# **'Sewage fungus'** A field and microscopic guide

Tim Geatches<sup>1</sup>, Julie Gething<sup>2</sup> and Graham Rutt<sup>2</sup>



<sup>1</sup> Environment Agency
 <sup>2</sup> Cyfoeth Naturiol Cymru / Natural Resources Wales

Version 3 March 2014

# Contents

- 4 Introduction
- 5 References
- 6-7 Main sewage fungus taxa and growth forms
- 8 Assessment methodology
- 9 Examples of sewage fungus densities
- 10 Sewage fungus recording sheet

<u>Main sewage fungus taxa</u>

- 12-13 Sphaerotilus natans
- 14-15 Beggiatoa alba
- 16-17 Zoogloeal bacteria
- 18-19 Fusarium aquaeductuum
- 20-21 Geotrichum candidum
- 22-23 Leptomitus lacteus
- 24-25 Carchesium polypinum

Other sewage fungus taxa

- 27 *Flexibacter* spp.
- 28-29 Thiothrix II
- 30 Achyla spp.
- 31 Flavobacterium spp.

Taxa commonly mistaken for sewage fungus

- 33 Didymosphenia spp.
- 34 Leptothrix ochracea

# Introduction

- Mainly heterotrophic micro-organisms
- Sensitive indicators of organic pollution
- Matrix of filamentous bacteria, fungi and/or stalked protozoa
- Can be present either as an almost pure monoculture of one species or as a mixed growth of several species
- Growth form varies due to species type and severity of organic pollution
- Species present can indicate type of organic pollution
- Colour varies widely from white to brown or pink
- Can be used to identify organic pollution sources several kilometres away
- Assessment below substrate particularly useful as lack of competition with autotrophic organisms
- Microscopic examination required to confirm identification

# References

Curds CR, Gates MA and Roberts DMcL (1983) British and Other Freshwater Ciliated Protozoa: Part II, Synopsis of the British Fauna No. 23.

Curtis EJC (1969) Sewage fungus: its nature and effects, Water Research, Vol. 3, 289-311.

Eikelbloom DH (2000) Activated Sludge Information Systems <u>www.asissludge.com</u>

Gray NF (1982) A key to the major slime-forming organisms of 'sewage fungus', J. life Sci. R. Dubl. Soc. 4, 97-102.

Gray NF (1985) Heterotrophic slimes in flowing waters, Biol. Rev., 60, 499-548.

Tomlinson TG and Williams IL (1975) Fungi. In Ecological Aspects of Used Water Treatment. I. The Organisms and their Ecology, pp 93-152.

Van Veen WL, Mulder EG and Deinema MH (1978) The Sphaerotilus-Leptothrix Group of Bacteria, Microbiological Reviews, 329-356.

# Main sewage fungus taxa and growth forms

The taxa below are the main sewage fungus organisms found in freshwaters and typical growth forms. One or more taxa can be present in sewage fungus outbreaks and microscopic examination is required to confirm identification. Further information on growth forms, environmental conditions and key identification features can be found on pages 12 to 25.





**Sphaerotilus natans** Filamentous bacterium Slimy fronds

**Zoogloeal bacterium** Ill defined taxonomically

Jelly-like gelatinous mass

**Beggiatoa alba** Filamentous bacterium Thin white film

# Main sewage fungus taxa and growth forms



#### Fusarium aquaeductuum

Filamentous fungus Imparts pink or red colouration



## Geotrichum candidum

Filamentous fungus Soft texture loosely following contours of stones





Leptomitus lacteus

Filamentous fungus Overlapping cotton wool-like streamers

**Carchesium polypinum** Stalked protozoan Short 2-3mm tufts

# Assessment methodology

Record cover and density above and below substrate as follows:

<u>Cover</u> None Local - <30% Widespread - 30 – 60% Extensive - >60%

**Density** 

Trace - Present but only just detectable Thin - Obvious presence but substrate not obscured Thick - Thick enough to fully obscure substrate Massive - Occupies a significant proportion of the water column

#### **Recording**

It is also useful to record associated parameters on the recording sheet shown on page 10. All parameters follow BIOSYS methods. Take photographs to show cover, density and growth form. The standard issue Pentax Optio WG series camera is particularly useful, as it can take close up underwater photographs. Photographs can also be used as exhibits in witness statements.

#### Samples for analysis

Take representative samples of sewage fungus in a small amount of water. A 30ml plastic polypropylene vial (shown right) with a conical base is ideal for samples. Write sample site details on side of vial with a permanent pen. If immediate analysis is not possible keep samples refrigerated.



