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Norwegian Institute of Food, Fisheries and Aquaculture Research

Carbon bioavailability and nitrate determine H_2S production potential in sludge collected from Atlantic salmon in RAS

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CtrlAQUA

Relevance for the industry

Sudden, “unexplained” mass mortalities in brackish/marine RAS – H_2S?

undercurrentnews
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Land-based salmon farmer hit by die-off after securing funding for US expansion

On June 29 Atlantic Sapphire -- which has secured $130 million of financing for a massive land-based salmon farm project in Florida -- lost a quarter of its budgeted harvest volume in its farm in Denmark, Langsand Laks. The news was first reported by *iLaks.no*.

According to Andreassen, this was 250 metric tons. The farm was “struck with a sudden and unexpected mortality of fish”, according to a statement.

SalmonBusiness

Head of aquaculture insurance firm on RAS: “some insurers don’t want to insure those kind of farms”

News by **Owen Evans** - 11 September 2018

The Health Situation in Norwegian Aquaculture 2018

… As expected, problems related to hydrogen sulphide were identified much more commonly in RAS systems compared to through-flow farms. Only 6.75% of respondents reported this type of problem in through-flow farms while 57.2% had experienced H2S associated problems in RAS farms.

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Updated numbers for 2021

Only 11% reported H_2S problems in RAS

Norwegian Veterinary Institute

H_2S in RAS – Suspected cause for sudden mass mortalities?

H_2S toxicity

- H_2S binds with iron in mitochondrial cytochrome enzymes, prevents cellular respiration
  - $LC_{50}96$ for marine species: 50-500 µg/L (Boyd, 2014)
  - Chronic effects in 40-55 g smolts: ~270 µg/L (Kiemer et al., 1995)
  - Acute stress & damage in 150-200 g smolts: ~950 µg/L (Kiemer et al., 1995)
  - Recommendation: H_2S below 2-5 µg/L (Timmons et al., 2007; Boyd, 2014)

