Transformation of the Emerging Insensitive Munitions Compound 3-Nitro-1,2,4-triazol-5-one (NTO) by Soil Microorganisms

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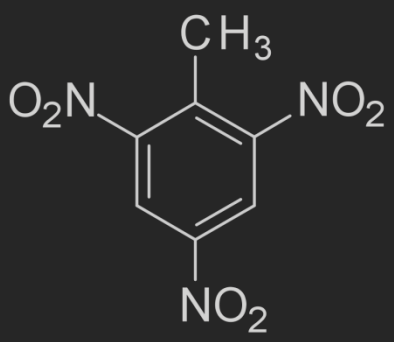
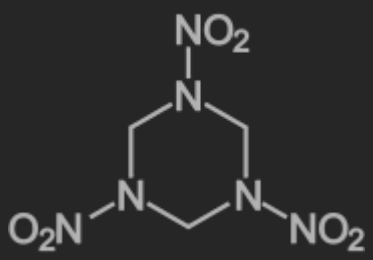
Insensitive Munition Compounds (IMCs)

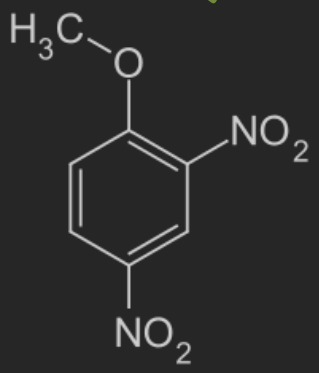


TNT

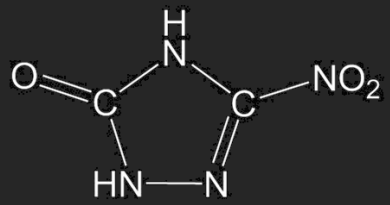


RDX

Conventional Explosive Compounds



DNAN



NTO

Insensitive Munitions Compounds

IMCs are less sensitive to shocks and high temperature, reducing risk of accidental explosions.

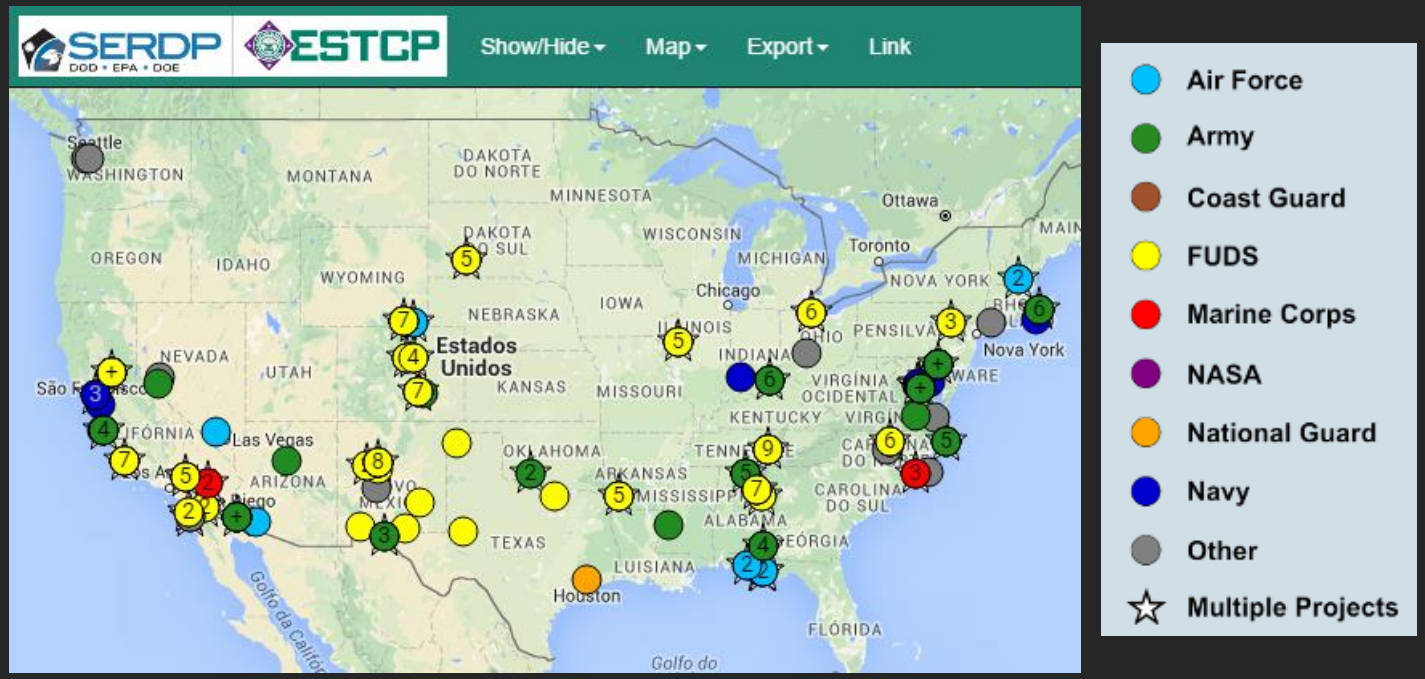
Insensitive Munition Compounds (IMCs)

Incomplete detonations lead to soil contamination.

Knowledge about NTO’s fate in the environment is still scarce.

Insensitive Munition Compounds (IMCs)

Munition Response Sites (MRS): known or suspected to contain unexploded ordnance, discarded military munitions, or munitions constituents.



