Filamentous organisms and foam formers

Caroline Kragelund & Per Halkjær Nielsen
Section of Biotechnology, Aalborg University, Denmark

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Content of talk

- Background
- Identity of filamentous bacteria
- Ecophysiology methods
  - What do they eat
  - Under what electron acceptor conditions
  - Storage potential, enzymatic degradation
- Ecophysiology of gene probe defined filamentous bacteria
- Formulating control strategies
  - Specific for a certain filamentous bacteria
  - Non specific

Cause of bulking and/or foaming incidences
- Hydraulic overload
- Construction
- Overload of lipids
- Toxication
- Physio-chemical conditions
- Non-filamentous bacteria
- Filamentous bacteria

Measurements to detect bulking
- Sludge volume index (SVI)
- Filament index (scale 0-5)
- Filamentous bacteria
  - Abundance of filaments (scale from 1-5)
  - Identity based on cell shape, cell diameter, shape of filament, staining properties
- 26 different types in municipal WWTP based on morphological characters
- 20 new types from industrial WWTP

- Conventional identification
  - Noli
  - Ppx3
  - Meg968
  - MC2
  - Nostoc649

- Molecular approach
  - Use bacteria DNA material to determine identity
  - FISH or other molecular methods

- Identity of filamentous bacteria in activated sludge

- Filamentous bacteria in Danish WWTP

- Chloroflexi/TM7/Nocardioforms
- Alphaproteobacteria
- Nostocoida limicola
- Type 1851, 0092, 0803
- Type 1863
- Haliscomenobacter hydrossis
- Nocardioforms

- Type 1701
- Alphaproteobacteria
- Chloroflexi
- TM7
- Nocardioforms

- Type 021N
- Alphaproteobacteria
- Chloroflexi
- TM7
- Nocardioforms
Abundance of filamentous bacteria in WWTP

**Municipal WWTP**
1. Microthrix parvicella
2. Chloroflexi (Type 0041, 0803, 0092, 1851)
3. TM7 related (Type 0041)
4. H. hydrossis and H. hydrossis-like
5. Mycolata

Based on Microbial database, determined by morphology and FISH. 50 full scale WWTP

**Industrial WWTP**
1. filamentous Alphaproteobacteria (N. limicola)
2. Thiothrix sp.
3. Mycolata
4. Chloroflexi (Type 0041, 0803, 0092, 1851)
5. Morphotype 0041 (TM7)

Based on Dynafilm data; 126 different industrial WWTP samples. Determined by morphology and FISH

Different biological processes (nitrification, denitrification, phosphorous removal)
- Aerobic, anoxic and anaerobic

Substrates present in municipal WWTP
- Carbohydrates (18%)
- Lipids (31%)
- Protein (30%)
- Volatile fatty acids (acetate and propionate up to 95%)

Municipal WWTP: slowly biodegradable organic matter (SBCOD)

Industrial WWTP: more easily biodegradable compounds (RBCOD)
- Slaughterhouse, dairy or fishing industry

Methods for ecophysiological studies

- Microautoradiography, MAR
- Types of substrates (simple substrates)
- Electron acceptor conditions (oxygen, nitrate or nitrite, no oxygen)
- Storage capacity (poly-β-hydroxyalkanoates)
- Excretion of exo-enzymes
- Surface properties
Ecophysiology on filamentous bacteria

Filamentous Alphaproteobacteria or N. limicola

5 species of filamentous Alphaproteobacteria

Very common in industrial WWTP

<table>
<thead>
<tr>
<th>MAR</th>
<th>Group 1*</th>
<th>Group 2**</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-acceptor</td>
<td>O₂, NO₂, NO₃</td>
<td>O₂, NO₂, NO₃</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sugars</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>(+)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>-</td>
<td>(+)</td>
</tr>
</tbody>
</table>

*Synechococcus: Monilibacter bovaceus, Allysosphaera europaea & Boltonicola proteobacter
**Alphaproteobacteria: Alysiosphaera europaea & Meganema perideroedes

