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Development of a high throughput method for screening readily biodegradable chemicals

Background

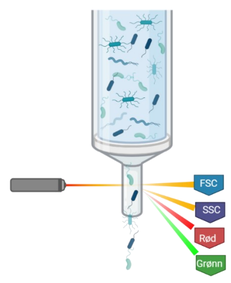
- High throughput method for **down the drain substances**
- Screening for **indication** of readily biodegradability to identify safe and **green chemicals**
- **Representative** and **reproducible** inoculum
- Increase understanding of **microbial dynamics**
- Activate **biodegradation potential** of microbial community

Approach

Bacterial growth is used to meassure degradation of chemicals

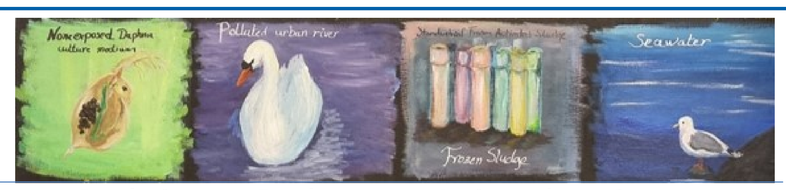
	1	2	3	4	5	6	7	8	9	10	11	12
A: Aniline	A	Blank control			5 mgC/L		25 mgC/L		125 mgC/L		empty	empty
B: Sodium Benzoate	B											
C: 4-nitrophenol	C											
D: Benzotriazole	D											
E: Trimethoprim	E											
F: Oxipurinol	F											
G: Acesulfam	G											
H: Sucralose	H											

X 24 plates with different inoculums



Flowcytometer for bacterial cell count

- Four sources of bacterial inoculum
- Eight reference chemicals
- Different treatments of inoculums



Treatment	Inoculum dilution	Daphnia media	Freshwater	Activated sludge	Seawater
	Dilution medium 301: 1.1 mgC/L	7.8 mgC/L, 9 µgN/L	3.7 mgC/L, 950 µgN/L	9.1 mgC/L, <10 µgN/L	2.1 mgC/L, 110 µgN/L
Untreated	0, 10, 100	3	3	3	3
Co-substrate: Yeast extract 1 mg/L	10, 100	Not done	2	2	2
Pre culture: Yeast extract 100 mg/L	10, 100	Not done	2	2	2

Results and discussion

- **Readily biodegradable chemicals** (sodium benzoate and aniline) had positive growth responses, other chemicals did not give reliable results.
- **Optimum cell density**: Growth responses were higher at low cell densities, but with possible lower probability of biodegradation, and this varied between inoculum sources. Diluting the inoculum also increased the variability between replicates.
- **Chemical test concentration**: growth response increased with increased chemical concentration, and 5 mg C/L was not sufficient to give a significant results.

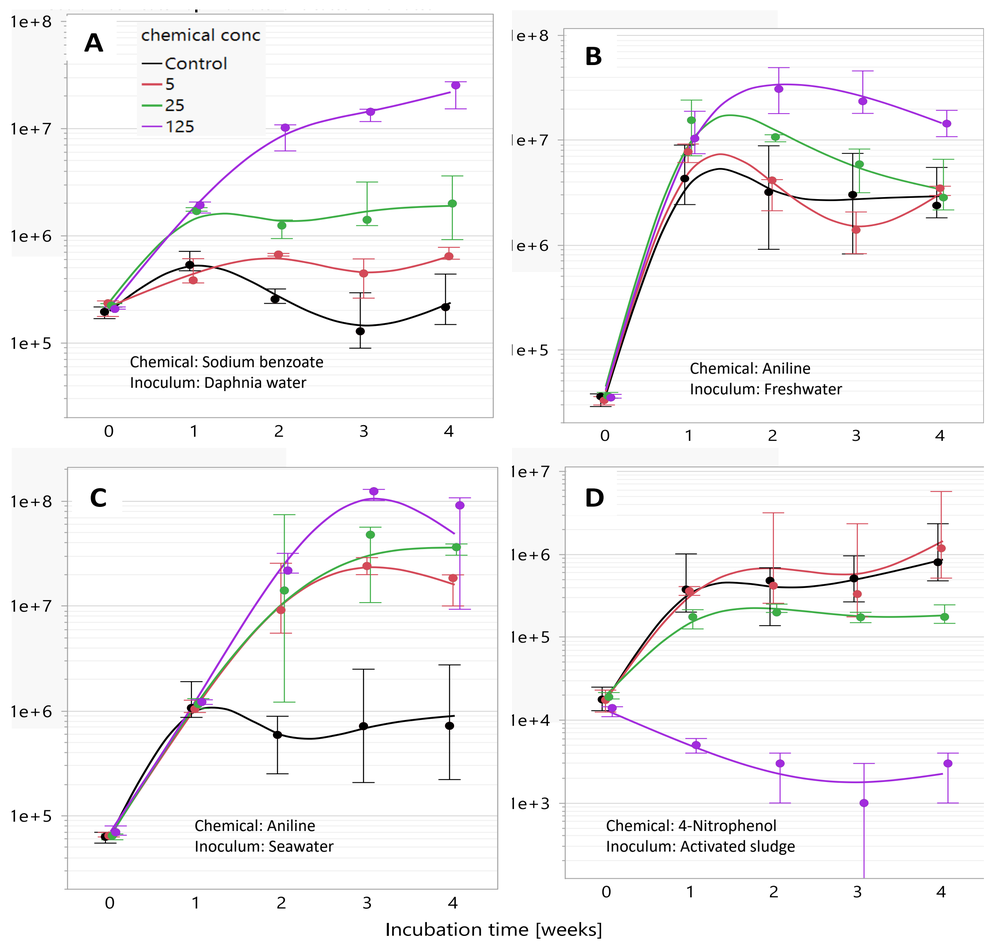


Figure: Examples of time series.

A: positive growth response increasing with increasing chemical concentration and time

B: Positive growth response on week 1-2 followed by decline

C: High positive growth response for all test concentrations

D: No growth response for lowest chemical concentration and inhibitions of higher concentrations

- **Inoculum treatments to enhance activity:** The use of yeast extract as co-metabolite or pre-culture with yeast extract for acclimatizing and conditioning the inoculum is not recommended as this gave variable results and is not permitted in standard biodegradation screening tests.
- **Replicates:** As the use of growth as an indicator of biodegradation includes more sources for variability than other methods based on respiration, the use of replicates is essential, and more replicates should be used.

High throughput potential: 576 experiments were run in triplicates by one operator over 7 weeks.
Positive controls gave significant growth response in 76% (109 of 144) experiments.
Potential as a pre-screening method for developing new green chemicals.

More stringent than standard screening tests.
Does not correlate to percent or rate of degradation.
Might not work for slowly degrading chemicals.
Need to define applicability domain.
Refinements are needed.

Future perspectives

- Optimize conditions and correlate to regulatory endpoints.
- Use FCM “fingerprint” data for understanding bacterial community dynamics in biodegradation tests.
- A follow up experiment was done to look at:
  - Effect of protozoa
  - Higher inoculum concentrations
  - Results not evaluated yet

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Further reading: Wennberg et al., 2022 https://doi.org/10.1016/j.chemosphere.2022.134476

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