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Harbin Institute of Technology



城市水資源與水環境國家重點實驗室
State Key Laboratory of Urban Water Resource & Environment

Micro-aerobic enhanced performance of denitrifying sulfide removal with high C/N ratios

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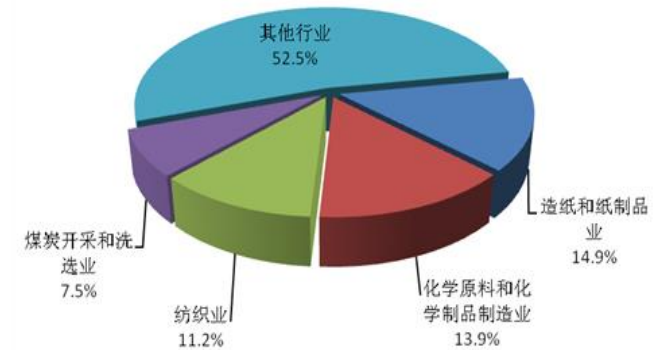
Outline

- ◆ What is denitrifying sulfide removal process (DSR)
- ◆ Performance of DSR
- ◆ Micro-aerobic enhanced performance of DSR in high C/N ratios
- ◆ Why is micro-aerobic enhanced performance ?
- ◆ Summary and future challenges

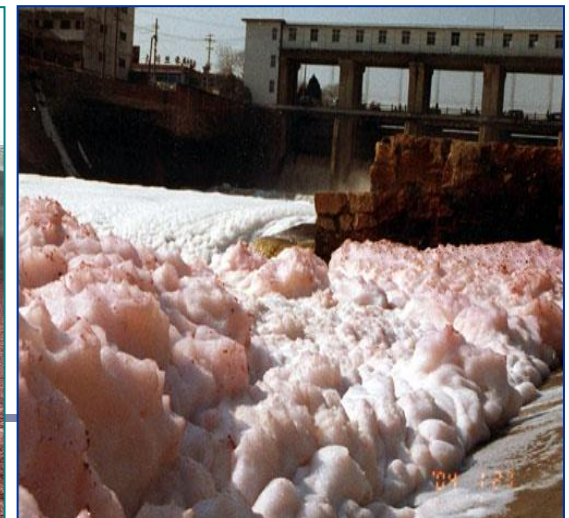


Background

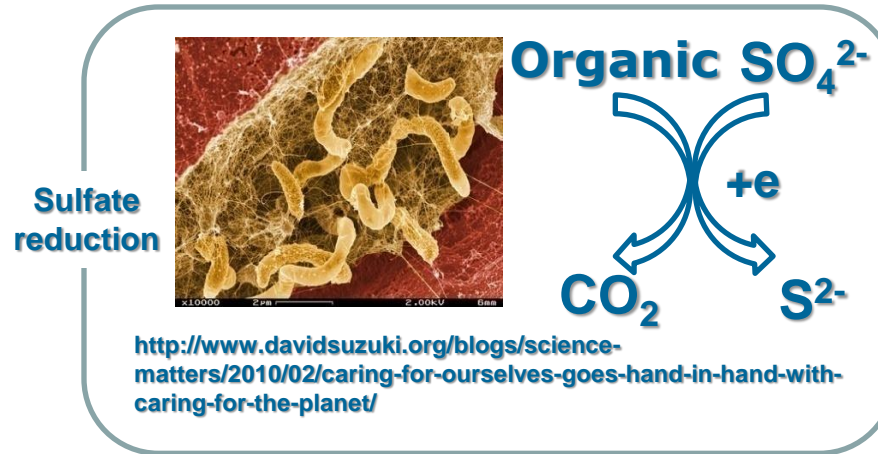
- ➔ Total industrial wastewater discharge over twenty billions tons per year
- ➔ Wastewaters discharged from paper, chemical engineering, textile and mining over nine billions tons (47.5% in total)
- ➔ Contain sulfate, ammonia, nitrate and COD
- ➔ More and more stringent discharge standards implemented



Based on **Environmental statistic annual report of China, 2013**

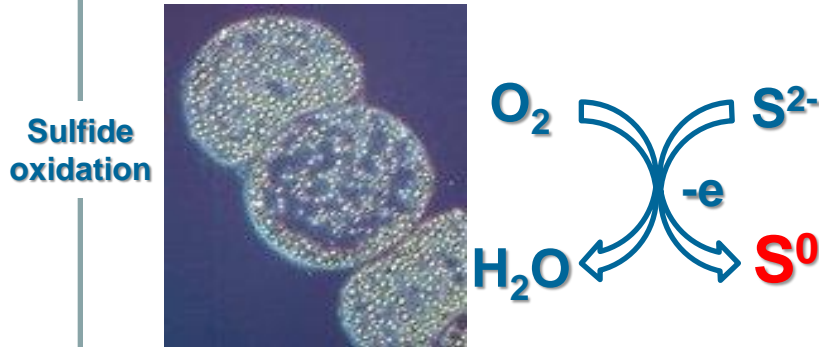


◆ Sulfate reduction and sulfide-oxidation



• Aerobic

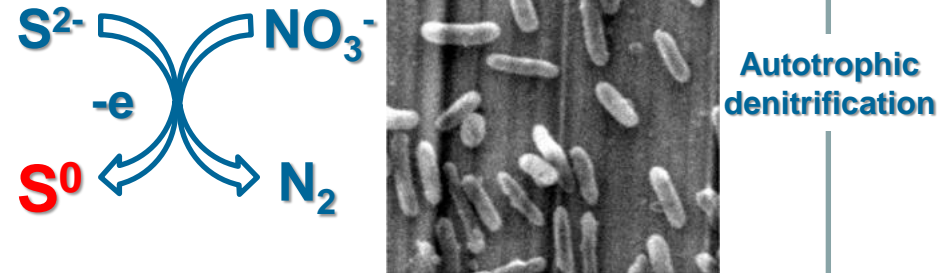
Sulfur accumulated intracellularly with low rate, Practical application restricted



