Off-flavours in rainbow trout production systems with water recirculation:

Occurrence, production and removal

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Recirculated systems in Denmark:
- Water source is well or stream water.
- Typically 95% water reuse.





Water treatment:
50 micron screens and biofilter

Taste-and-odour (TOC) problems in recirculated aquaculture systems (RAS) with rainbow trout in Denmark:

Results from analysis of 200 rainbow trouts from 8 RAS:

- ***Evaluation by sensory panel:***

63% of the fish from production basins had detectable content of TOCs

- ***Chemical analysis:***

About 90% of fish from production basins had a TOC level >0.1 µg/kg (human detection limit for geosmin)

- ***Supermarkets:***

Consumer complaints on TOC-tainted fish



HOME | PRODUCTS | CURRENT PRODUCTION | FARMING OPERATION | CONTACT | INVESTOR RELATIONS - QUAN HỆ CỔ ĐÔNG

ABT on current stock quotes (click here)

Products

WHITE CLAM

PANGASIUS FISH

White Pangasius - Unique Fully Integrated System in VN



Imported fish, e.g., Pangasius from Vietnam, inform about TOCs

1. General information:
- Commercial name: FROZEN PANGASIUS HEADLESS & GUTTED
- Latin name: Pangasius Hypophthalmus

2. Product information:
- Headless, gutted, fin off, tail off, skin on cleaned, bone in.
- 5%, 10% or protective glazing depend on clients' order.
- 300-500, 500-700, 700-1000 gram per piece or sizing by customers' order.

4. Ingredient declaration (%): Pangasius 100%

5. Supply stability: Stably for whole year round

6. Raw material: 100% raw fish is from Aquatex Bentre's farms where are all GLOBAL G.A.P certified

6. GM status: Product is free from GMO

7. Organoleptic characteristics:

Appearance:	Frozen
Colour:	Natural colour
Flavour:	No muddy taste
Smell:	No off odour
Texture:	Firm

8. Usage information:
- Storage conditions: < - 18^{0}C
- Life time: 24 months from production date
- Consumption advice: Fully cooked before consumption

9. Packaging information:

Brand name:	By Aquatex Bentre or by client's brand
Inner packing:	+ Individually wrap packing, 5 Kg net or with glazing per PE bag/Carton + Or individually wrap packing, 10 Kg net or with glazing per PE bag/Carton Or may be changeable to clients orders.
Packing material:	Inner bag: PE bag and outside: master carton

Known off-flavour compounds in freshwater fish:

- **Earthy**: Geosmin
- **Musty**: 2-MIB (methylisoborneol)
- **Rancid**: aldehydes, e.g. 2,4-heptadienal (from oxidation of lipids)
- **Woody**: Terpenes
- **Petroleum**: Environmental pollution

1. WHO ARE THE PRODUCERS OF OFF-FLAVOURS?

- **If light, N and P are present:**

  Cyanobacteria (bluegreen algae) and some algae

- **If organic matter and oxygen are present:**

  Streptomycetes (bacteria)

  Myxobacteria (slime or gliding bacteria)