## Examples of sewage fungus densities



Trace Present but only just detectable

Thin Obvious presence but substrate not obscured

Thick Thick enough to fully obscure substrate

Massive Occupies a significant proportion of the water column

# Sewage fungus recording sheet

Water Body:			Site ID:		Date:		Time:		
Site Name:			NGR (GPS):						
Photos: Yes / No			Sampler:						
TURBIDITY: Tick box	ODOUR: Tick box SEWAGE LI			E LITTER: Tick box		GENERAL COMMENTS:			
CLEAR: Water not visibly turbid	NONE: No discernible odour	NONE: No litter present							
SLIGHT : Visible turbidity but no effect on light penetration	SLIGHT: Odour detectable within the channel	LOCAL: <30% of possible area							
MODERATE: Significant effect on light penetration	STRONG: Odour obvious within the channel or noticeable away from it	WIDESPRE possible a	SPREAD: 30 - 60% of the ble area						
HIGH: Visibility limited to 10cm depth		GROSS: > area	>60% of possible						
VERLAYING SILT COVER: Tick box for cover and density			OCHRE: Tick box for cover and density						
NONE: None present	TRACE: Just detectable by eye		NONE: None present			TRACE: Just detectat	ole by eye		
LOCAL: Occasional patches: <30% of area	THIN: Obvious presence but fine details of substrate not obscured		LOCAL: Occasional patches: <30% of area			THIN: Obvious presence but fine details of substrate not obscured			
WIDESPREAD: 30 - 60% of area	THICK: Coats stones and obscures fine details of substrate		WIDESPREAD: 30 - 60% of area			THICK: Coats stones details of substrate	and obscures fine		
EXTENSIVE: >60%	MASSIVE: Fills interstices between gravel sized particles		EXTENSIVE: >60%			MASSIVE: Fills interst sized particles	tices betw een gravel		
SEWAGE FUNGUS ABOVE STONES: Tick box for presence and density			SEWAGE FUNGUS BELOW STONES: Tick box for presence and density						
NONE: None present	TRACE: Present but only just detectable		NONE: None present			TRACE: Present but o	only just detectable	5	
LOCAL: Occasional patches:	THIN: Obvious presence but substrate not		LOCAL: Occasional patches:		THIN: Obvious presence but substrate no		nce but substrate not		
WIDESPREAD: 30 - 60% of	THICK: Thick enough to fully obscure the		WIDESPREAD: 30 - 60% of			THICK: Thick enough to fully obscure the substrate			
EXTENSIVE: >60%	MASSIVE: Occupies a significant proportion of the water column		EXTENSIVE: >60%		MASSIVE: Occupies a significant proportion of the water column				
SEWAGE FUNGUS ABOVE STON	SEWAGE FLINGUS BELOW STONES: Field comments								

Lab use only:

Analyst:	Laboratory:		Date:	Time:				
SEWAGE FUNGUS ABOVE STONES: Lab analysis	SEWAGE FUNGUS BELOW STONES: Lab analysis							

# Main sewage fungus taxa





### Sphaerotilus natans

- Filamentous bacterium
- Requires aerobic conditions

- Requires flowing water
- Prefers pH between 6.8-9.0
- Growth form variable from short tufts to slimy fronds
- Tolerates a wide range of organic conditions





#### Sphaerotilus natans

•

•

•

- Filamentous bacterium
- **Unbranched filaments** with rod shaped cells
- 1-2 µm wide filaments
- Characteristic beaded • appearance especially at ends of filaments
  - Cells within a closely fitting sheath
  - Sheath varies in thickness and can obscure beaded appearance
  - False branching can be present
  - In lower organic conditions false branching common and thin sheath typical
  - In higher organic conditions false branching weakly developed or absent and thick sheath typical
  - Zigzag growth may indicate an intermittent discharge



### Beggiatoa alba

- Filamentous bacterium
- Forms a thin white film on surface of substrate
- Prefers low dissolved oxygen
- Prevalent in high organic conditions
- Oxidises hydrogen sulphide

- Characteristic of slow flowing waters
  - Can be found as a monoculture in faster flowing waters where it forms very long fine filaments
  - Tolerant of saline conditions



## Beggiatoa alba

- Filamentous bacterium
- Unbranched filaments with rod shaped cells
  - 3-4 µm wide filaments
- Motile filaments
  - Sulphur granules stored within cells increasing in number with age