http://www.mpi-bremen.de/en/Ecophysiology_Group.html

• Anaerobic

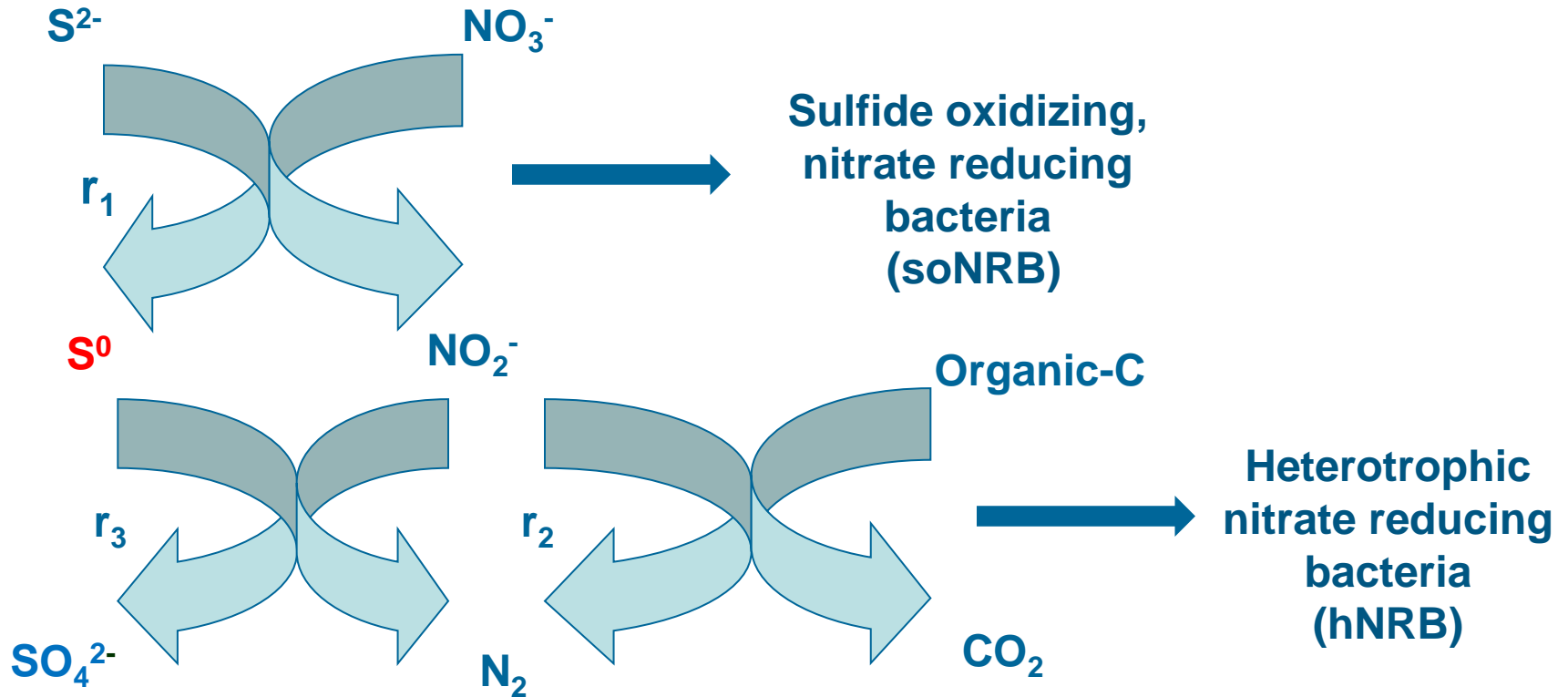
Sulfur accumulated extracellularly with high rate, Suitable for Practical application



genome.jgi-psf.org/thide/thide.home.html



Denitrifying sulfide removal (DSR)



Reaction rate argued by **Reyes-Avila *et al.*** (water res., 2004) and **Chen *et al.*** (Appl. Microbiol. Biotechnol. 2008,78(6): 1057-1063)



The optimum performance of DSR

The maximum capacity of EGSB-DSR process

Autotrophic bacteria: $S^{2-} \rightarrow S^0$

Heterotrophic bacteria: $NO_3^- \rightarrow N_2$



Biological breakdown of denitrifying sulfide removal process in high-rate expanded granular bed reactor

Chuan Chen · Aijie Wang · Nanqi Ren · Hongjing Kan · Duu-Jong Lee

Maximum sulfide loading
6.09 kg/m³·d

Received: 8 August 2008 / Revised: 12 September 2008 / Accepted: 14 September 2008 / Published online: 30 October 2008
© Springer-Verlag 2008

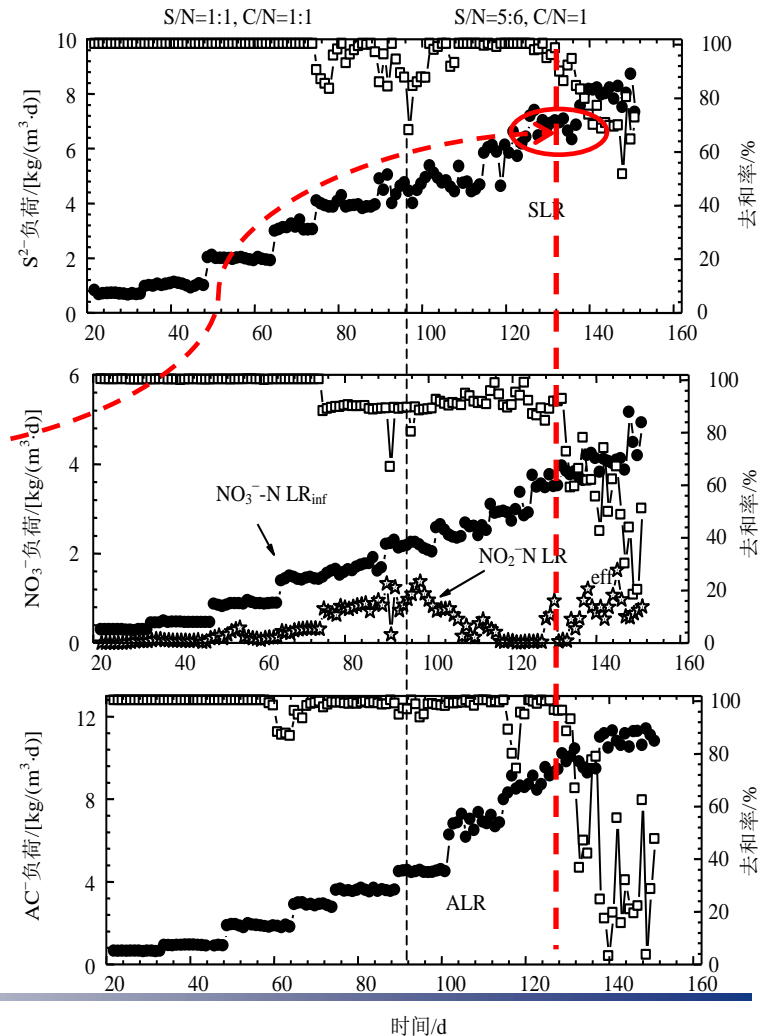
Abstract This work conducted a denitrifying sulfide removal (DSR) test in an expanded granular sludge (EGSB) reactor at sustainable loadings of 6.09 kg m⁻³ day⁻¹ for sulfide, 3.11 kg m⁻³ day⁻¹ for nitrate-nitrogen, and 3.27 kg m⁻¹ day⁻¹ for acetate-carbon with >93% efficiency, which is significantly higher than those reported

