Microbiology in sewer systems

Catherine A. Biggs
Henriette S. Jensen
Interdisciplinary approach:
Chemical Engineering, Civil Engineering & Microbiology

Funding:
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Project investigators:
Catherine A. Biggs, Joby B. Boxall, Simon Tait & A. Mark Osborn

Post doctoral fellows:
Sekar Raju, Peter Deines & Henriette S. Jensen
Conveyance system

Public Health
Both conveyance system and ecosystem

Sewer atmosphere

Sewer “dry” biofilm

Different sewer ecosystems

Bulk water/wastewater

Sewer “wet” biofilm
UWS - an Ecosystem

- Complex environment
  - Biodiversity – who is there, the good, bad and ugly?
  - Spatial distribution – where are they? (biofilms, wastewater, solids)

- Internal processes
  - Cell-cell and cell-environment interactions – what are they doing, who are they doing it with, group ><individual behaviour?

- External drivers
  - How do changes in operation (e.g. hydraulic conditions, temperature, nutrient loads) influence biological activity?
Both conveyance system and ecosystem

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>740</td>
<td>gCOD m(^{-3})</td>
</tr>
<tr>
<td>BOD</td>
<td>350</td>
<td>gBOD m(^{-3})</td>
</tr>
<tr>
<td>BOD dissolved</td>
<td>70</td>
<td>gBOD m(^{-3})</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>80</td>
<td>g N m(^{-3})</td>
</tr>
<tr>
<td>Ammonia</td>
<td>50</td>
<td>g N m(^{-3})</td>
</tr>
<tr>
<td>Nitrate+Nitrite</td>
<td>0.5</td>
<td>g N m(^{-3})</td>
</tr>
<tr>
<td>Organic Nitrogen</td>
<td>30</td>
<td>g N m(^{-3})</td>
</tr>
<tr>
<td>Total phosphorous</td>
<td>14</td>
<td>g P m(^{-3})</td>
</tr>
<tr>
<td>Ortho phosphate</td>
<td>10</td>
<td>g P m(^{-3})</td>
</tr>
<tr>
<td>Organic Phosphorous</td>
<td>4</td>
<td>g P m(^{-3})</td>
</tr>
</tbody>
</table>

Henze et al., 2000
Key Research Questions -
Changes in biodiversity, spatial distribution and behaviour in engineering context?

Do we have the right tools?

MUWS
Developing our portfolio of Tools
Measurements Techniques
Culture based techniques

- **Spread-plate method**
  - Sample is pipetted onto surface of agar plate (0.1 ml or less)
  - Sample is spread evenly over surface of agar using sterile glass spreader
  - Incubation
  - Surface colonies

- **Pour-plate method**
  - Sample is pipetted into sterile plate
  - Sterile medium is added and mixed well with inoculum
  - Incubation
  - Subsurface colonies
  - Surface colonies

**Bacterial type 1**

**Bacterial type 2**

**Bacterial type 3**

**Bacterial type 4**

**Bacterial type 5**
Culturable bacteria in comparison with total (microscopic) cell counts

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Culturability (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>0.001 - 0.1</td>
</tr>
<tr>
<td>Freshwater</td>
<td>0.25</td>
</tr>
<tr>
<td>Mesotrophic lake</td>
<td>0.1 - 1</td>
</tr>
<tr>
<td>Unpolluted estuarine waters</td>
<td>0.1 - 3</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>1 - 15</td>
</tr>
<tr>
<td>Sediments</td>
<td>0.25</td>
</tr>
<tr>
<td>Soil</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Culturable bacteria are measured as colony forming units (CFU)

Analytical approaches

Sewer
- Wastewater
- Biofilms
- Solids

Community analysis
- DNA extraction
  - T-RFLP
  - DGGE
  - FISH
  - Overall Community profile
  - Detailed community composition
  - Cell counts identification

Substrate utilization
- Biolog EcoPlates™
- Specialised reactor setups
  - Carbon source screening
  - Kinetics and stoichiometry for specific substrates
DNA based techniques

DNA extraction kit

[Image of DNA extraction kit]

[Image of DNA double helix]

[Image of a witch with a broom and a cauldron]

[Image of a printer]
Terminal restriction fragment length polymorphism (T-RFLP)

- Microbial profiling method
- High throughput
- Reproducible
- Semi-quantitative analysis of the diversity
- Overall community profile

Fluorescence intensity

Fragment length
Denaturing Gradient Gel Electrophoresis (DGGE)

- Electrophoresis separation method
- Possible to get sequences
- Provides detailed community structure and composition

Samples: 1 2 3 4 5 6
Fluorescence *in situ* hybridization (FISH)

Amann & Fuchs, (2008)
Analytical approaches

Sewer

Wastewater  Biofilms  Solids

Community analysis

DNA extraction

T-RFLP  DGGE  FISH

Overall Community profile  Detailed community composition  Cell counts identification