Estimated cost to clean up MRS sites: > $13 billion

Objectives

Investigation of bioremediation as a method to clean up sites contaminated with NTO

Sequential anaerobic-aerobic degradation of NTO by soil microorganisms in batch reactor

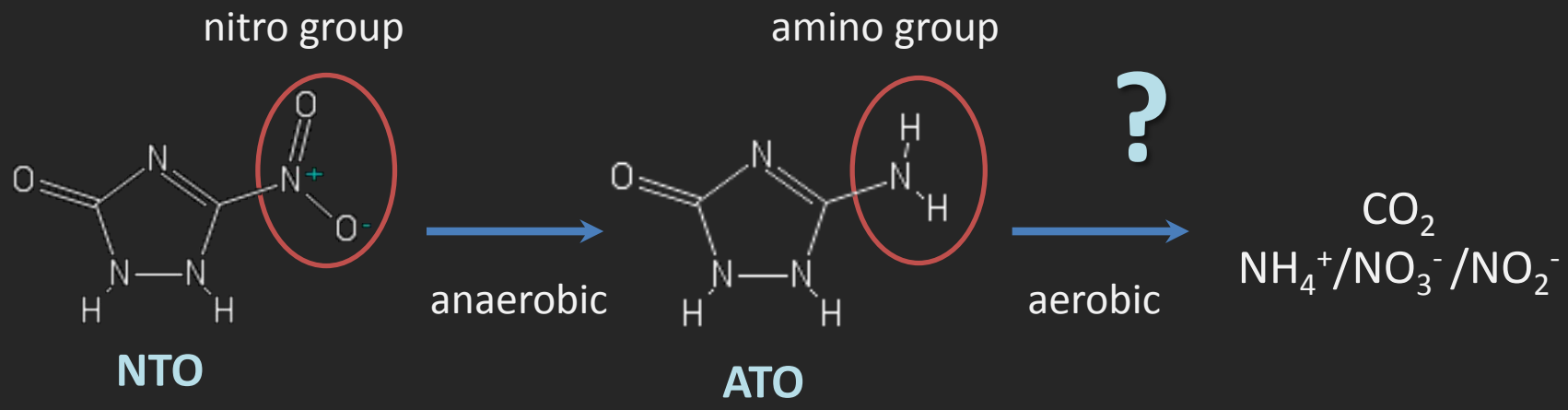
Study of NTO transformation products and mineralization