Introduction

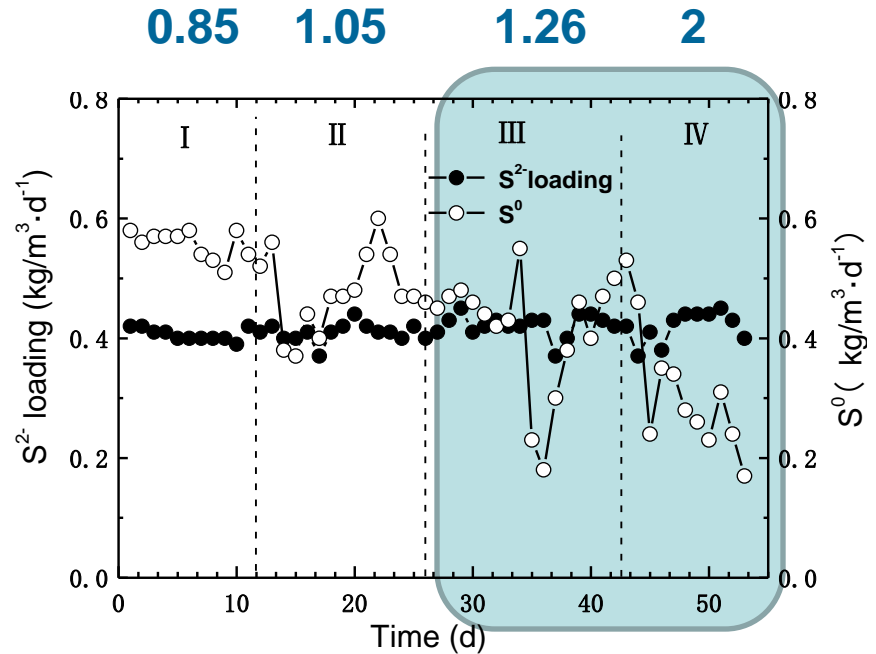
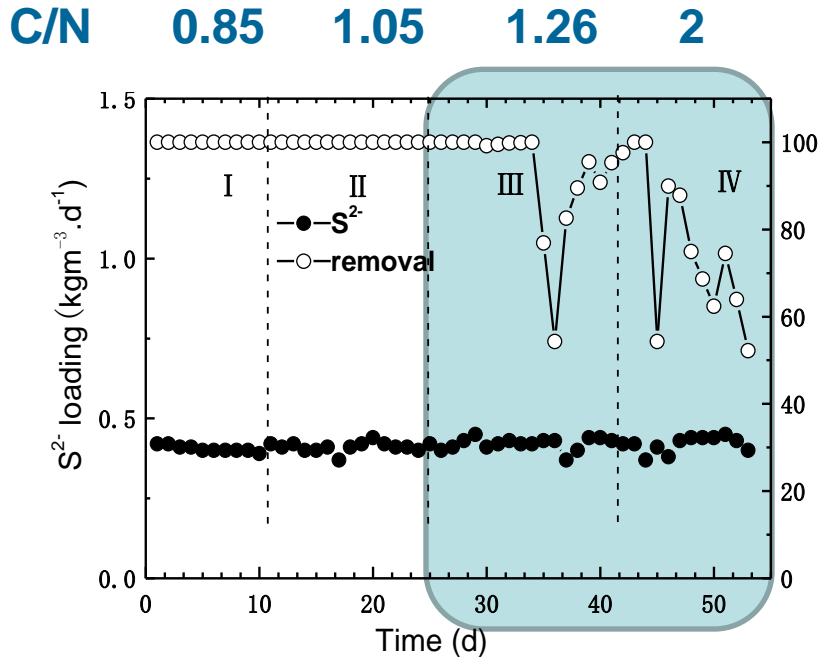
Denitrifying sulfide removal (DSR) simultaneously converts sulfide, nitrate, and chemical oxygen demand (COD) from industrial wastewater into elemental sulfur (S⁰), nitrogen gas (N₂), and carbon dioxide. Using only

Maximum sulfide loading: sulfide loading
0.294 kg/m³·d

----- Reyes-Avila Jesus et al., *Wat Res.*, 2004, 38: 3313-3321



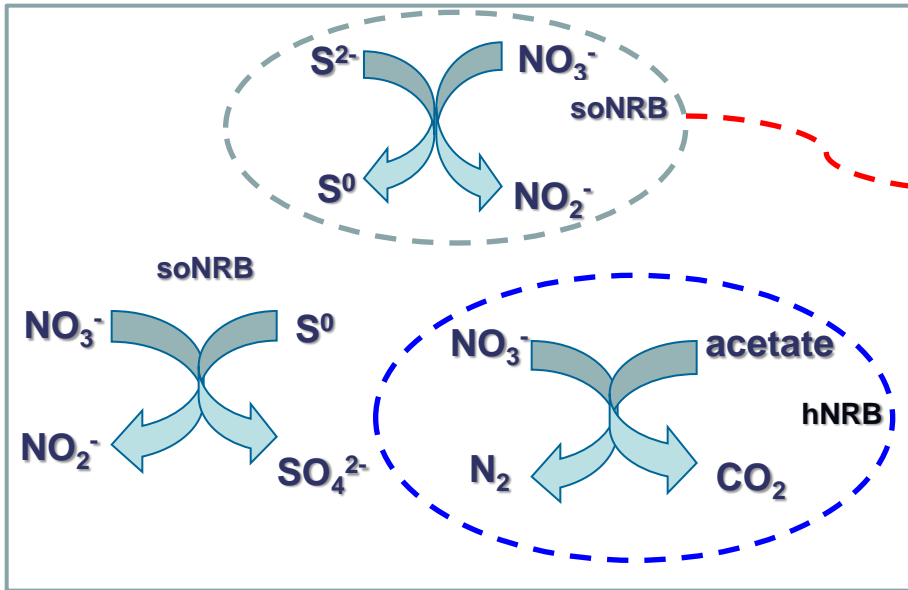
◆ The problem of DSR in high C/N ratios



- With high C/N ratio, **low S^{2-} removal and S^0 recovery** achieved
- Regulation strategy to solve the unbalance between soNRB and hNRB.