Substrate utilization

Biolog EcoPlates™

Carbon source screening

Specialised reactor setups

Kinetics and stoichiometry for specific substrates
Substrate Utilisation

- Corrosion products (e.g., Gypsum)
- Sulfuric acid, \( \text{H}_2\text{SO}_4 \)
- Sulfide oxidation
- Sulfide adsorption
- Gaseous sulfide, \( \text{H}_2\text{S} \)
- Sulfide emission

- Dissolved sulfide, \( \text{S}_{\text{d,III}} \)
- Precipitation
- Sulfide oxidation

- Metal sulfide, \( \text{X}_{\text{S,III}} \)
- Sulfate reduction
- Elemental sulfur, \( \text{S}_{\text{Sv}} \)
- Sulfate, \( \text{S}_{\text{sv}} \)

- Air-water interface
- \( \text{CO}_2 \)
- Heterotrophic growth and maintenance

- Dissolved oxygen, \( \text{O}_2 \)
- Reaeration

- Readily biodegradable substrate, \( \text{S}_r \)
- Fermentable substrate, \( \text{S}_f \)
- Fermentation
- Fermentation products, \( \text{S}_a \)

- Fast hydrolyzable substrate, \( \text{X}_{\text{St,fast}} \)
- Slowly hydrolyzable substrate, \( \text{X}_{\text{St,slow}} \)
- Biomass, \( \text{X}_{\text{bio, X}_{\text{mf}}} \)

- Sulfur cycle
- Carbon cycle

- Aerobic processes
- Anaerobic processes
- Interfacial exchange
<table>
<thead>
<tr>
<th>Category</th>
<th>Carbon sources</th>
<th>Well designation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reference well</strong></td>
<td>Water (no carbon)</td>
<td>A1</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td>β-Methyl-D-Glucoside</td>
<td>A2</td>
</tr>
<tr>
<td></td>
<td>i-Erythritol</td>
<td>C2</td>
</tr>
<tr>
<td></td>
<td>D-Mannitol</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td>D-Cellobiose</td>
<td>G1</td>
</tr>
<tr>
<td></td>
<td>α-D-Lactose</td>
<td>H1</td>
</tr>
<tr>
<td></td>
<td>N-Acetyl-D-Glucosamine</td>
<td>E2</td>
</tr>
<tr>
<td></td>
<td>D-XYlose</td>
<td>B2</td>
</tr>
<tr>
<td><strong>Polymers</strong></td>
<td>Tween 40</td>
<td>C1</td>
</tr>
<tr>
<td></td>
<td>Tween 80</td>
<td>D1</td>
</tr>
<tr>
<td></td>
<td>α-Cyclodextrin</td>
<td>E1</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>F1</td>
</tr>
<tr>
<td><strong>Carboxylic acids</strong></td>
<td>D-Galactonic Acid Lactone</td>
<td>A3</td>
</tr>
<tr>
<td></td>
<td>D-Galacturonic Acid</td>
<td>B3</td>
</tr>
<tr>
<td></td>
<td>γ-Hydroxybutyric Acid</td>
<td>E3</td>
</tr>
<tr>
<td></td>
<td>D-Glucosaminic Acid</td>
<td>F2</td>
</tr>
<tr>
<td></td>
<td>Itaconic Acid</td>
<td>F3</td>
</tr>
<tr>
<td></td>
<td>α-Ketobutyric Acid</td>
<td>G3</td>
</tr>
<tr>
<td></td>
<td>D-Malic Acid</td>
<td>H3</td>
</tr>
<tr>
<td></td>
<td>2-Hydroxybenzoic Acid</td>
<td>C3</td>
</tr>
<tr>
<td></td>
<td>4-Hydroxy Benzoic Acid</td>
<td>D3</td>
</tr>
<tr>
<td><strong>Phosphorylated chemicals</strong></td>
<td>Glucose-1-Phosphate</td>
<td>G2</td>
</tr>
<tr>
<td></td>
<td>D,L-α-Glycerol Phosphate</td>
<td>H2</td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td>L-Asparagine</td>
<td>B4</td>
</tr>
<tr>
<td></td>
<td>L-Phenylalanine</td>
<td>C4</td>
</tr>
<tr>
<td></td>
<td>L-Serine</td>
<td>D4</td>
</tr>
<tr>
<td></td>
<td>L-Threonine</td>
<td>E4</td>
</tr>
<tr>
<td></td>
<td>Glycyl-L-Glutamic Acid</td>
<td>F4</td>
</tr>
<tr>
<td></td>
<td>L-Arginine</td>
<td>A4</td>
</tr>
<tr>
<td><strong>Amines</strong></td>
<td>Pheny lethylamine</td>
<td>G4</td>
</tr>
<tr>
<td></td>
<td>Putrescine</td>
<td>H4</td>
</tr>
<tr>
<td><strong>Esters</strong></td>
<td>Pyruvic Acid Methyl Ester</td>
<td>B1</td>
</tr>
</tbody>
</table>
Reactor setup