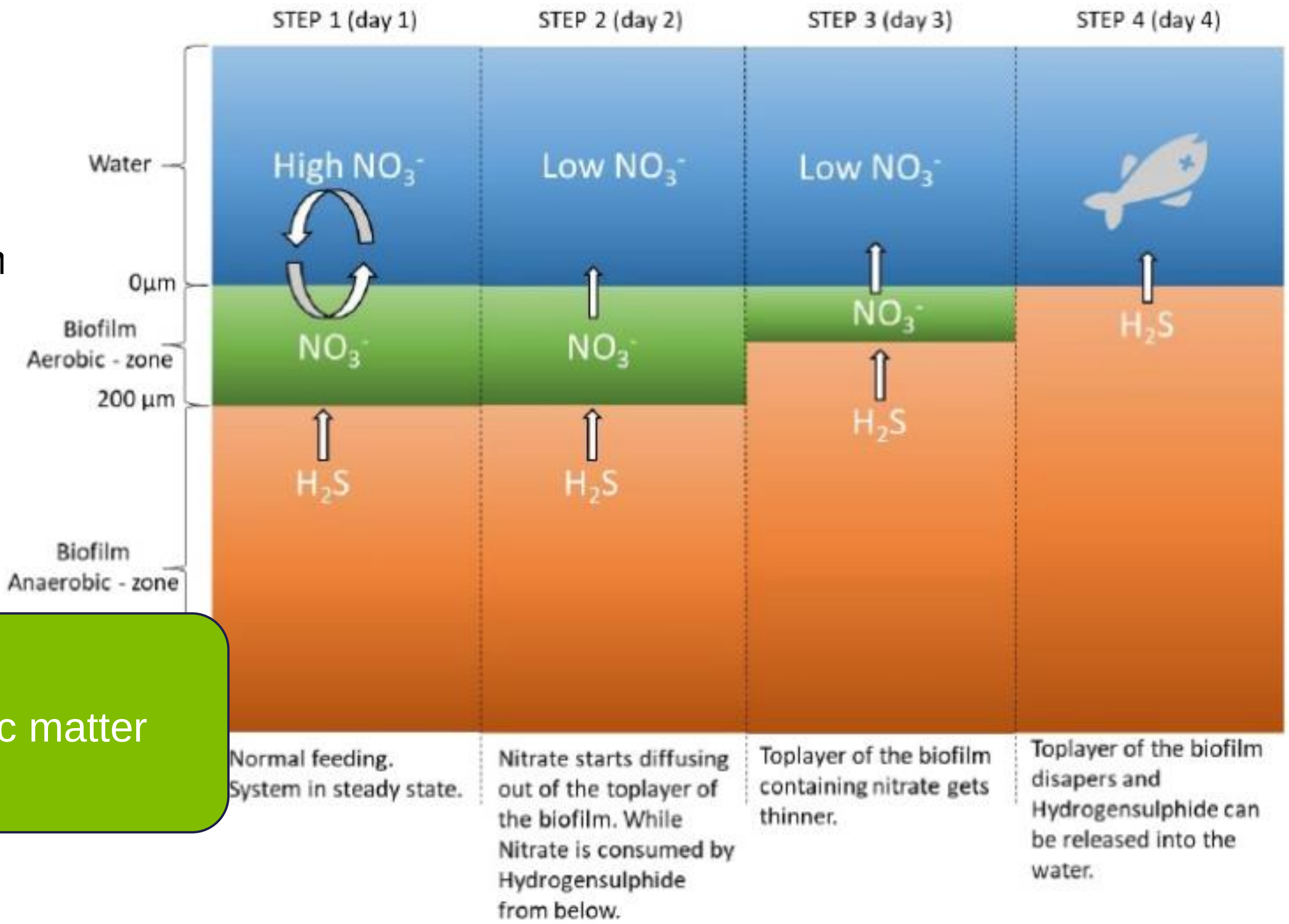
RAS-specific risks

- RAS biologically very active, rich in nitrate, organic matter and biofilms
  - Sulphate in sea water (~2600 mg/L)
  - Denitrification & sulphate reduction in oxygen-free niches (sediments/biofilms)
  - Operational conditions fluctuate (feed, water, biomass, etc.)

Current hypothesis on H_2S in RAS

Dynamics in H_2S generation in RAS

- Organic matter consumption & sulphate reduction in anaerobic zones
- Nitrate is consumed to oxidize H_2S in top layers
- Drop in nitrate → less H_2S oxidation
- H_2S release into water



"Mass mortality in RAS – Solved?" - Højgaard, 2017 (salmonbusiness.com)

Balance of two key processes

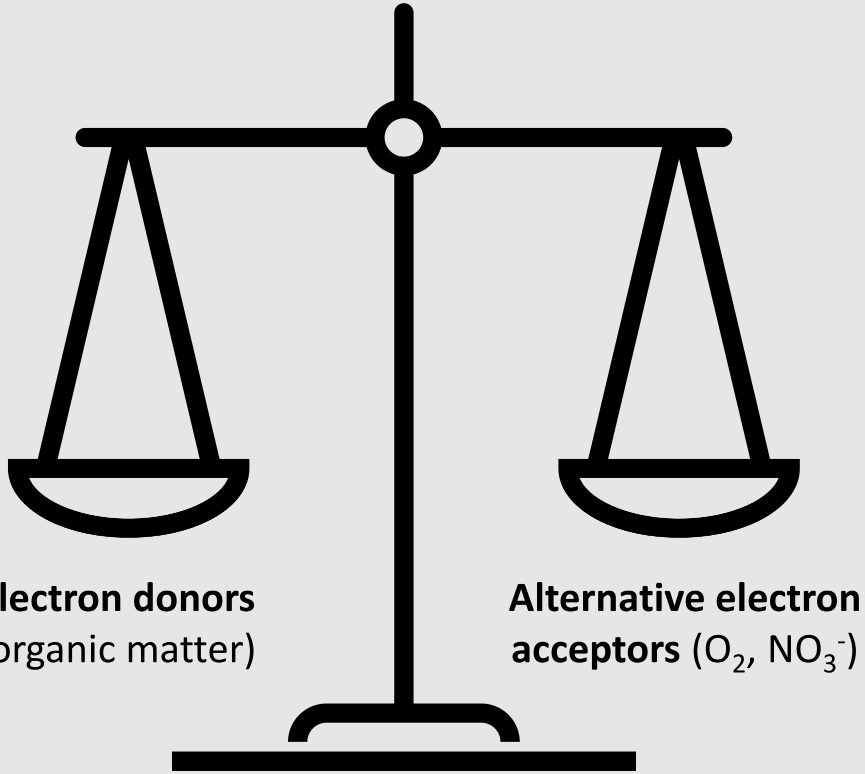
- Sulphate (SO_4^{2-}) reduction with organic matter
- H_2S oxidation with nitrate (NO_3^-)

Central hypotheses

- The ratio of biodegradable organic matter (BOD) to sulfate determines the total potential for H_2S generation

- The ratio of BOD to alternative electron acceptors determines net “release” potential for H_2S

“Equilibrium”
(Zero net H_2S production)



$$\text{Net } H_2S \text{ release} = H_2S_{production} - H_2S_{oxidation}$$

Carbon-to-nitrogen ratios are key to quantify the potential for H_2S generation in RAS

What do we want to find?

