ml (episodic high) (Siegrist 2001)(no reduct.)
- bacteria depending upon sources/media size:
 - reduced to 10^3 - 10^4 fecal coliform 100 ml
 - reduced to 10 - 100 fecal coliform / 100 ml
 - from 10^6 - 10^8 to 10 - 10^3 FC (Siegrist 2001)
 - (under drains) reduced to < 200 cfu/100ml Gustavson etal.(works with high cleaning rate)
 - June 2001 - Lake Wash and Duluth MN -
 - » 4 sand filters <200 cfu/100ml



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

SAND FILTERS - single pass (cont)

- Protozoa (assume pore size < protozoan)
 - Use for Giardia in water supplies
 - Used for Cryptosporidium in water supplies - careful with the backwash (Milwaukee)
- Helminth eggs <1/L Mexico City study(1999)



FIXED BIOFILM WASTEWATER TREATMENT Pathogen Reduction

RMF Recirculating Media Filters

- Bitton: Because of the larger media size - does not remove fecal coliforms as effectively as single pass. Need coarse media for higher loading rates.
- Christopherson, Gustavson, Anderson. Found sand RMF reduction from $10^9 - 10^{12}$ to 5,000 - 10^5 fecal coliform - still need be applied to soil infiltration systems (no biomat forms)
- recirculating sand - 2 systems MN2001 <200 fc/100ml.



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

PEAT FILTERS

- Gustavson (MN) < 1,000 cfu/100ml FC
- Modular peat- Geerts, et al,2001
 - fc 94-99% reduction
 - viral 0-20% reduction
- N. Small Flows -2001 10^6 to 10^3 99%FC
- Lake Washington/Duluth 2001 4 peat <200fc/100ml
- City of Austin 2001 3-4 log reduction



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

FOAM : TEXTILE FILTER EFFLUENT

– Viruses

- 0-10⁷pfu/100ml tank
- 0 - 10⁷ pfu / 100ml filter effluent
- episodically high

– bacteria FC -

- 10⁵ - 10⁸ /100ml in tank to
10 - 10³/100 ml in filter effluent

® Fuzzy Film for helminth eggs Mexico city study effluent <1 egg/l



FIXED BIOFILM WASTEWATER TREATMENT

Pathogen Reduction

- ✍ Sequential units
 - fixed biofilm
 - suspended biofilm



Some final thoughts.....

- ✍ Adsorptions to solids most effective in reducing viral loads
- ✍ Larger organisms better reduced with smaller pore sizes - like sand filtration
- ✍ Minnesota as of June 2001 - developing protocols for viral pathogen surrogates and bacterial surrogates....for methods to address need more of the comparatives studies
- ✍ Need information on infectivity of pathogens after treatment, not just the numbers.
- ✍ Developing pathogen mimics for all pathogens a new study in NC.

Pooper Scooter Highboy - - - ->

Racing model
for the daring senior