Identification of microorganisms related to NTO and ATO degradation

Sequential anaerobic-aerobic degradation in batch reactors

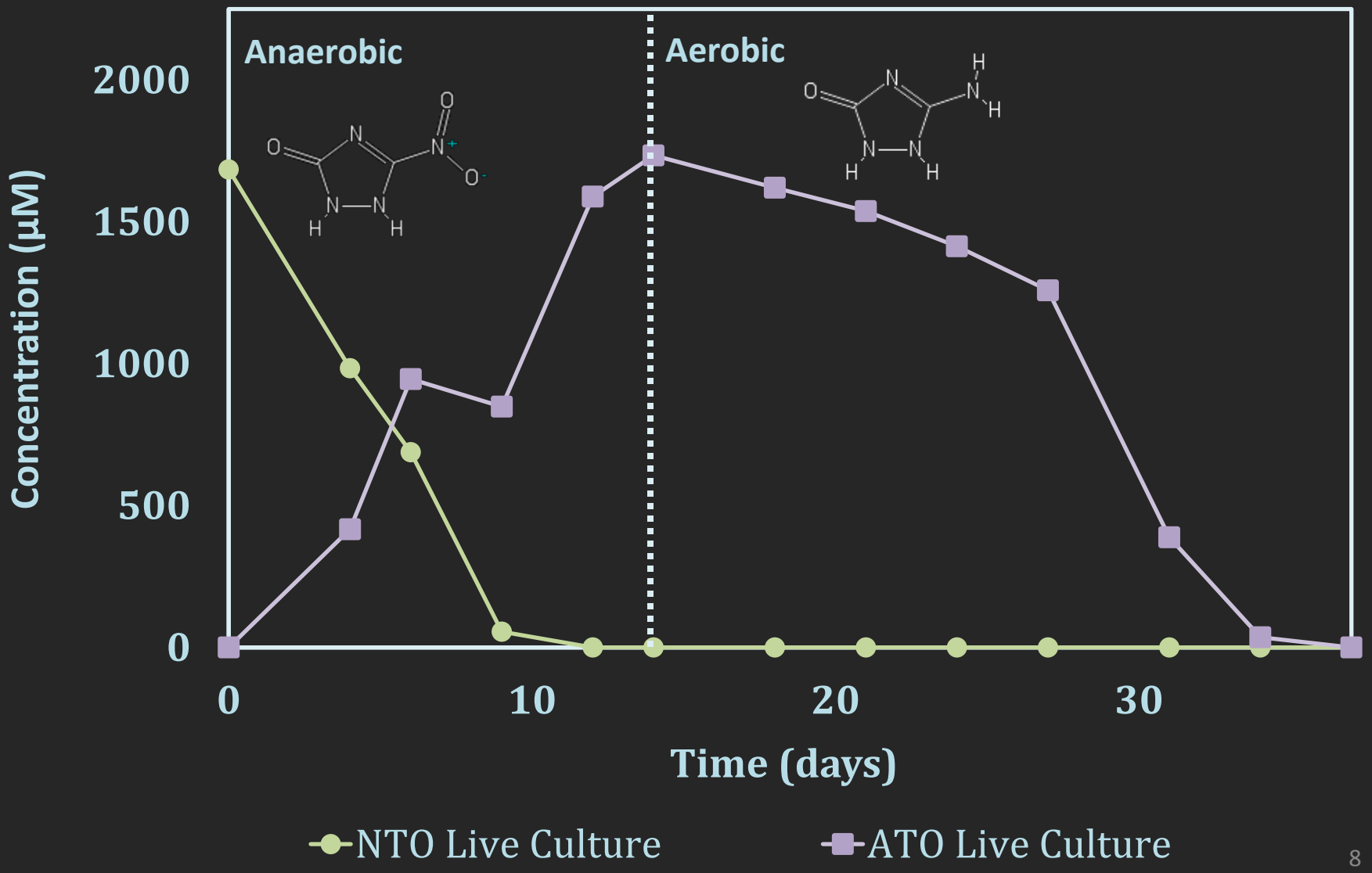
Sequential anaerobic-aerobic approach

Previous studies: NTO is primarily reduced to ATO (3-amino-1,2,4-triazol-5-one) by bacterial culture

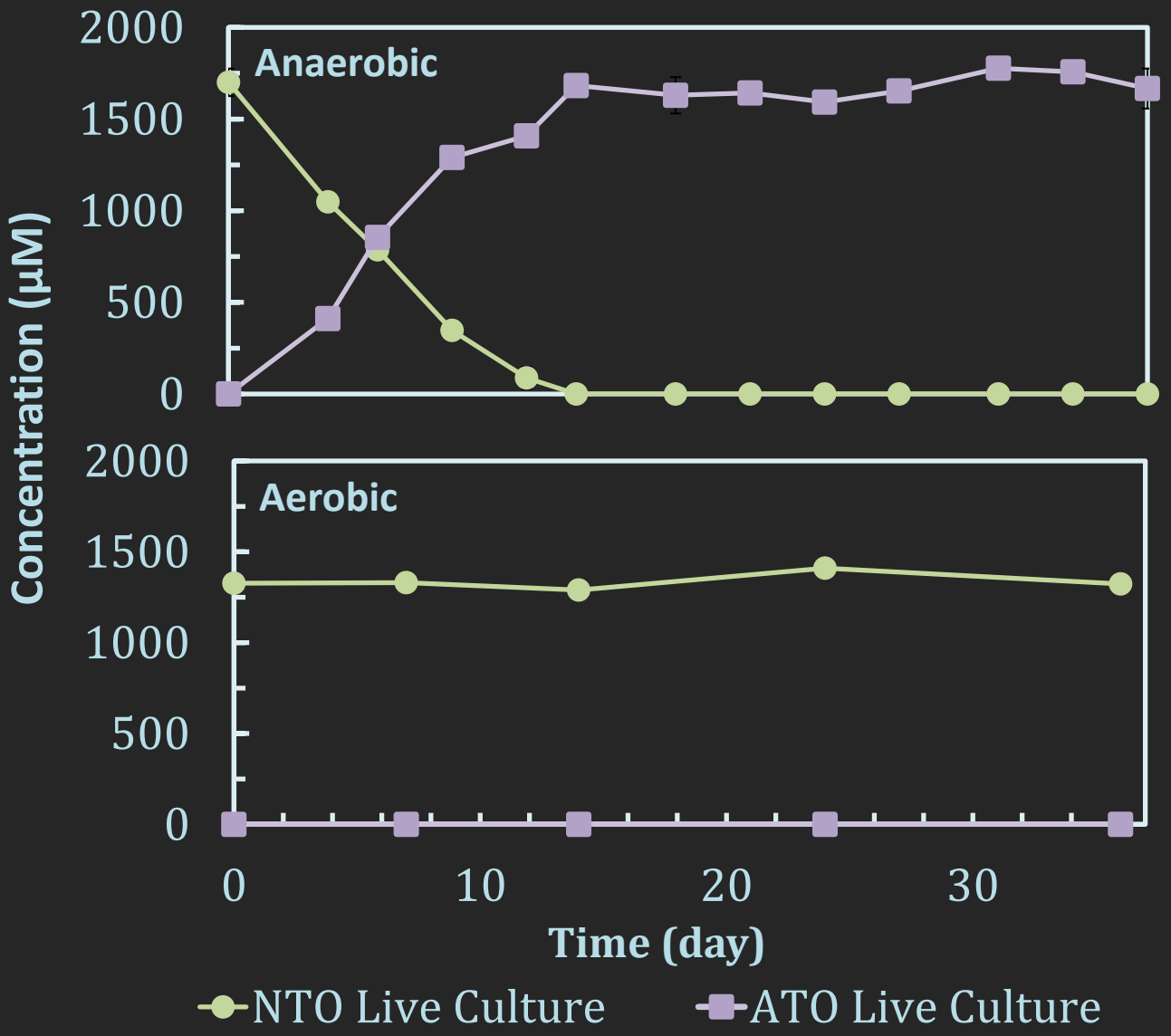


10 g/L Soil
1.5 mM NTO
2.7 mM Na-pyruvate
Mineral Medium

Results


Anaerobic
Aerobic
2000
1500
1000
500
0
Concentration (µM)
0
10
20
30
Time (days)
NTO Live Culture
ATO Live Culture