- Focus on organic matter properties and how it is used, to assess H_2S production potential in sludge
  - How is bioavailable carbon allocated between denitrification and sulphate reduction?
  - Stoichiometry & kinetics of H_2S generation

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aque

Increased sulfate availability in saline water promotes hydrogen sulfide production in fish organic waste

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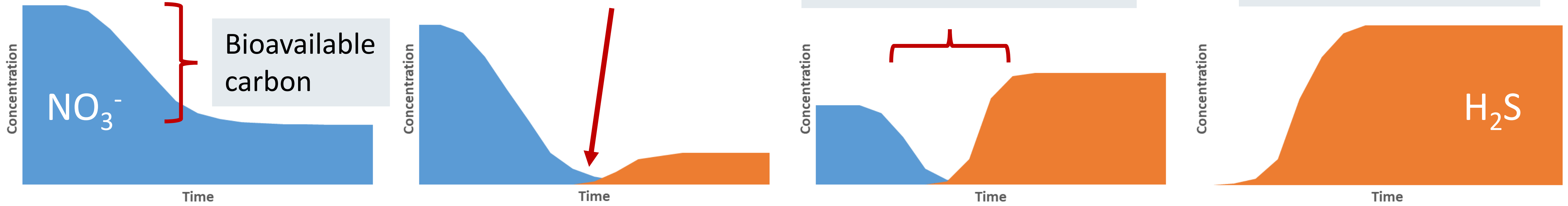
Critical nitrate concentrations?

Delay in H_2S response & reaction speed

Total H_2S production potential?



No H_2S, sludge & excess nitrate

Decrease in NO_3^- concentration → increase in H_2S response

Sludge only

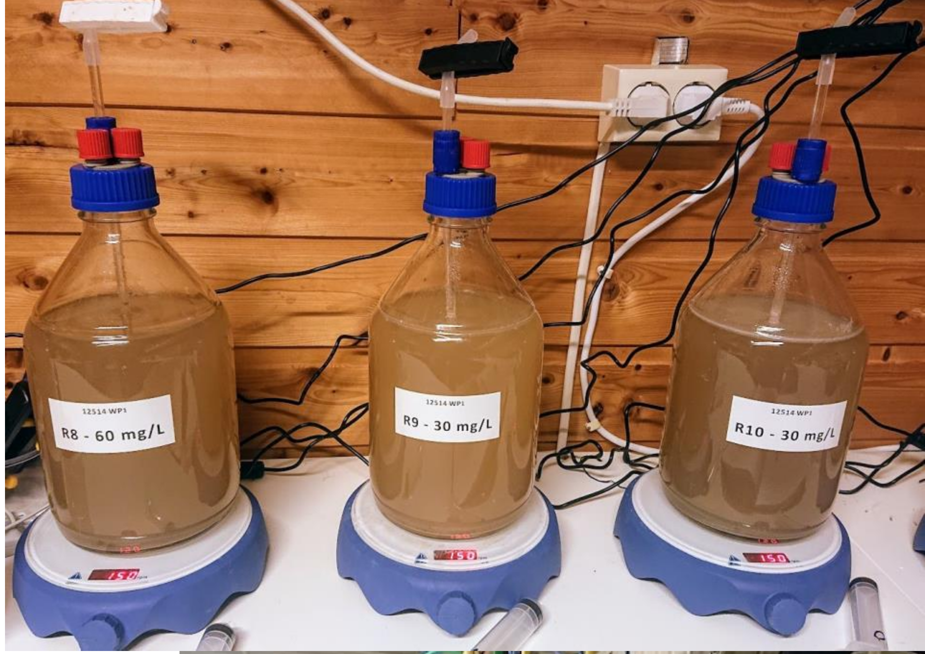
Experimental design

Sludge collection

- Compound sample of sludge from 3 swirl separators in brackish RAS (12 ppt, 6 x 3.3 m^3 tanks, 12 °C, 30-40 kg/m^3)
  - Atlantic salmon post-smolts of 200-300 g
  - Collection & homogenization of sludge (feces and feed spill)
  - COD/BOD to determine biodegradability of organic matter

Reactor design

- Similar setup as Letelier-Gordo et al. (2020)
- Continuously stirred batch reactors with brackish water (V = 2 L, T = 12.4 °C ± 0.2, 12.1 ppt)
  - Fixed sludge dose: 1% sludge (V/V)
  - Variable nitrate dose: 0, 30, 60, 120 mg/L NO_3-N (3 replicates)
  - Deoxygenated with N_2 before inoculation