Filamentous Alphaproteobacteria or N. limicola

Storage capacity, PHA

Surface charge

Versatile filaments

Large PHA storage capacity

No exoenzyme activity

Hydrophobic cell surface

Occurring in 60% of the industrial samples

Only rarely in municipal WWTP

Responsible for bulking in 25%
Morphological representatives in
Actinobacteria
Firmicutes
Chloroflexi
Planctomycetes

Very limited knowledge exist on their physiology
Industrial samples mainly Alphaproteobacteria
Municipal WWTP, only rarely identity unknown

**Curvibacter related filaments or Type 1701**

- Distributed in both municipal and industrial WWTP
- Present in relatively low abundance
- Substrates: Some sugars, amino acids and proteins
- Conditions: Aerobic, anoxic with nitrate and nitrite
- Surface properties: Hydrophilic
- Exo-enzymatic activity: Protease

**Chloroflexi sp. (Type 1851)**

- Distributed in both municipal and industrial WWTP
- Present in relatively low abundance
- Ecophysiology:
  - Substrates: sugars, glucose, few short chain fatty acids and N-acetylglucosamine
  - Conditions: aerobic
  - No significant PHA storage
  - Exoenzymes: galactosidase, glucuronidase, chitinase
  - Surface properties: Hydrophilic

Filaments with attached growth

- Distributed in both municipal and industrial WWTP
- Present in relatively low abundance
- Belong to different phyla: TM7, Betaproteobacteria, Chloroflexi
**Type 0092 and 0803 (Chloroflexi)**

- Distributed in both municipal and industrial WWTP (different species?)
- Present between 2-8% of municipal samples

**Ecophysiology for 0803:**
- Substrates: glucose, and some N-acetylglucosamine
- Conditions: aerobic, also anoxic and anaerobic
- Exoenzymes: many different including protease (chitinase, esterase etc)

0803 Hydrophobic (can be found in foam), no significant PHA

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**TM7 positive filaments (Type 0041)**

- Found frequently in municipal and industrial WWTP
- Approx. 15% are TM7 probe positive others unidentified
- Rarely cause bulking. Forming backbone of sludge flocs

**Ecophysiology**
- Amino acids and sugars
- Under all conditions $O_2$($NO_3$, anaerobic)
- Protease activity
- No significant PHA storage

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**Bacteroidetes sp. H. hydrossis**

- Haliscinemobacter hydrossis
- Distributed in both municipal and industrial WWTP
- Industrial WWTP: Present in 42%
  - High abundance in 16%
- Municipal WWTP: average 2%
- Epiflobacter: epiphytic bacteria on filaments, abundance 6%

**Ecophysiology:**
- Substrates: sugars, glucose and N-acetylglucosamine
- Conditions: aerobic
- No significant PHA storage
- Surface properties: Hydrophobic
- Exoenzymes: galactosidase, glucuronidase, chitinase
- Epiflobacter: utilize proteins and express protease
  - Are often on filaments affiliated to Chloroflexi, TM7 and Curvibacter
Distributed in mainly plants without full nitrogen and phosphorus removal
Thiothrix present in 38% of 126 industrial WWTP
Morphotype 021N only in 4%
Municipal WWTP 1%
Correlation with the presence of sulfide
Different species, defined by gene probes
Thiothrix: probe Tni
Morphotype 021N: Thiothrix eikelboomii probe 021N

<table>
<thead>
<tr>
<th>Thiothrix</th>
<th>021N</th>
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</thead>
<tbody>
<tr>
<td>$O_2$</td>
<td>$O_3$</td>
</tr>
<tr>
<td>few or no S granules</td>
<td>S granules</td>
</tr>
<tr>
<td>short chain fatty acids</td>
<td>long chain fatty acids</td>
</tr>
<tr>
<td>sugars</td>
<td>amino acids</td>
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</tbody>
</table>

Two species; M. parvicella & M. calida
Present in municipal WWTP (ca. 6%)
Responsible for both bulking and foaming