Cyanobacteria: filaments or free cells

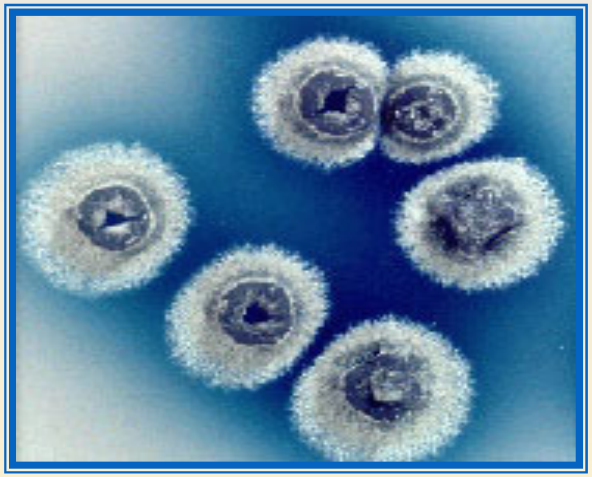


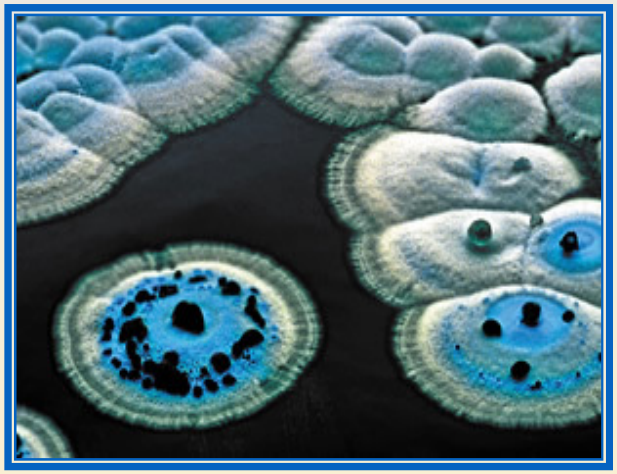




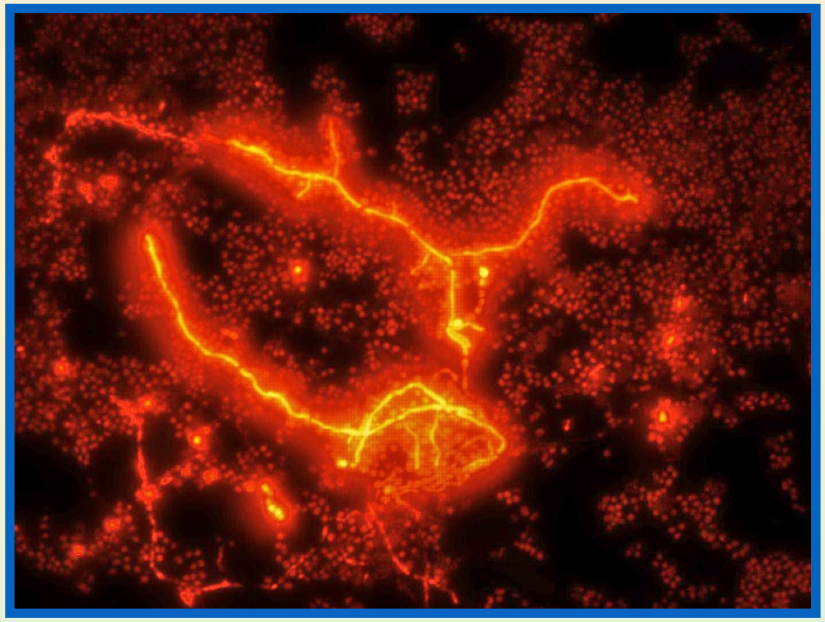


Streptomycetes: Gram-positive, filamentous (soil) bacteria



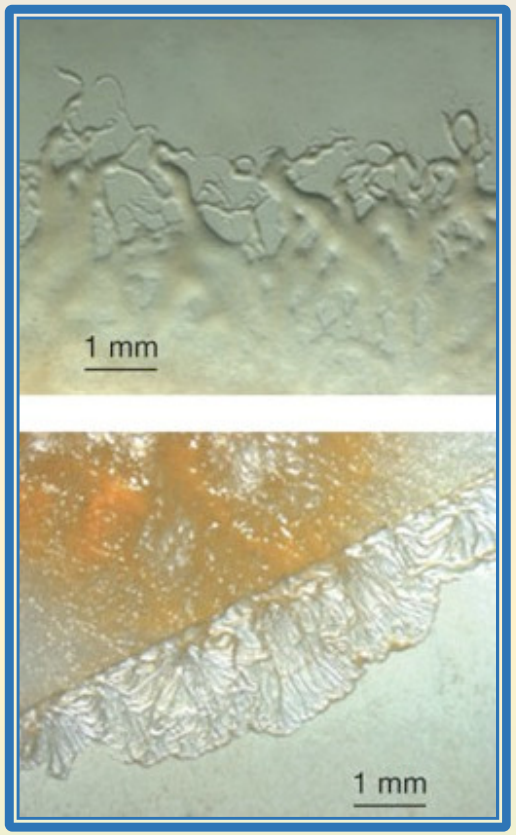






Myxobacteria (*Sorangium* and *Nannocystis*): Gram-negative, slime- and spore-forming bacteria in RAS





Molecular method indicate capacity to produce geosmin (presence of the gene encoding the geosmin synthase enzyme)*