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Results

Concluded after 90 days of incubation

Elimination of 5 reactors, due to air leaks

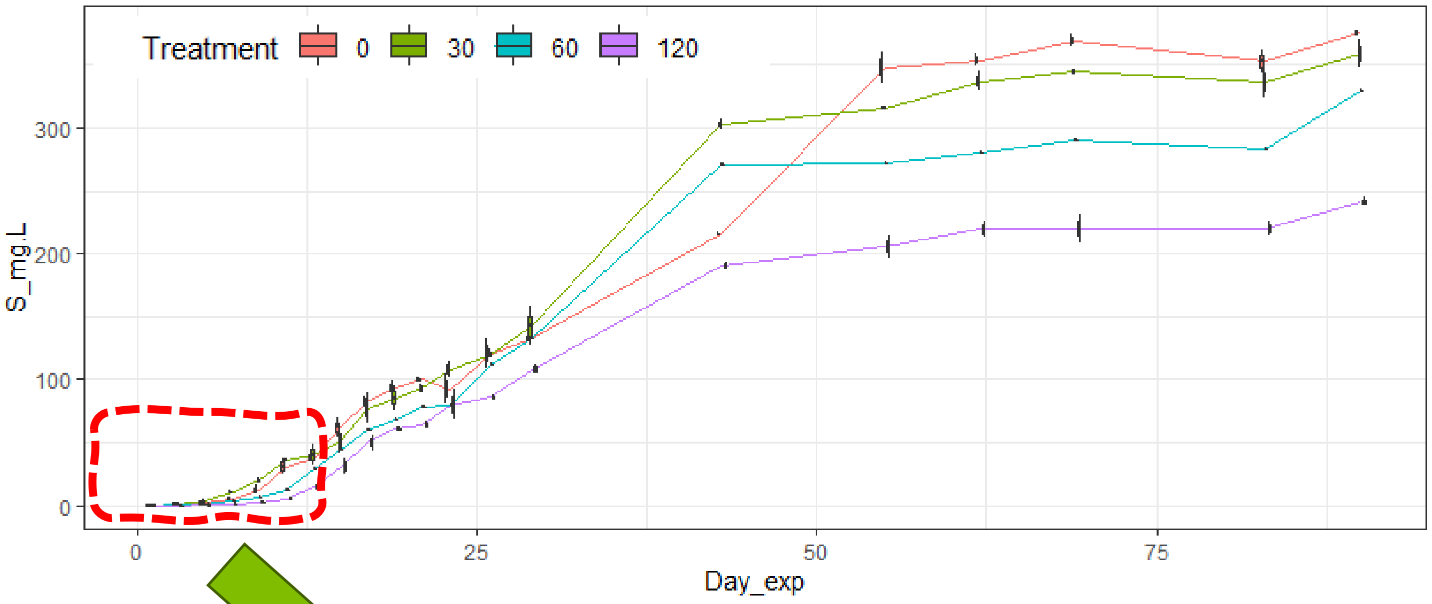
Results: Nitrate depletion and S^{2-} response