Micro-aerobic condition increases S^0 conversion



Process Biochemistry 45 (2010) 1007–1010

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Journal homepage: www.elsevier.com/locate/procbio

Short communication

Enhancing denitrifying sulfide removal with functional strains under micro-aerobic condition

Chuan Chen^{a,b}, Aijie Wang^{a,b}, Nanqi Ren^{a,b,*}, Qingliang Zhao^{a,b}, Lihong Liu^b, Sunil S. Adav^c, Duu-jong Lee^{a,c,d}, Jo-Shu Chang^d

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^c Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan
^d Department of Chemical Engineering, National Cheng Kung University, Tainan 801, Taiwan

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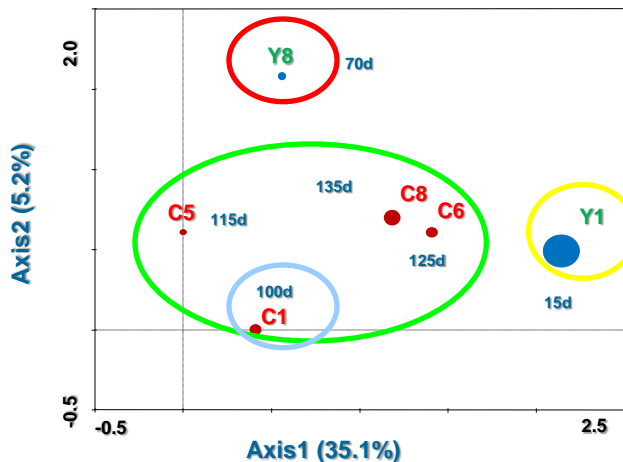
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ABSTRACT

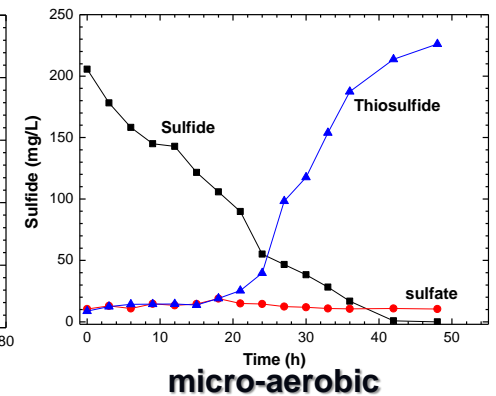
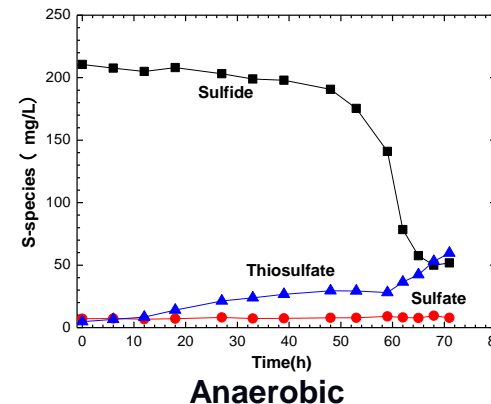
The biological denitrifying sulfide reaction (DSR) frequently proceeds in an anaerobic environment since excess oxygen inhibits the activity of the denitrifier. This study isolated a consortium (H7), comprising two strains, *Parabacterium* sp. and *Neutrophilum* anaerobitoxiens, that indicated that the DSR performance with the H7 in a nitrifying medium insignificantly enhanced at high sulfide concentrations. The H7 was inhibited by adding 200 mg l^{-1} of S^{2-} under anaerobic conditions. However, when 280 mg l^{-1} of S^{2-} was added, H7 could still degrade some of the sulfide, nitrate and acetate under micro-aerobic conditions. Micro-aerobic conditions stimulated the activity of sulfide oxidase and increased the removal rate of highly concentrated sulfide, reducing the inhibition of sulfide on denitrifier and improving DSR performance.

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Keywords:
 Denitrifying sulfide removal
 Micro-aerobic
 Sulfide oxidase
 Inhibition



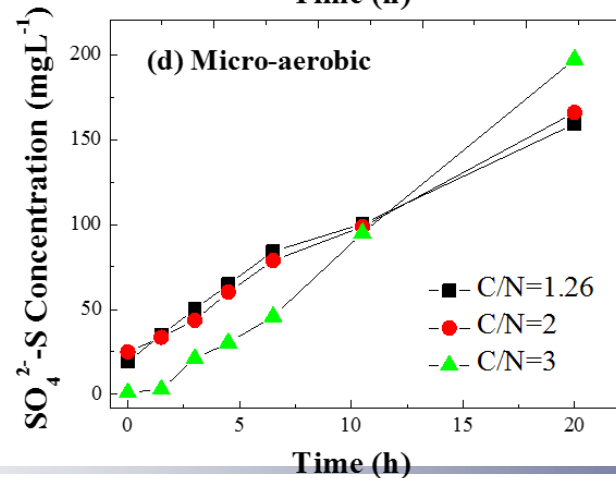
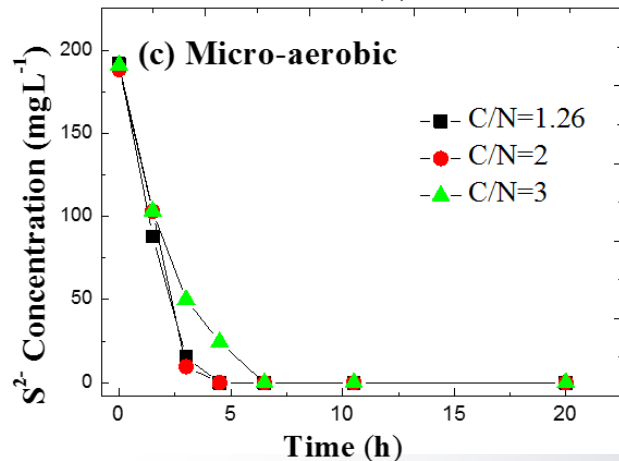
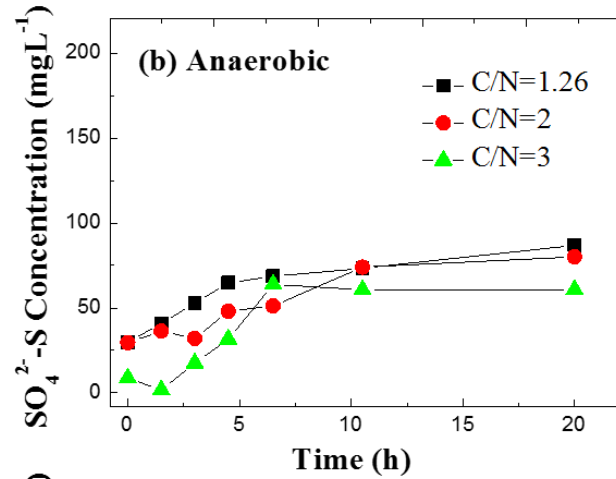
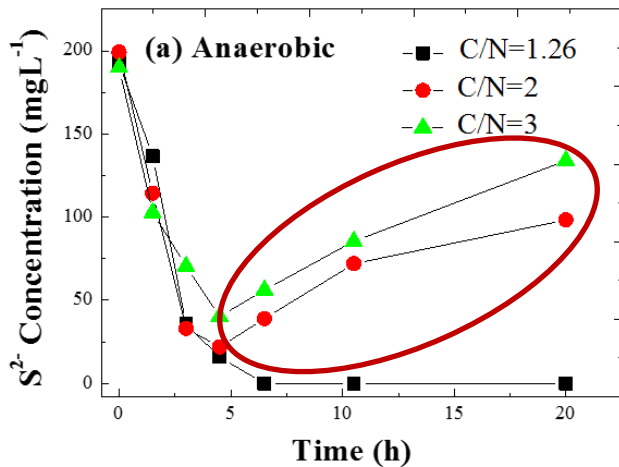
Under DO of 0.2-0.4mg/L, sulfide oxidation enhances significantly