*Auffret et al. (2013). Impact of water quality on the bacterial populations and off-flavours in recirculating aquaculture systems. FEMS Microbiology Ecology 84, 235-247

2. WHICH ENVIRONMENTAL FACTORS CONTROL OFF-FLAVOUR PRODUCTION?

Surfaces are important for production of off-flavours:
In RAS, most off-flavour producers occur in biofilm on surfaces on biofilter beads and tank walls





Test for growth of biofilm in depuration basin in rainbow trout RAS in Denmark



Transparent chambers on cement walls



After 3 weeks

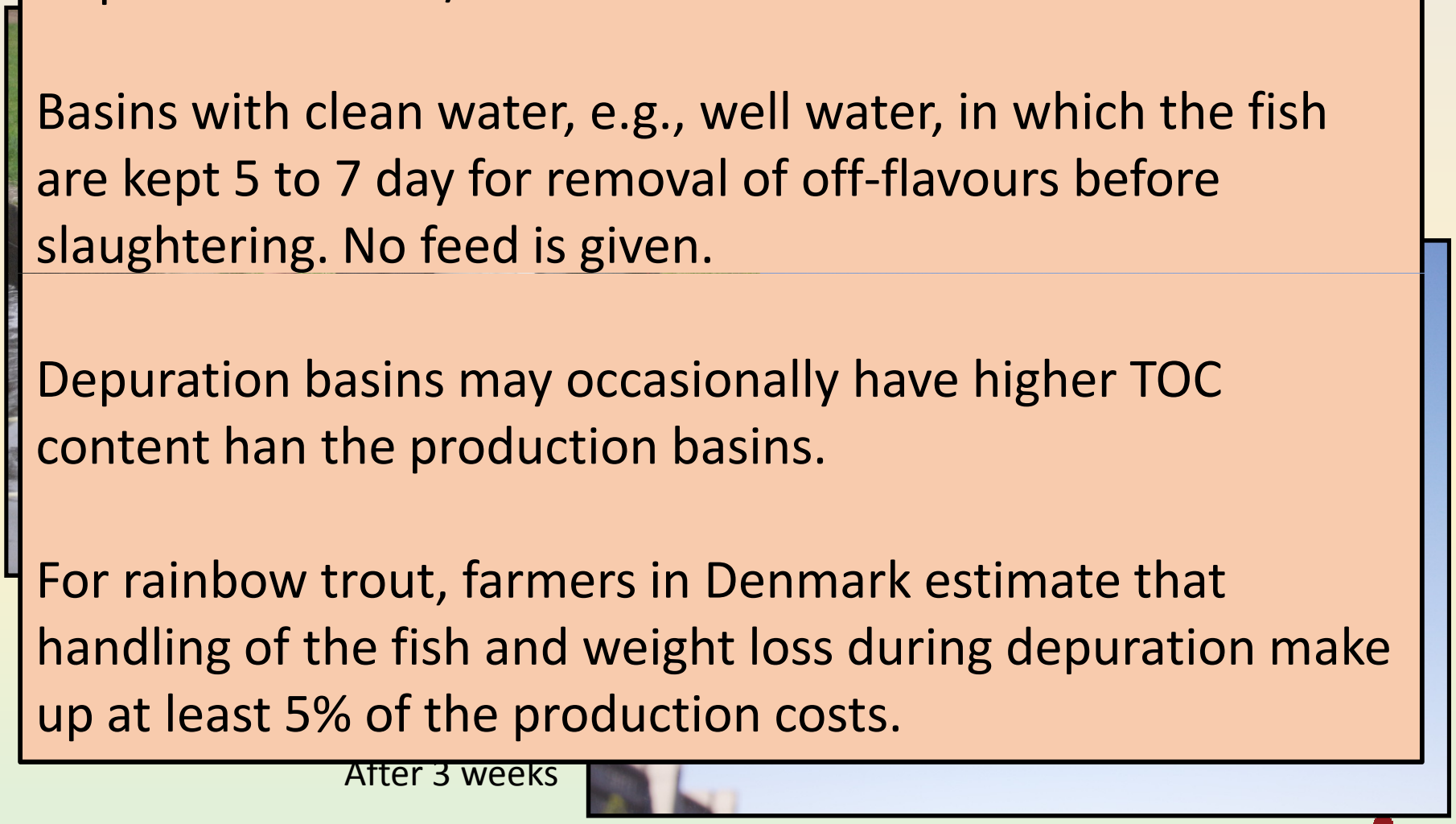
Test for growth of biofilm in depuration basin in rainbow trout RAS in Denmark

Depuration basins/tanks:

Basins with clean water, e.g., well water, in which the fish are kept 5 to 7 day for removal of off-flavours before slaughtering. No feed is given.