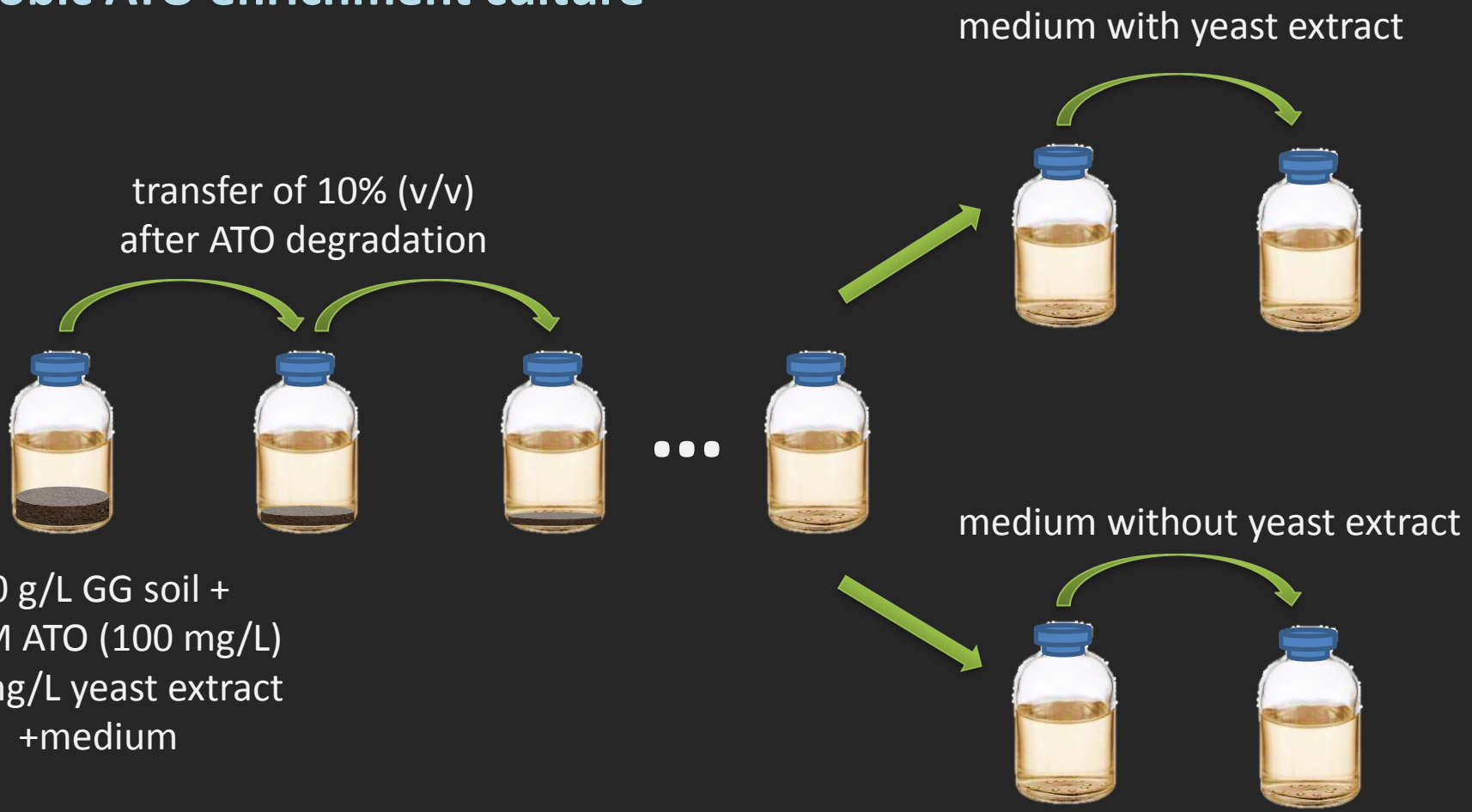
Results


Anaerobic
Aerobic
Concentration (µM)
2000
1500
1000
500
0
0
10
20
30
Time (day)
NTO Live Culture
ATO Live Culture

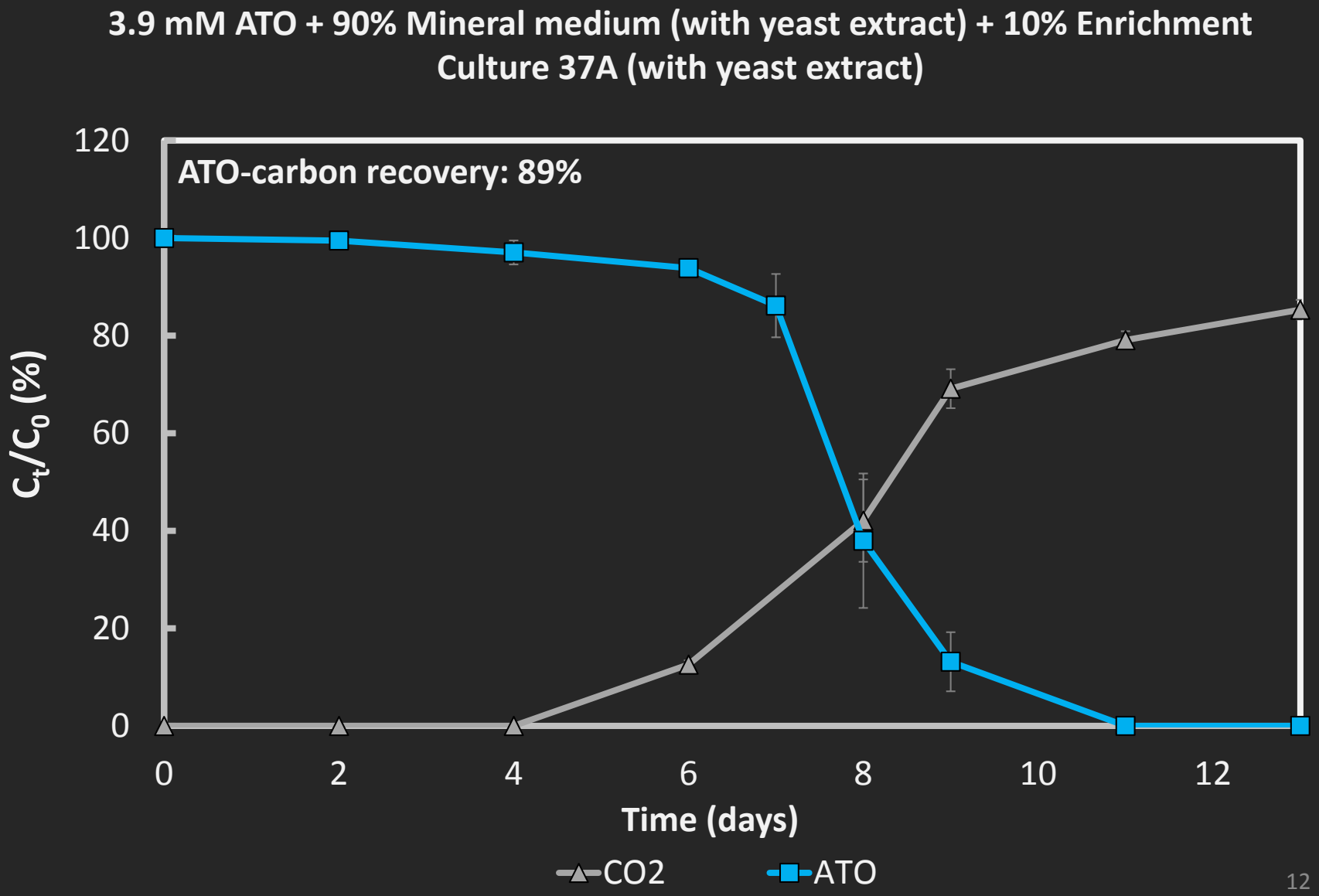
ATO mineralization by enrichment culture