Ecophysiological studies:
- Only long chain fatty acids
- Lipase and esterase activity
- Activity under all conditions
- Anaerobic conditions only storage

Mycolata; morphological definition
Many different species, not all are known
Foam formation but seldom bulking

GALO: Gordonia amarae
PTLO: Pine tree-like organisms
Different Families: Corynebacteriaceae, Williamsiaceae, Tsukamurellaceae, Mycobacteriaceae, Dietziaceae, Nocardiaceae, Gordoniaceae
Identification of Mycolata by FISH

Gene probes:
- Myc657 (most Mycolata)
- Gor596 (most of the family Gordonia)
- Gam205 (the species Gordonia amarae)
- Spin1449 (the species Skermania piniformis)

Indicate different families

Mycolata sp. or nocardia

Die quickly in foam - remain there

Many species, different specialized populations
Some long chain fatty acids
Others soluble substrates
Different exo-enzymes
Some can be chemically controlled

Unidentified species

Abundant filaments
- Remaining filaments with attached growth
  - Many affiliated to Chloroflexi but no probes exist
- Still many morphotypes of Chloroflexi
  - Some inside sludge flocs and others outside
- Other unidentified are not very abundant

Physiology of filamentous bacteria

Substrate types
- Specialized on a single type
  - Sugars, lipids, proteins
- Versatile - use many substrate types
  - Short chain fatty acids, long chain fatty acids, sugars, amino acids, alcohols

E-acceptor conditions
- Different conditions (oxygen, nitrate, nitrate)
  - Only oxygen

Storage capacity
- Starvation sensitivity

Excretion of exo-enzymes
- Complex substrates

Surface properties
- Related to some extent to substrate uptake
Versatile filaments

Short chain fatty acids, long chain fatty acids, sugars, amino acids, alcohols

<table>
<thead>
<tr>
<th>Identity</th>
<th>E-acceptor conditions</th>
<th>Exo-enzymes</th>
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</thead>
<tbody>
<tr>
<td>Filamentous Alphaproteobacteria (N. limicola)</td>
<td>O₂, (NO₃ and NO₂)</td>
<td>No</td>
</tr>
<tr>
<td>Thiothrix (021N)</td>
<td>O₂</td>
<td>No</td>
</tr>
<tr>
<td>Mycelia (nocardioforms)</td>
<td>O₂, (NO₃ and NO₂), anaerobic</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Specialized filaments

<table>
<thead>
<tr>
<th>Identity</th>
<th>Substrate</th>
<th>E-acceptor conditions</th>
<th>Exo-enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroflexi Type 1851 thin filaments (H. hyphogea)</td>
<td>Sugars</td>
<td>O₂</td>
<td>Polysaccharide degradation</td>
</tr>
<tr>
<td>Chloroflexi Type 0803</td>
<td>Sugars</td>
<td>O₂, (NO₃ and NO₂)</td>
<td>Polysaccharide degradation</td>
</tr>
<tr>
<td>Coriobacter-related (1-701) TMT filaments (0041)</td>
<td>Amino acids</td>
<td>O₂, (NO₃ and NO₂)</td>
<td>Protein degradation</td>
</tr>
<tr>
<td>Microaerol</td>
<td>Lipoates</td>
<td>O₂, (NO₃ and NO₂)</td>
<td>Lipid degradation</td>
</tr>
<tr>
<td>Thiothrix sp. (021N)</td>
<td>Sulfides</td>
<td>O₂, (NO₃ and NO₂)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Formulating control strategies

- What filaments are present?
- What triggers the excessive proliferation of filaments
- What causes foam formation
- Correlations with filament identity and process conditions (WWTP type etc.)

Research needs

- Identification of the remaining important filaments
- Some morphotypes with attached growth
- Gene probes for new filamentous species
- Ecophysiology of new filamentous species
- Better understanding of the ecosystem “activated sludge”
- Full scale experiments for control strategies
People involved

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Yunhong Kang
Marianne Stevenson
Susanne Bieltz
Jane Aili