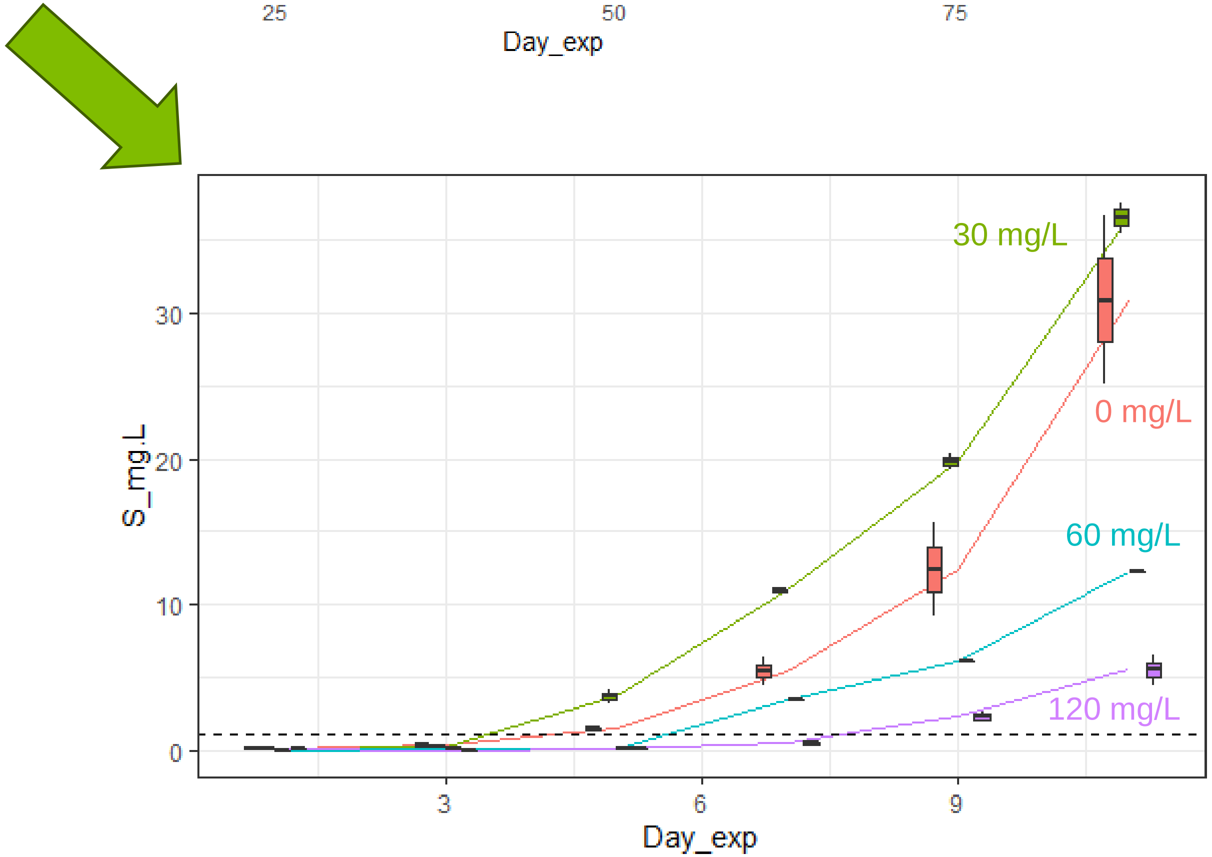
Nitrate depletion confirmed

- 3 days in 30 and 60 mg/L NO_3N
- 5 days in 120 mg/L NO_3N

H_2S response ($S^{2-} \geq 1$ mg/L)

- 5 days in 0 and 30 mg/L, 9 days in 120 mg/L
- Fastest increase with 30 mg/L
- 2-5 days delay in H_2S response after depletion of electron acceptor