Material and Methods

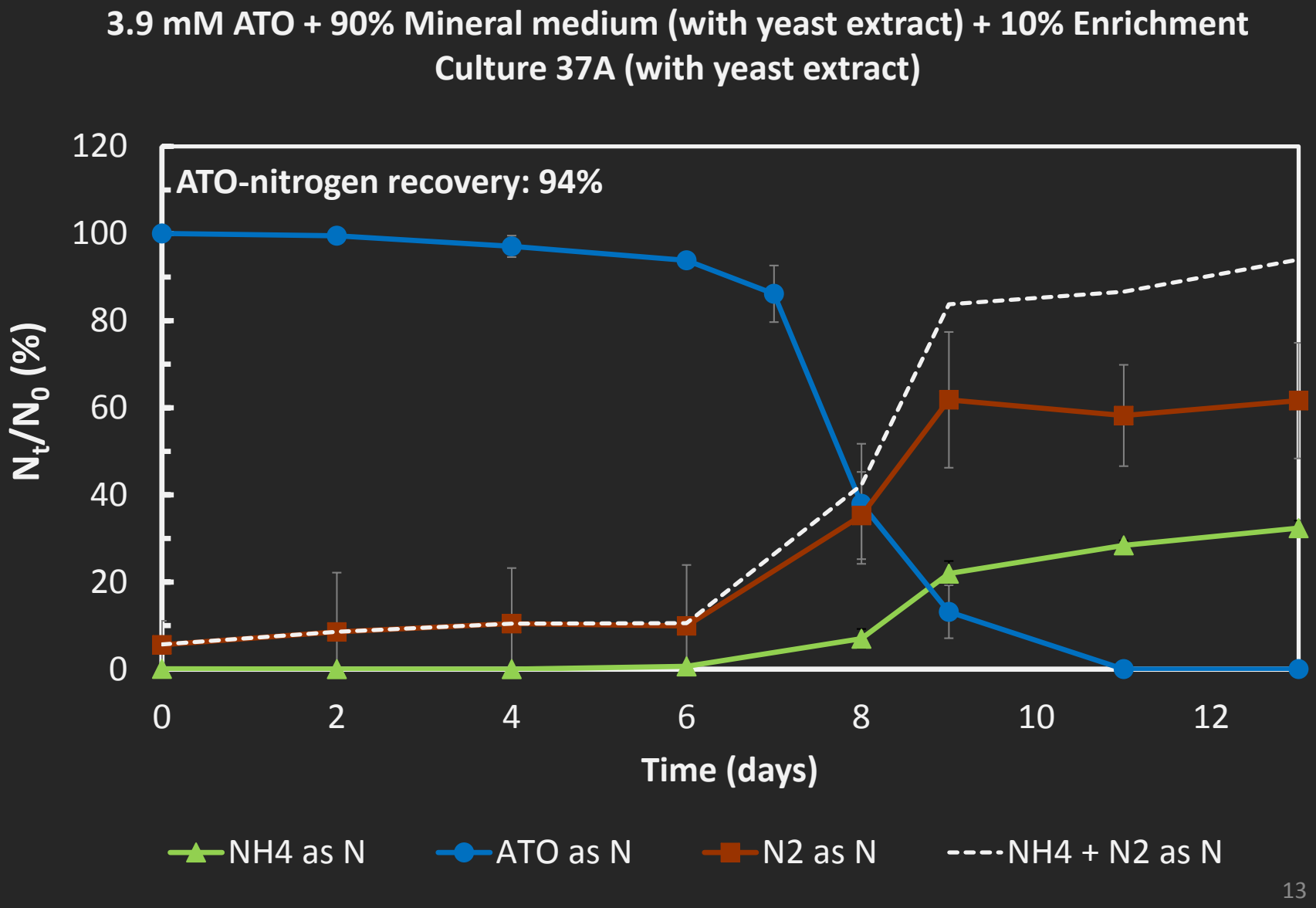
Aerobic ATO enrichment culture



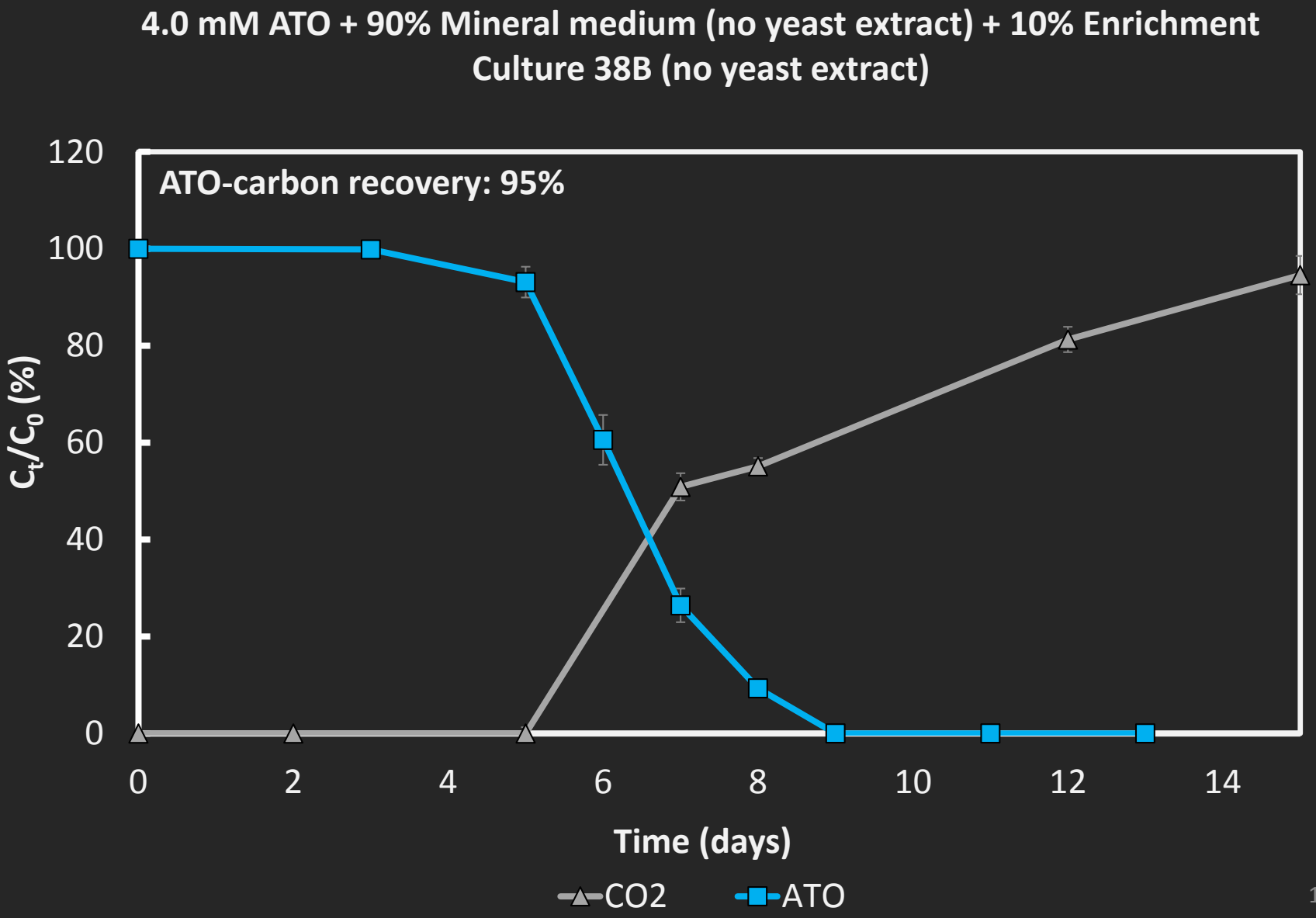
Results


3.9 mM ATO + 90% Mineral medium (with yeast extract) + 10% Enrichment Culture 37A (with yeast extract)
ATO-carbon recovery: 89%
120
100
80
60
40
20
0
C_t/C_0 (%)
0
2
4
6
8
10
12
Time (days)
CO2
ATO