Depuration basins may occasionally have higher TOC content han the production basins.

For rainbow trout, farmers in Denmark estimate that handling of the fish and weight loss during depuration make up at least 5% of the production costs.



Test for growth of biofilm in depuration basin in rainbow trout RAS in Denmark

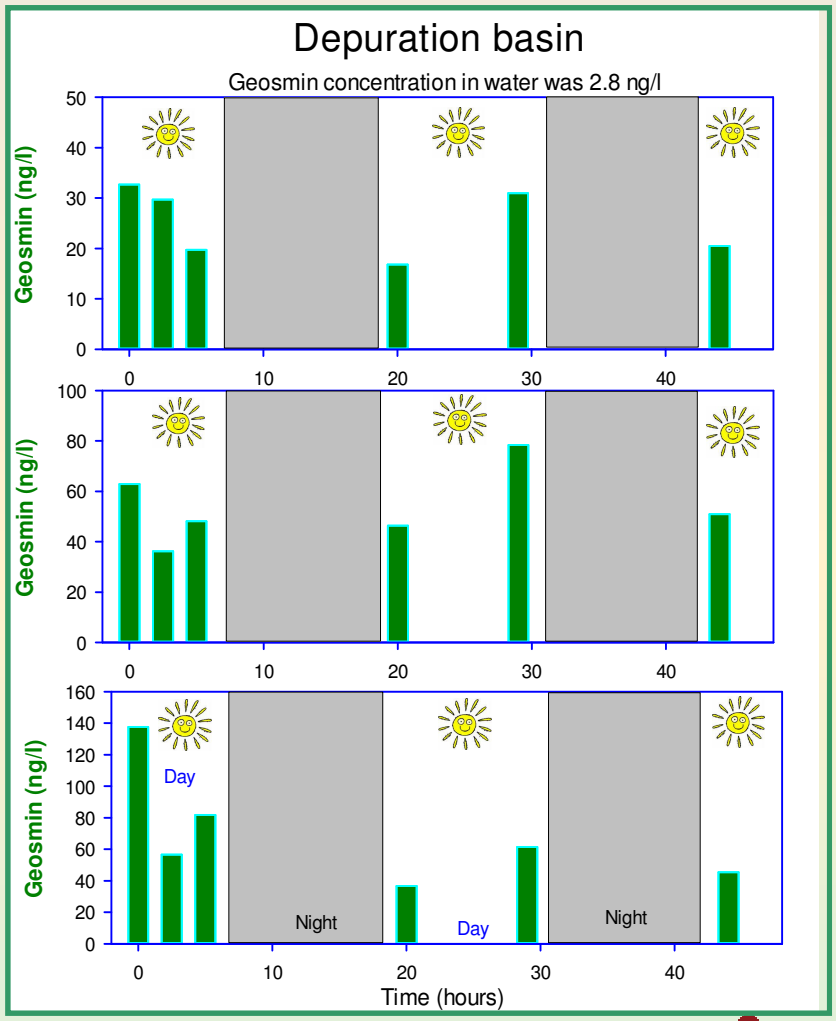


Transparent chambers on cement walls

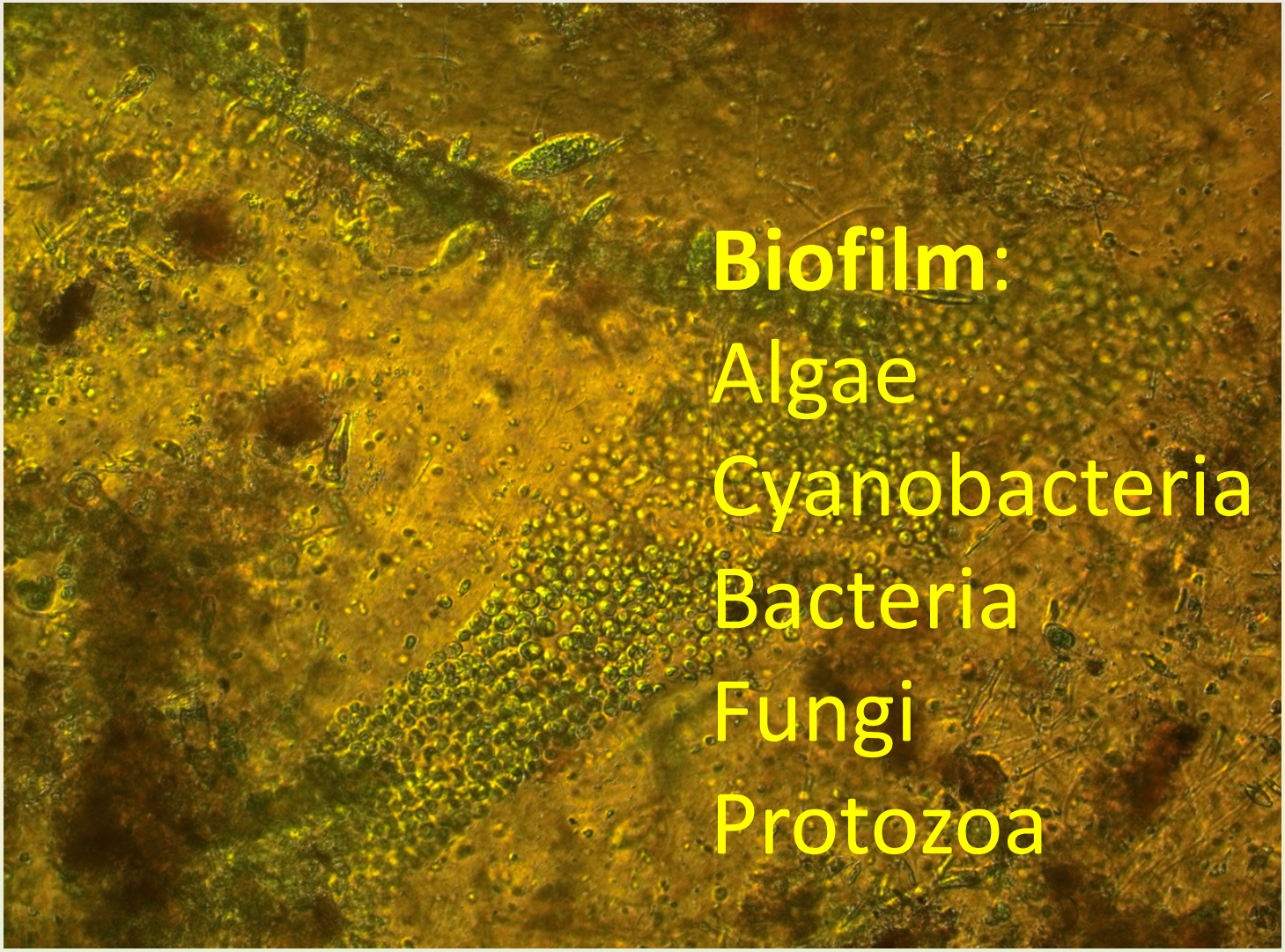


After 3 weeks

Geosmin concentrations after 3 weeks:
- 30 - 135 ng/l in chambers
- 3 ng geosmin/l in water outside chambers



RAS (biofilm microscopy, 20x magnification)



2. WHICH ENVIRONMENTAL FACTORS CONTROL OFF-FLAVOUR PRODUCTION?

Content of phosphorus in feed may control the off-flavour level:

Sarker et al. (2014)* observed a close correlation between (1) content of P in feed and (2) concentrations of geosmin in water and in fish (rainbow trouts) in Canadian RAS

P in feed is released as ortho-P that stimulate growth of off-flavour producing microorganisms

*Aquaculture Environment Interactions, vol. 6: 105-117

3. HOW CAN WE DETECT OFF-FLAVOUR PRODUCING MICROORGANISMS?

1. Isolation and cultivation of organisms in the laboratory, combined with measurements of off-flavour production.

Risk: laboratory conditions differ from the environmental conditions (nutrients, temperature, oxygen, light) and off-flavours may not be reflect in-situ production.

Thus, potential producers of off-flavours may not produce or over-produce off-flavours in the laboratory.