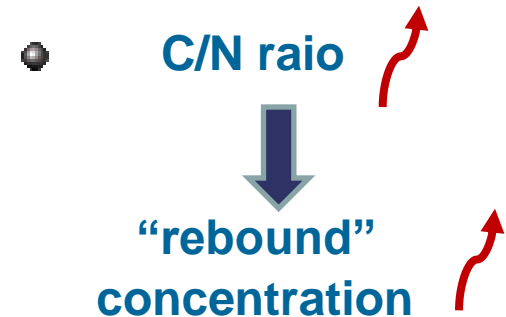
Enhanced performance of DSR in high C/N ratios

Batch tests

S/N=5/6



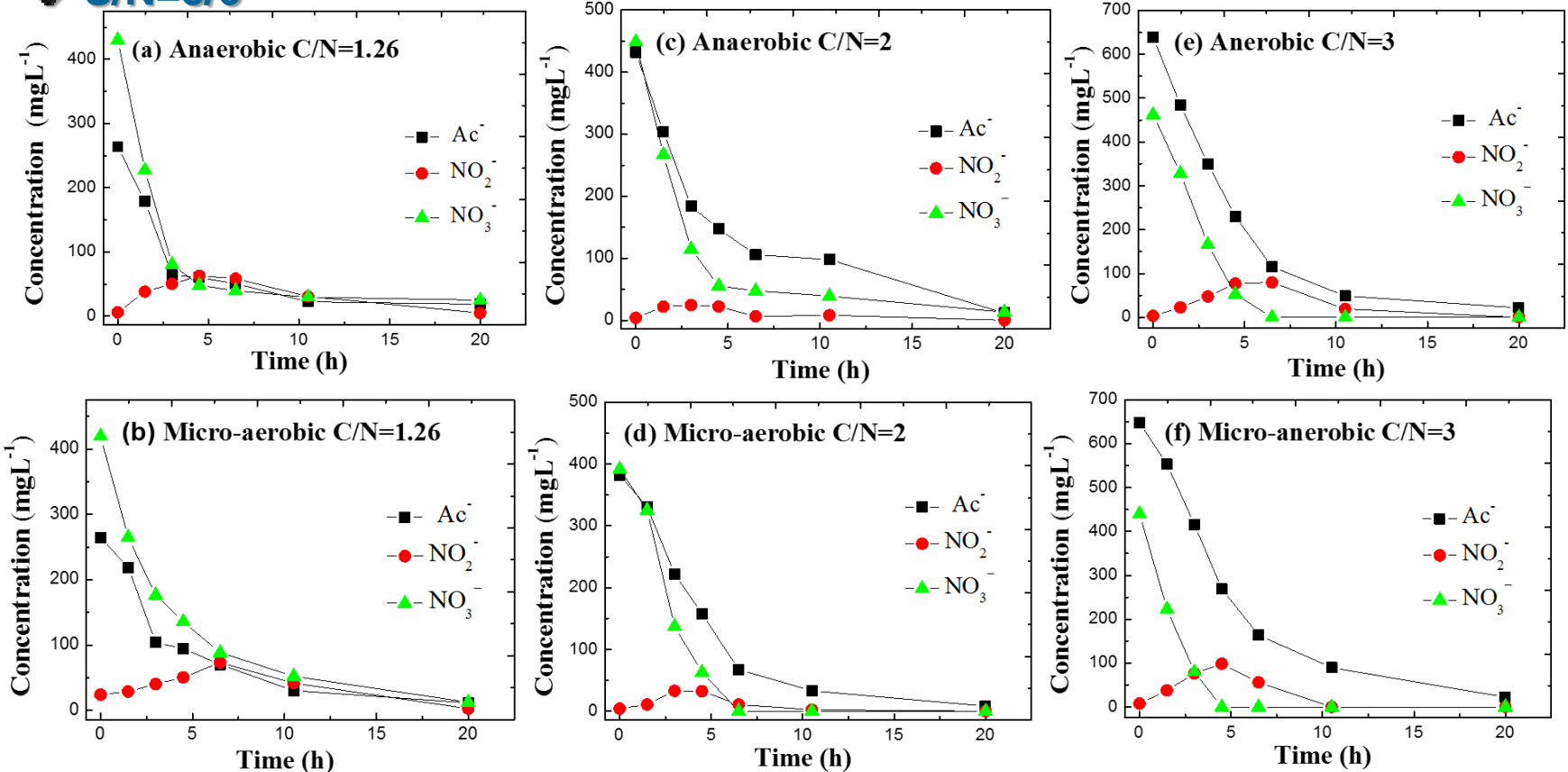
sulfide concentration “rebound” under C/N=2 and 3