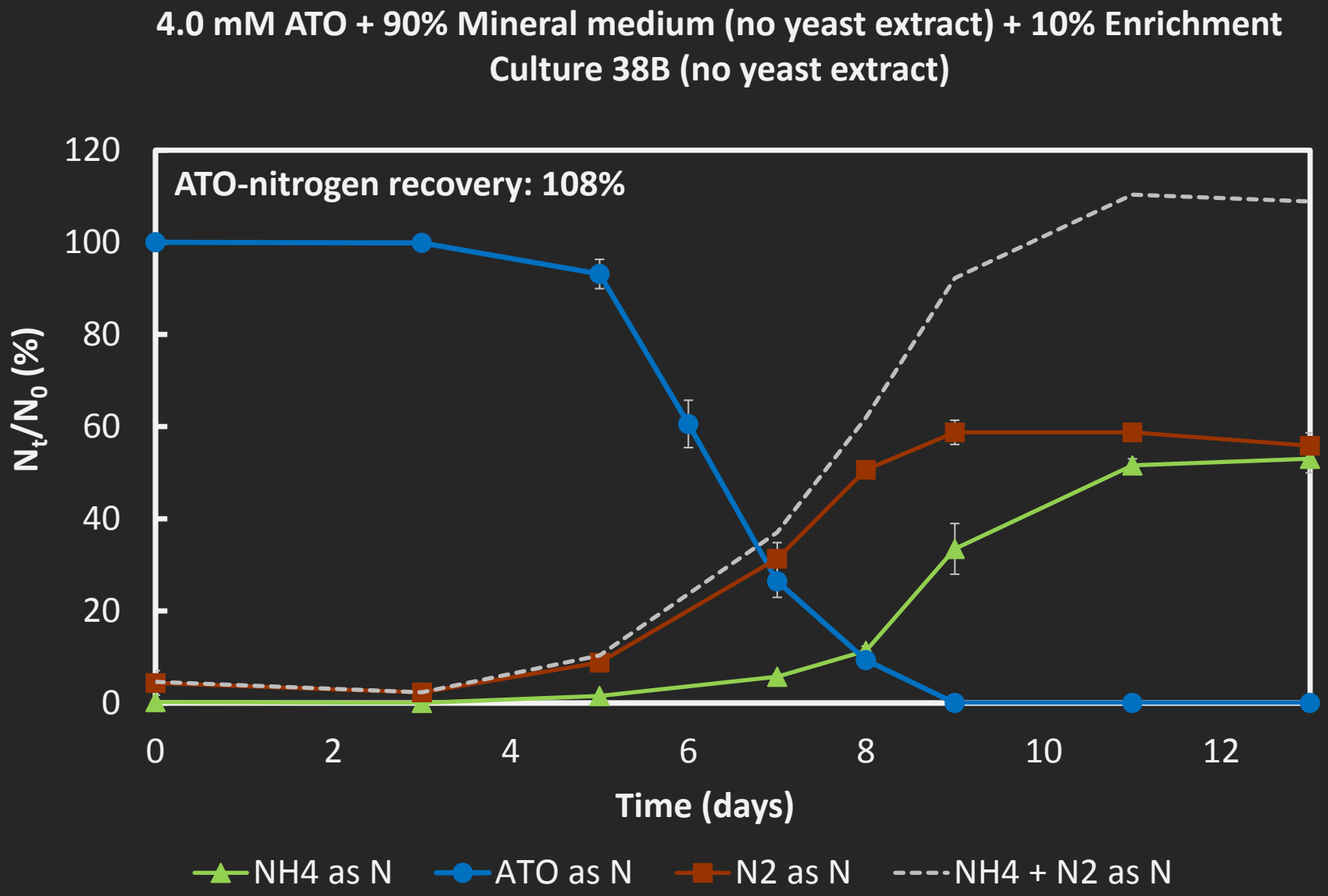
Results


3.9 mM ATO + 90% Mineral medium (with yeast extract) + 10% Enrichment Culture 37A (with yeast extract)
ATO-nitrogen recovery: 94%
120
100
80
60
40
20
0
N_t/N_0 (%)
0
2
4
6
8
10
12
Time (days)
NH4 as N
ATO as N
N2 as N
NH4 + N2 as N

Results


4.0 mM ATO + 90% Mineral medium (no yeast extract) + 10% Enrichment Culture 38B (no yeast extract)
ATO-carbon recovery: 95%
120
100
80
60
40
20
0
C_t/C_0 (%)
0
2
4
6
8
10
12
14
Time (days)
CO2
ATO

Results


4.0 mM ATO + 90% Mineral medium (no yeast extract) + 10% Enrichment Culture 38B (no yeast extract)
ATO-nitrogen recovery: 108%
N_t/N_0 (%)
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Time (days)
NH4 as N
ATO as N
N2 as N
NH4 + N2 as N

Results

Summary of Mass Balance

Enrichment culture	C_t/C_o (%)	N_t/N_o (%)
A (with yeast extract)	89	94
B (no yeast extract)	95	108