Experimental setup

Hydrogen sulfide sensor

Oxygen sensor

Computer for data sampling

Experimental setup
Analytical approaches

Sewer

Wastewater Biofilms Solids

Community analysis

DNA extraction

T-RFLP DGGE FISH

Overall Community profile Detailed community composition Cell counts identification

Substrate utilization

Biolog EcoPlates™ Specialised reactor setups

Carbon source screening Kinetics and stoichiometry for specific substrates
Results and Applications
Community Profiles within Real Systems
Sampling Nantes
DGGE profiles

[Image of DGGE profiles with bands labeled Denmark, Nantes 1, Nantes 2, Nantes 2-b, Nantes 2-a, Nantes 2-c, Nantes 1-b, Nantes 1-a, Nantes 1-c, Denmark c, Denmark a, Denmark b for similarity values ranging from 20 to 100.]

[Image of DGGE profiles with bands labeled Nantes 2 November, Nantes 2 May, Nantes 2 July, Nov c, Nov a, Nov b, May b, May a, July b, July d, July c, May d, May c, July a for similarity values ranging from 20 to 100.]
Substrate Utilisation in Lab and Field Studies
Hydrogen sulphide oxidation
Concrete corrosion

\[ \text{Ca(OH)}_2 \rightarrow \text{H}_2\text{SO}_4 \rightarrow \text{O}_2 \rightarrow \text{H}_2\text{S} \rightarrow \text{CaSO}_4 \]

\[ \text{H}_2\text{S} \rightarrow \text{H}_2\text{SO}_4 \rightarrow \text{O}_2 \rightarrow \text{O}_2 \]

\[ \text{H}_2\text{SO}_4 \rightarrow \text{Ca(OH)}_2 \rightarrow \text{H}_2\text{S} \rightarrow \text{CaSO}_4 \]
Determination of reaction pathway

Possible pathways:

\[ \text{O}_2/\text{S} = 0.61 \]
Substrate utilization profiles

Biolog EcoPlates™

Graph showing absorbance over time at different temperatures and sites.
Future Work
### Present / future work

### Well monitored catchment

Effect of velocity on the microbial communities

<table>
<thead>
<tr>
<th>Velocity [m/s]</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
<th>3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time [h]</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- FM001
- FM004
- FM012
- FM019
Implications

..... various microbiological tools which can be applied to study the microbial ecology in sewer networks and give new insight....

• **In-sewer processes**
  • which are dominant and which are in control.
• **Dynamic Behaviour**
  • consequence of and response to perturbations

into the effect of in-sewer processes and potentially improved risk assessment in the event of sewer faiiures........
All this MUWS work ...

... will facilitate the sustainable operation and management of urban water systems into the future, maximizing water quality performance and minimizing environmental impact.
Thank you!
Discussion of

Microbiology in sewer systems

Catherine A. Biggs and Henriette S. Jensen

MUWS is EU funded.

Nutrient concentrations affect in-sewer biological activity. For a catchment with a strong season variation (holiday town or one with a seasonal food processing campaign) the biofilms can take time to acclimate to the changed load, which can in turn affect the dose required for septicity control. Traditional sampling and plating and culture methods find fewer than 1% of the species in most media and at best 15% (page 11), whereas DNA techniques enable understanding or a much broader spectrum of those present.

There was greater similarity of species profile at a site than between site (page 25) but there was difference between sampling times at a particular site. Species profiles at the intertidal zone and the dry zone of a sewer were different.

There is clearly a lot we do not know about in-sewer processes. It is certain that what goes into sewers is not necessarily the same as what arrives at the WwTW, but we do not yet have the knowledge to estimate the dynamics.

Tim Evans presented supporting data that demonstrate the importance of in-sewer microbiology. The municipality reported that there had been no change in septicity or in sewer corrosion. Each sampling event was mid-week so there was no week-day / weekend bias. The paper is currently going through peer review. Recently he has learnt that anammox bacteria have been found in sewer films where they are presumably “protected” by layers of nitrifying bacteria (Nitrosomonas) just as they are in the granular biomass used in the ANAMMOX® catabolic ammonia-denitrification process.
WwTW influent monitoring data from Surahammar SE – 4-weekly 24-hour composite samples and the 13 period moving average from Jan’95 to Apr’09. In May 1997 the city offered food waste disposers as one of 3 options for kitchen food waste, by 1998 30% of households had installed FWD, by 2009 50% of households used FWD. There has been no significant change in trade effluent or in domestic population. Flow has not increased nor have the loads of BOD or COD but biogas has increased by more than 40%.
The loads of total-N and ammoniacal-N have not increased. The load of P has decreased which is probably because of the contemporaneous phasing out of P in domestic laundry and other products. Results of statistical analysis (Student’s T-test) comparing different periods would be consistent with gradual acclimation of sewer microbiology to the changing sewage composition. (paper in press)