Enhanced performance of DSR in high C/N ratios

Batch tests

S/N=5/6



Complete degradation of Ac⁻ and NO₃⁻

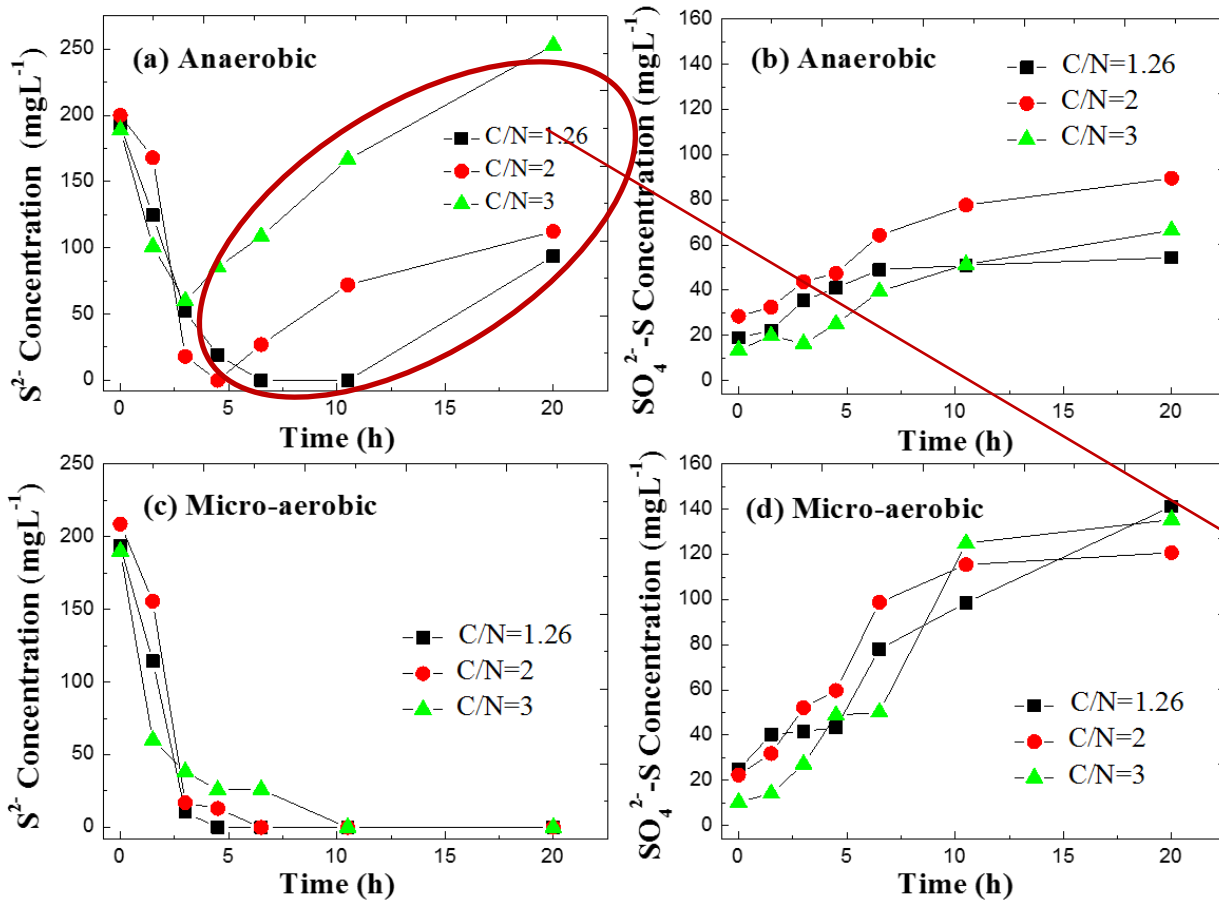
NO₂⁻ accumulation



Enhanced performance of DSR in high C/N ratios

Batch tests

S/N=5/8



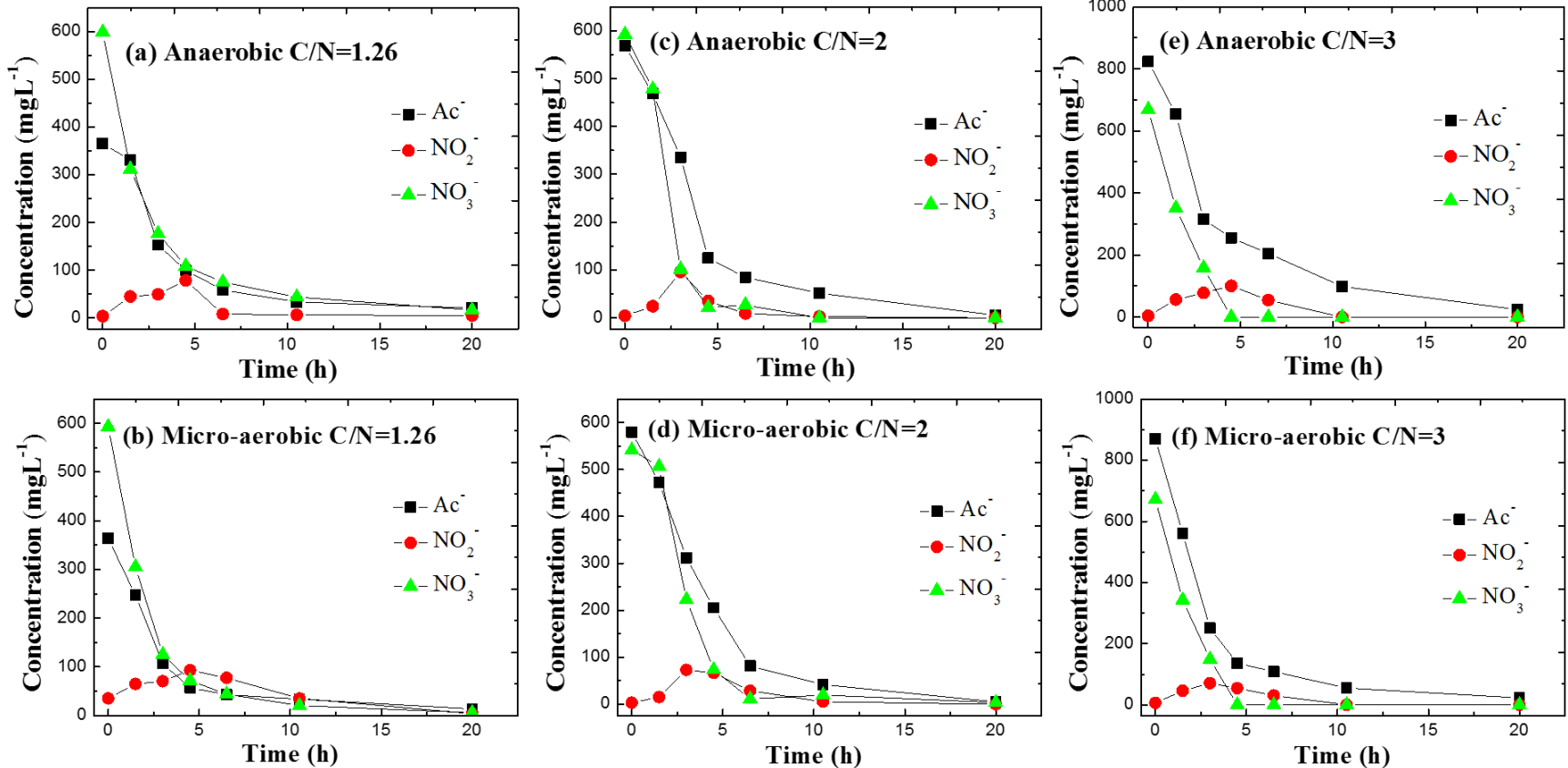
- Anaerobic: rebound
- Micro-aerobic: do not rebound
- More than initial level