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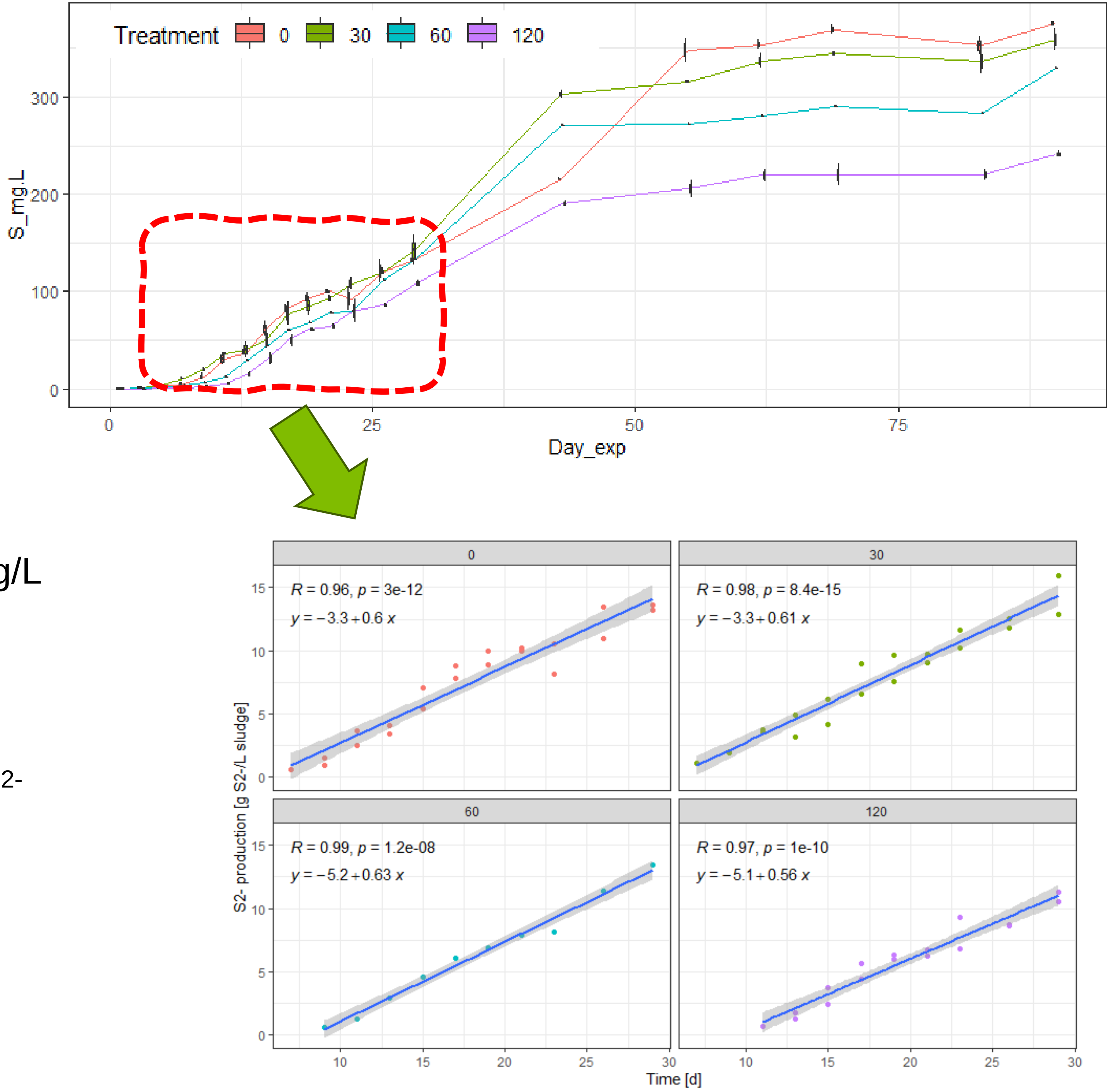
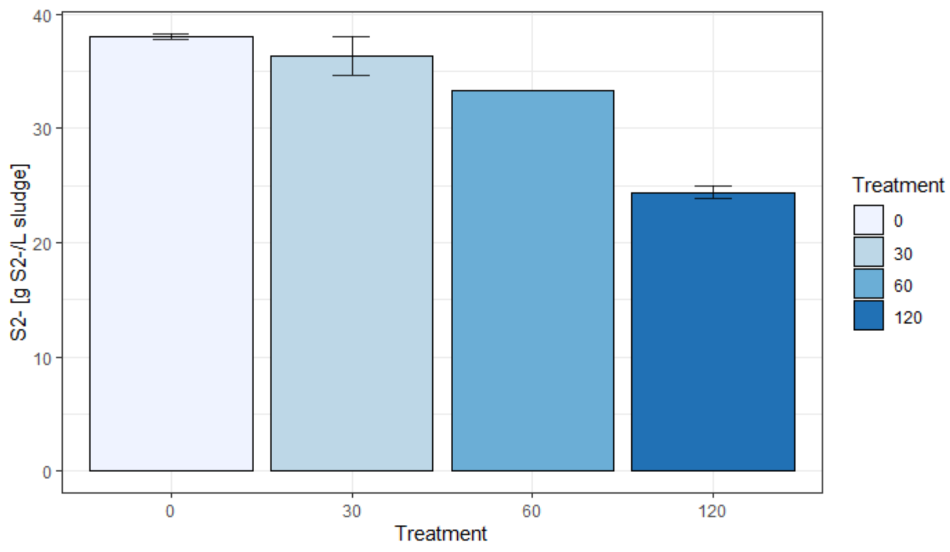
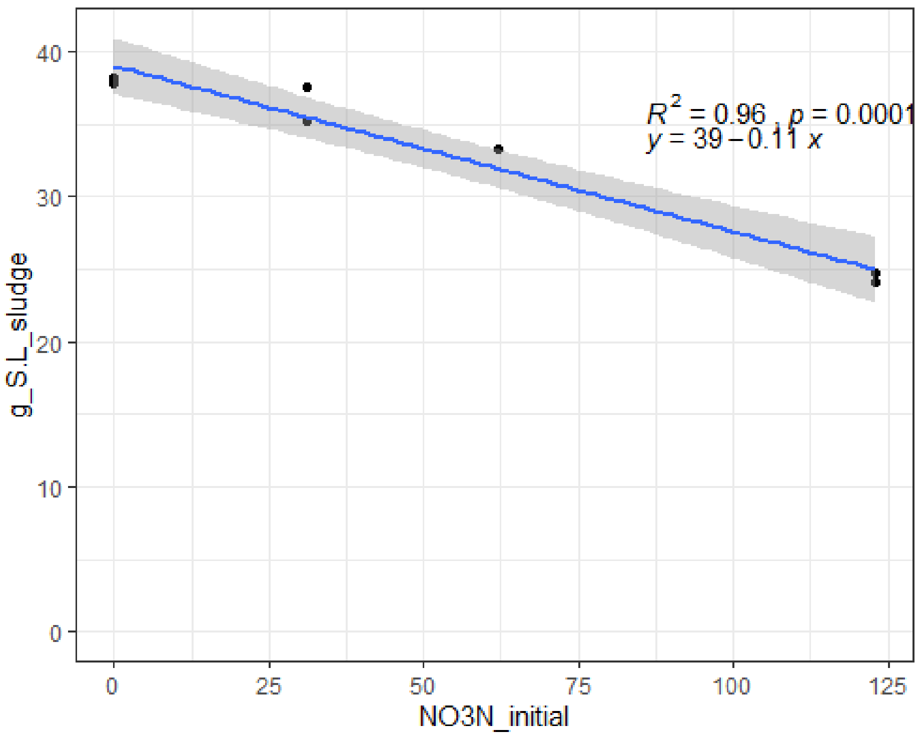
- Fastest increase with 30 mg/L
- 2-5 days delay in H_2S response after depletion of electron acceptor
- Average inital S^{2-} production rate ~0.6 g S^{2-}/L sludge/d