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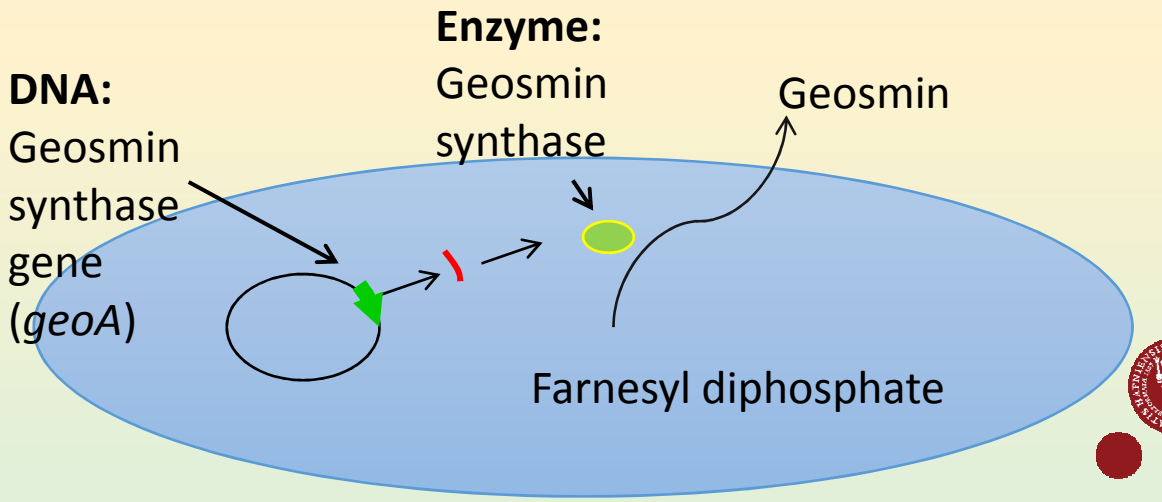
2. Examine molecular information behind the off-flavour production:
Extract DNA from biofilm or water samples; use PCR techniques to amplify the genes encoding enzymes involved in synthesis of geosmin (*geoA*, geosmin synthase) and MIB (*MIBS*, MIB synthase).



A short biochemistry lesson:

Synthesis of geosmin, MIB and terpenes is chemically related in microorganisms

For example:
Geranyl diphosphate → 2-MIB [and monoterpenes]
Farnesyl diphosphate → Geosmin [and sesquiterpenes]



A short biochemistry lesson:

Can *geoA* serve as ”fishing hook” to find geosmin-producing microorganisms?

Problem:
Many geosmin producers have slightly different *geoA* genes, or may even have two different *geoA* genes.

Example: Primers designed for PCR amplification of *geoA* in an Australian cyanobacterium (*Phormidium*) did not work for the same cyanobacterium in Denmark.

Universal PCR primers, targetting all geosmin producers, are complicated to design.

4. GEOSMIN AND MIB ARE NOT THE ONLY TOCs IN FISH

Pangasius and tilapia from ponds in Bangladesh:
Off-flavour in fish, but not caused by geosmin nor MIB

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Pangasius and tilapia from ponds in Bangladesh:
Off-flavour in fish, but not caused by geosmin nor MIB:

Organic solvents: Benzene, ethyl-benzene, 2-butanone, toluene, m-xylene (35 compounds found)

Terpenes (essential oils, e.g. in plants): Junipene, β-caryophyllene, α-humulene (18 compounds found)

Polyaromatic hydrocarbons (PAH, tar compounds or naphtalenes): 1,8-dimethyl-naphthalene, phenol, 1-methyl-naphthalene (9 compounds found)


EXPERIMENT:
- 8 ponds
- 4 types: Control, PondPlus, AquaPhoto, sand filter
- Pangas and tilapia kept in ponds for 6 months

4. GEOSMIN AND MIB ARE NOT THE ONLY TOCs IN FISH

Common source of protein in fish feed:

Mustard and **rape** seed cake.

Can the mustard flavour (isothiocyanate) cause off-flavour in fish?