Enhanced performance of DSR in high C/N ratios

Batch tests

S/N=5/8



Same degradation rule as shown in S/N=5/6

Micro-aerobic: faster Ac⁻ removal rate under C/N=3

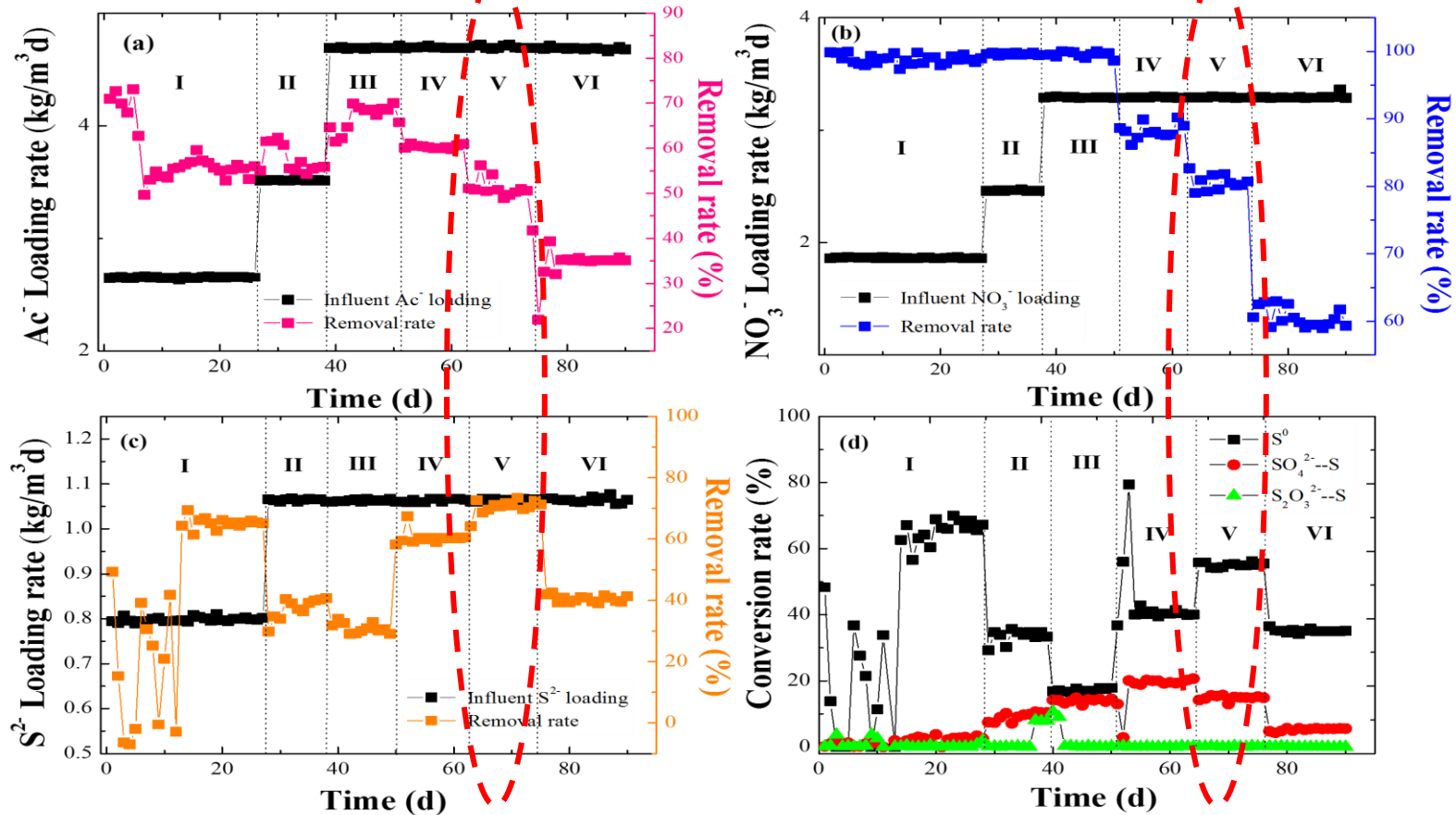


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Enhanced performance of DSR in high C/N ratios