## Zoogloeal bacteria

Not well defined taxonomically

•

- Jelly-like forming thick gelatinous mass
- Restricted to slow flowing waters
- Prevalent in high organic conditions



## Zoogloeal bacteria

- Not well defined taxonomically
- Cells embedded in a gelatinous matrix
- Forms lobed and unlobed spherical masses



## Fusarium aquaeductuum

- Filamentous fungus
- Requires high dissolved oxygen
- Prefers acid pH
- Imparts pink or red colouration to growth
- Rarely dominant



## Fusarium aquaeductuum

•

•

•

•

- Filamentous fungus
- Septate branched filaments
- 5 µm wide filaments
- Boat-shaped spores freely produced



R

10.00



#### Geotrichum candidum

- Filamentous fungus
- Grey to brown in colour
- Soft texture
- Growth loosely follows contours of stones
- Able to oxidise lactic acid
- Growth supported by ammonia, asparagine and urea
- Often associated with dairy products, silage and pickling wastes
- Prevalent in high organic conditions



#### Geotrichum candidum

- Filamentous fungus
- Septate branched filaments
- 5-10 µm wide filaments
- Dichotomous branching
- Brick-shaped arthrospores may be present







## Leptomitus lacteus

- Filamentous fungus
- Forms long characteristic streamers with overlapping cotton-wool like growths
- Requires high dissolved oxygen
- Prefers moderate to fast flow
  - Prefers acid pH



#### Leptomitus lacteus

- Filamentous fungus
- Non-septate coarse branching filaments
- 8-15 µm wide filaments
- Characteristic constrictions at intervals with spherical cellulin plugs
- Cellulin plugs block the constrictions and prevent cytoplasm flowing away from the growing apices of the filaments



## Carchesium polypinum

- Stalked protozoan
- Bacteria feeder
- Growth form of short tufts of 2-3 mm
  - Prevalent in low organic conditions and recovery zones





**Discontinuous** 

Peristomial lip bulges out

Sinuous myoneme

#### Carchesium polypinum

- Stalked protozoan
- Inverted bell-shaped zooids at ends of stalks
  - Branched stalks
- Zooids can become separated from stalks, especially in degraded samples
- Stalks can contract independent of each other
- Stalks contract spirally
- Stalk with discontinuous myoneme
- Sinuous myoneme
- Zooids 100-125 µm long
- Zooid peristomial lip bulges out
- Zooid with C-shaped macronucleus
- Zooid with smooth surface

# Other sewage fungus taxa



## Flexibacter spp.

- Filamentous bacterium
  - Unbranched filaments
- Motile with whole filament bending and flexing
- No sulphur granules in cells
- Rarely dominant



#### Thiothrix II

- Gammaproteobacteria
- Uses low molecular carbon sources (shortchain fatty acids and alcohols) as well as reduced sulphur compounds
- Does not grow in anoxic conditions
- Found associated with cows bedded on waste gypsum in West Wales



## Thiothrix II

- Straight or bowed filaments
- 1 um wide filaments
- False branching and rosette formation
- Sulphur granules evident
- No obvious septa
- Not motile
  - Filaments do not taper

#### Achyla spp.

- Filamentous
  phycomycete fungus
- Similar to *Leptomitus* but wider filaments and no spherical cellulin plugs
- Tends to grow near source of silage effluent with which it is closely associated

#### Flavobacterium spp.

- Filamentous bacterium
- Unbranched filaments
- 0.5-1 µm wide filaments lying parallel to each other
- Filaments lying close together separated by 10-12µm
- Individual cells 0.5-1 µm wide by 10-50µm long
- Occasionally forming pink
  or yellow growths
- Requires organic nitrogen
  source for growth
- Rarely dominant

# Taxa commonly mistaken for sewage fungus



### Didymosphenia spp.

- Stalked diatom
- Generally occurs as offwhite cohesive lumps or mats
- Prefers cool oligotrophic waters
- Not slimy

•

- Feels spongy and scratchy, like cotton wool
- Does not indicate pollution
  - Obvious 'coke-bottle' shaped diatom cells embedded in a mass of extracellular stalks



### Leptothrix ochracea

- Filamentous iron bacterium
- 2-3 µm wide filaments
- Grows in slow flowing waters high in ferrous iron and low in organic matter
- Requires ferrous (iron) salts, oxygen and carbon dioxide for growth
- Growth of bacterium results in accumulation and sedimentation of orange-brown ferric hydroxide in sheath surrounding filament
- Sheath has smooth surface
- Growth results in many empty sheaths