4. GEOSMIN AND MIB ARE NOT THE ONLY TOCs IN FISH: DO ALGAE ALSO PRODUCE OFF-FLAVOURS?

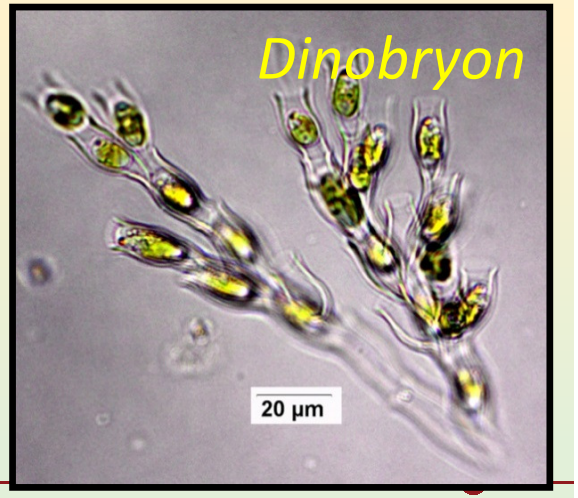
Water reservoir in China:

Unpleasant **fishy** odour was caused by dense growth of the golden alga *Dinobryon*, and the diatoms *Melosira* and *Cyclotella*

Odour compounds: **aldehydes** (*n*-hexanal, *n*-heptanal, 2,4-decadienal, 2,4-heptadienal)*

*Zhao et al. (2013). Journal of Environmental Sciences *25*:2361–2366





5. CONTROL OF GEOSMIN AND MIB (1)

PHYSICAL AND CHEMICAL METHODS

Particulate activated carbon (PAC), e.g. for drinking water

Advanced oxidation procedure (AOP): UV light + ozone



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Advanced oxidation procedure (AOP): UV light + ozone

BIOLOGICAL METHODS

Australia (drinking water): Bioreactor (sand column) with bacteria isolated from sewage treatment plant: 70% degradation of geosmin at 100 ng/l. Slow process, long contact time (5 ml/min in reactor).

China (RAS): Enrichment of bioflocs with *Bacillus* bacteria: 99% reduction of geosmin at 100 ng/l in 10 l biofloc reactor in 2 days.

Challenge: Geosmin removal from water phase in tanks

5. CONTROL OF GEOSMIN AND MIB (1)

PHYSICAL AND CHEMICAL METHODS

Alternative procedures for ”control” of off-flavours:

- Smoking

- Addition of spices: chili, lemon, lime, banana leaf ashes

- Treatment with saline solutions, plant extracts

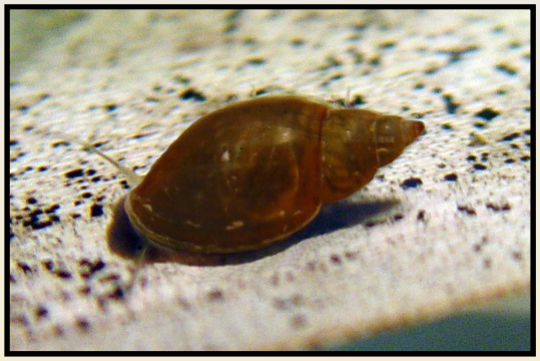
Only masking of off-flavours, not removal

China (RAS): Enrichment of bioflocs with *Bacillus* bacteria: 99% reduction of geosmin at 100 ng/l in 10 l biofloc reactor after 2 days.

Challenge: Geosmin removal from water in tanks

CONTROL OF GEOSMIN AND MIB (2)

BIOLOGICAL METHOD:
SNAILS GRAZING ON BIOFILM



Can snails reduce TOC in RAS?

Davidson and coworkers at Freshwater Institute, Shepherdstown:
500 l with 30 rainbow trouts (111 g)

Many s**treptomycetes occurred in biofilm on walls in aeration tank.**

Freshwater snail *Physa gyrina* living in aeration tank removed 2/3 of streptomycetes in biofilm.

Some reduction of geosmin in water in the tanks was observed.

INITIATIVE TO REDUCE OFF-FLAVOUR IN RAS IN DENMARK IN 2012:

Recommended treatments:

- Purging of fish for up to 7 days in water with <10 ng geosmin/l
- 6 annual analyses of geosmin in the water

Testing for TOC in fish

Selected fish from each party (loading) are cooked and tasted

Fish farmer request: Commercial facility for chemical analysis of TOC in fish


Fish production in Denmark in 2003:
Comments, questions?
Contact Niels Jørgensen
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