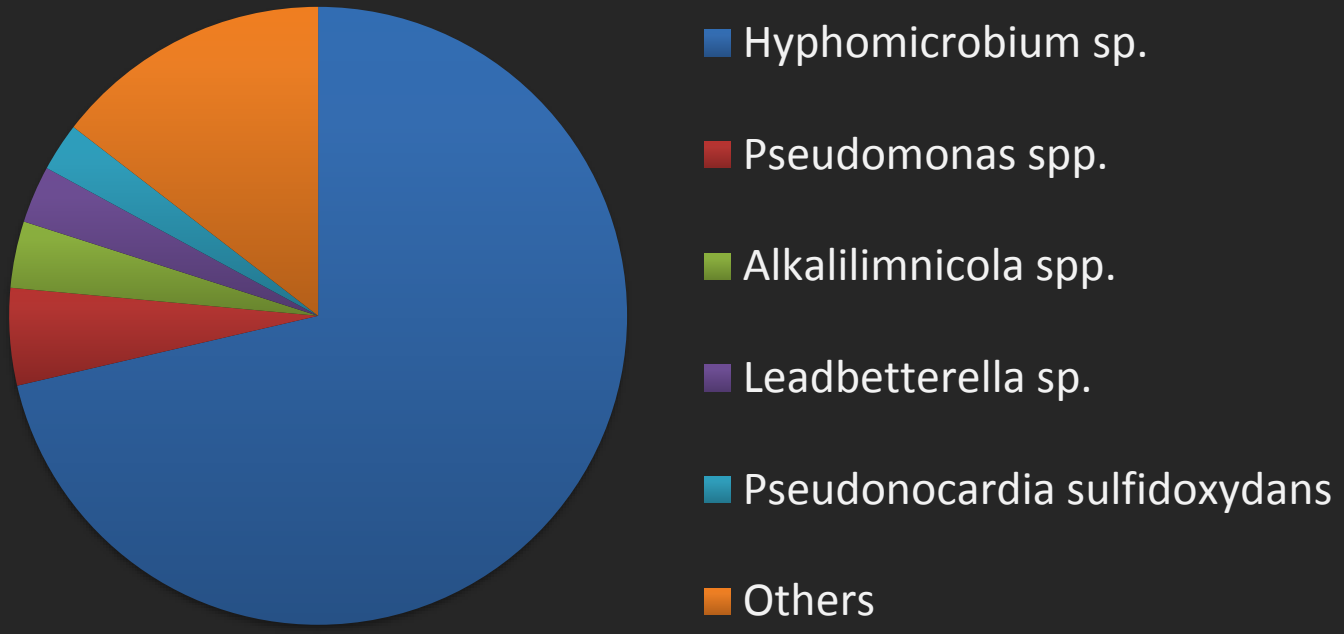
Stoichiometry

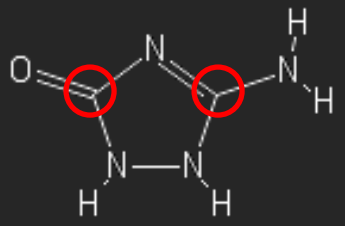
$$C_2N_4OH_4 + O_2 + H_2O \longrightarrow 2CO_2 + N_2 + 2NH_3$$

Results

Microbial Diversity of ATO enrichment culture (with yeast extract)

16S rRNA 454 Pyrosequencing

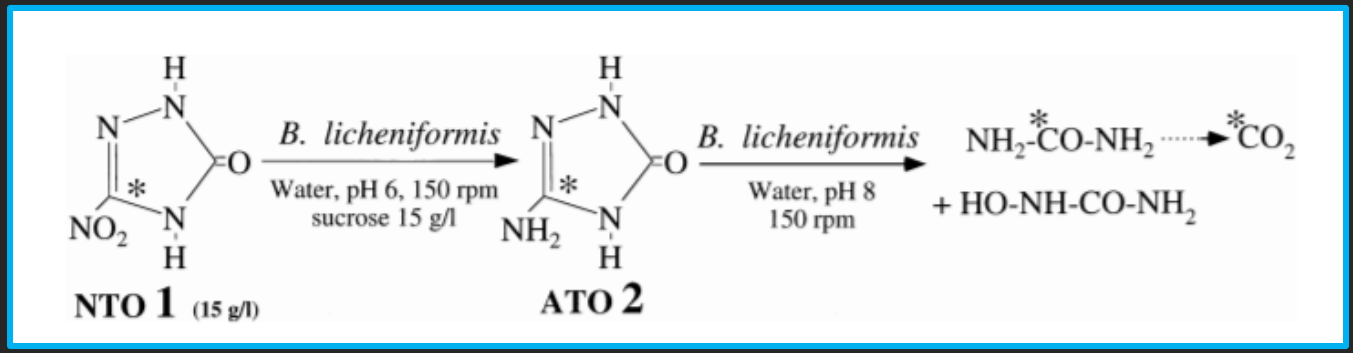




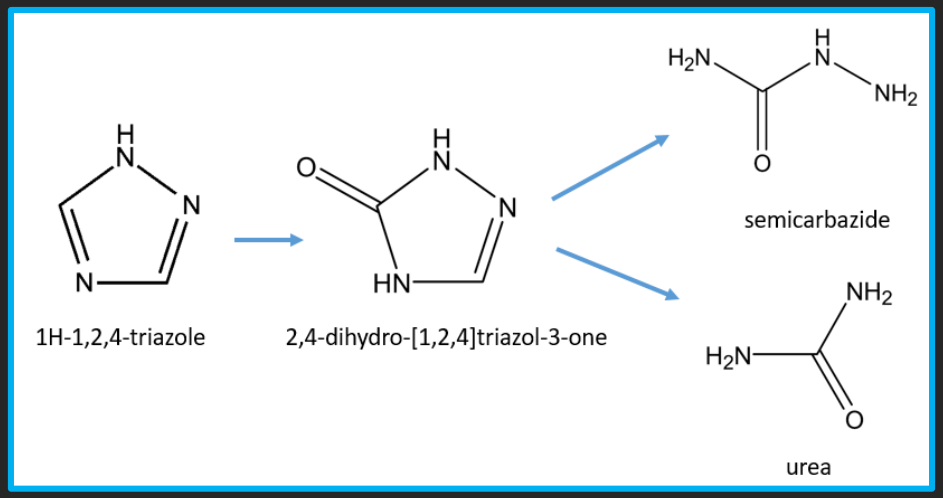
Hyphomicrobium sp.: related to transformation of C1 molecules (methylamine, methanol, chloromethane)

Results

Proposed degradation pathway of 3-nitro-1,2,4-triazole-5-one by *B. lichenniformis (Le Campion et al., 1999):*



Proposed degradation pathway of 1-H-1,2,4-triazole by *Shinella* sp. (Wu et al., 2016):



Conclusion

An anaerobic step is needed to activate NTO for further degradation.

Extensive mineralization of ATO was achieved by an enrichment culture using ATO as the sole carbon and nitrogen source, as evidenced by the complete recovery of ATO carbon and nitrogen as inorganic products. The formation of N_2 as a degradation product of a triazole was reported for the first time.

Hyphomicrobium sp. was the most abundant bacterial 16S rRNA ampliclon in ATO enrichment culture microbial analysis, being related to C1 metabolism.

Bioremediation was shown to be a potential alternative to promote the complete removal of NTO from the environment.

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