Results: Total S^{2-} production

- Maximum H_2S production for sludge without nitrate
  - 38 g S^{2-}/L sludge (0 mg/L NO_3N)
  - 24.4 g S^{2-}/L sludge (120 mg/L NO_3N)
- Significantly less H_2S with increasing nitrate concentrations

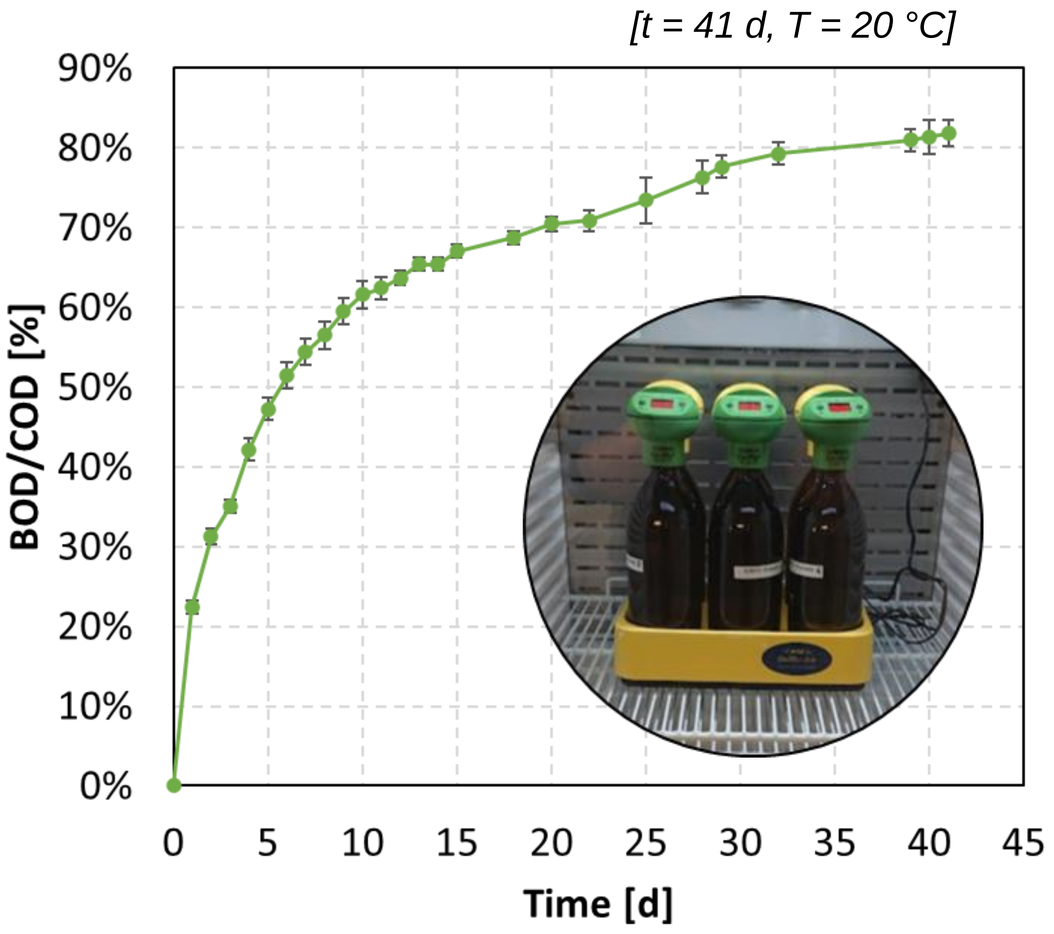




Results: Organic matter & H_2S production

Carbon bioavailability determined with respirometric cBOD test

- 82% of COD is degradable (41 d)
- BOD/N-ratios between 8 and 31 g/g

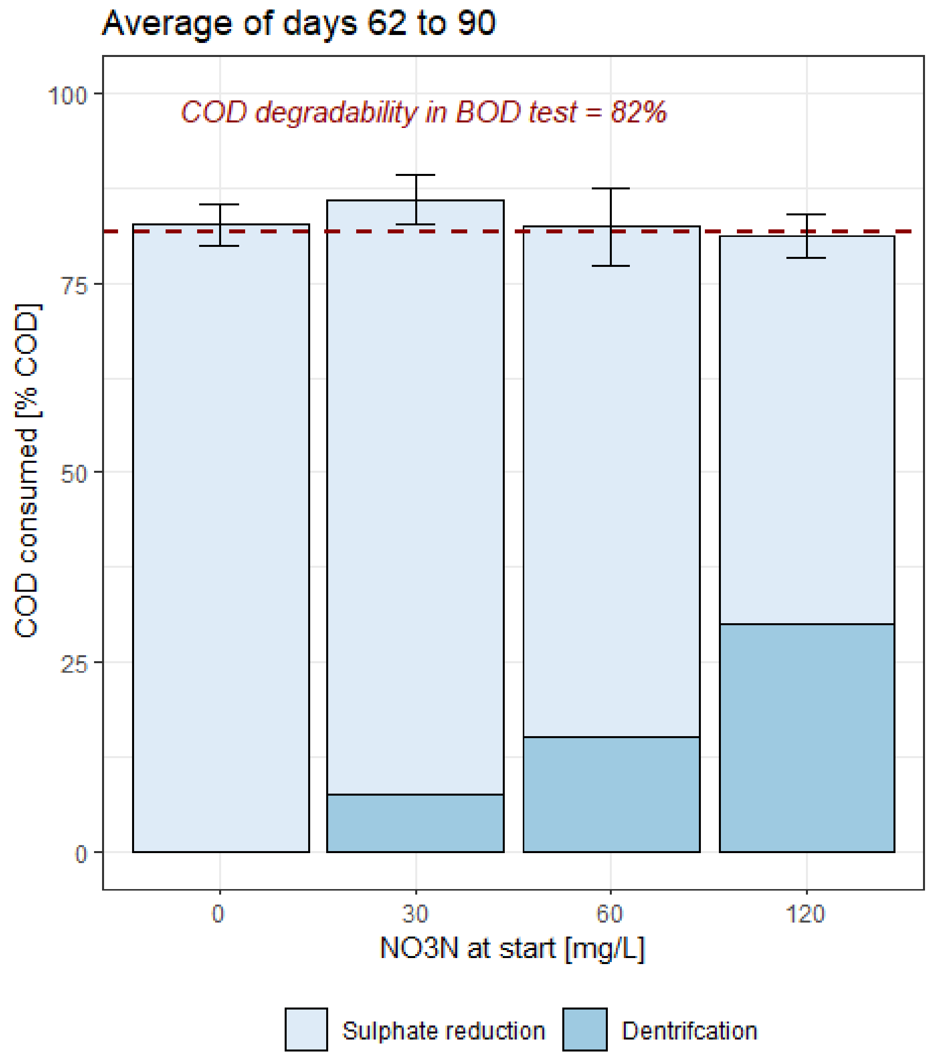


Treatment (NO_3N)	COD/N	$cBOD_{41}/N$
0		-
30 (31 mg/L)	37.7	30.9
60 (62 mg/L)	18.9	15.5
120 (123 mg/L)	9.5	7.8

Results: Organic matter & H_2S production

Calculating theoretical BOD demand, based on NO_3-N consumption and S^{2-} production

- 2.86 g BOD/ g NO_3-N (Metcalf & Eddy, 2004)
- 2.67 g BOD / g S^{2-} (Metcalf & Eddy, 2004)
- Our result: 2.67 ± 0.161 g BOD/ g S^{2-}



COD balance can be closed at ~100%, fate of biodegradable matter can be predicted, H_2S production potential calculated

Reference: Metcalf & Eddy Inc. (2004), *Wastewater engineering : treatment and reuse* (4th ed.). McGraw-Hill.

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Summary

- Bioavailability of carbon is key to understand risks for H_2S
- Nitrate delays/reduces H_2S response
- H_2S potential can be calculated

Knowledge gaps

- More work needed on diffusion-limited systems and H_2S oxidation rates relevant for RAS (sediments, biofilms)
- Degradation kinetics of carbon source could change reaction speed (e.g. feed spill vs. feces)

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