Continuous bioreactor

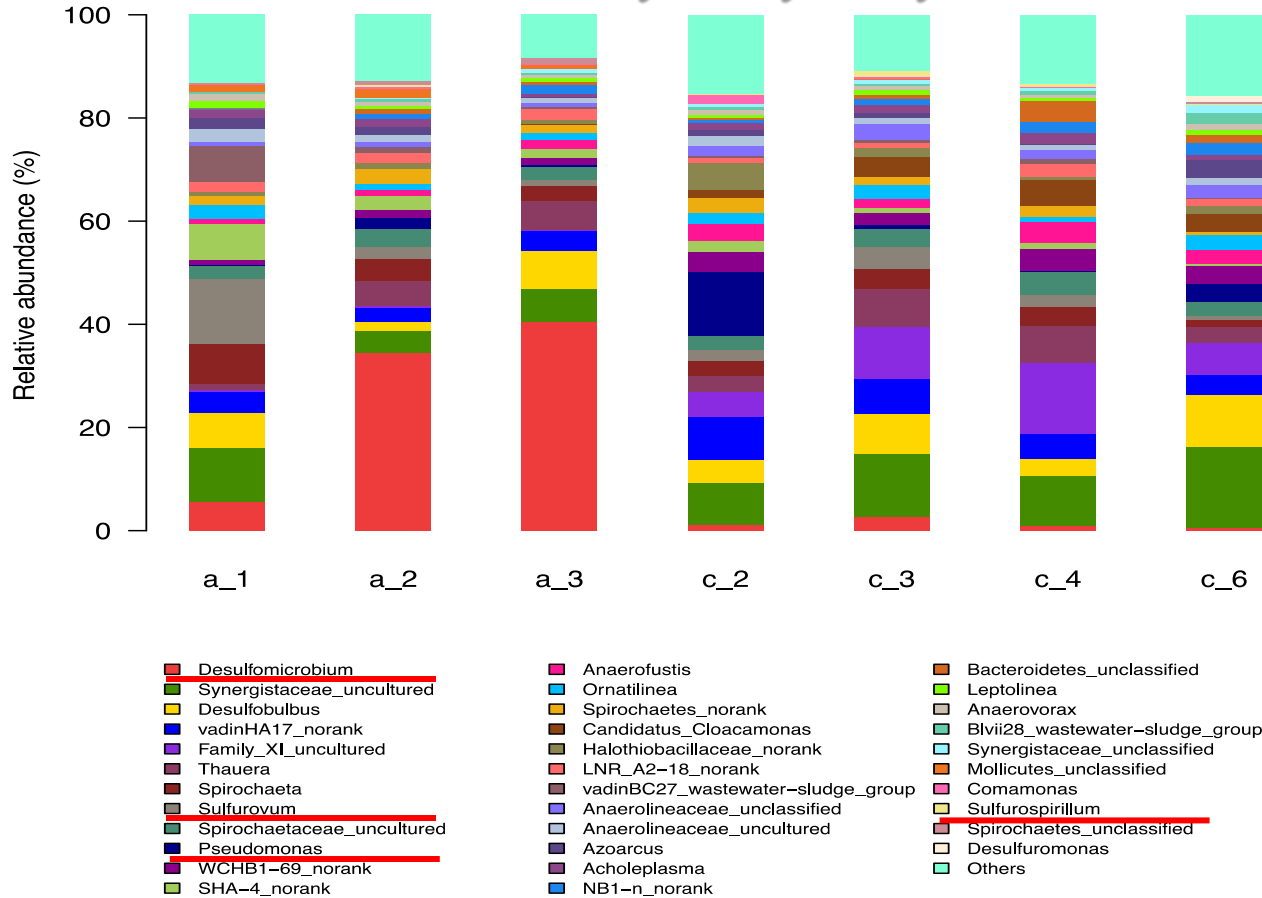


- 80% S²⁻ was removed and S⁰ recovery reached 55% at stage V (20 mL min⁻¹ L⁻¹, DO=0.2-0.3 mg/L)



Why is micro-aerobic enhanced performance obtained

Microbial community analysis by Illumina

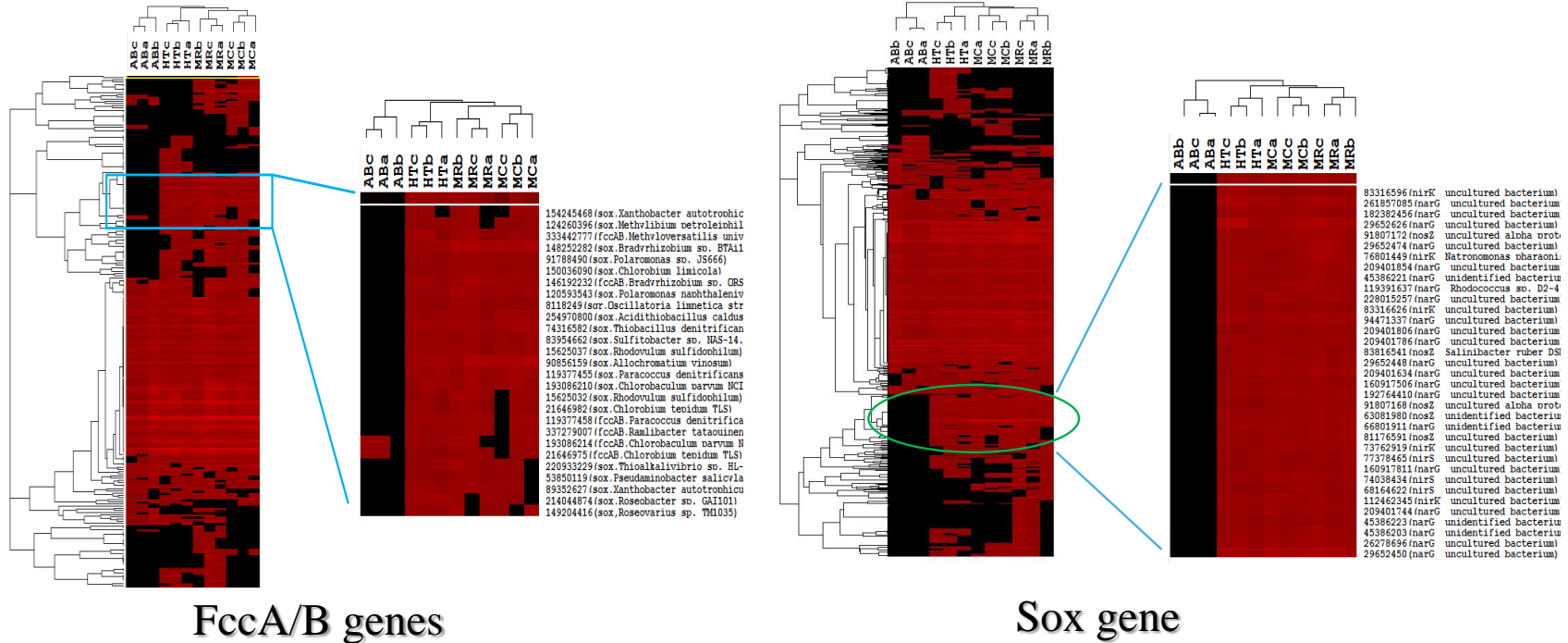


soNRB, including *Sulfurovum*, *Pseudomonas*, *Sulfurospirillum* were much higher in proportion in micro-aerobic condition than in anaerobic condition



Why is micro-aerobic enhanced performance

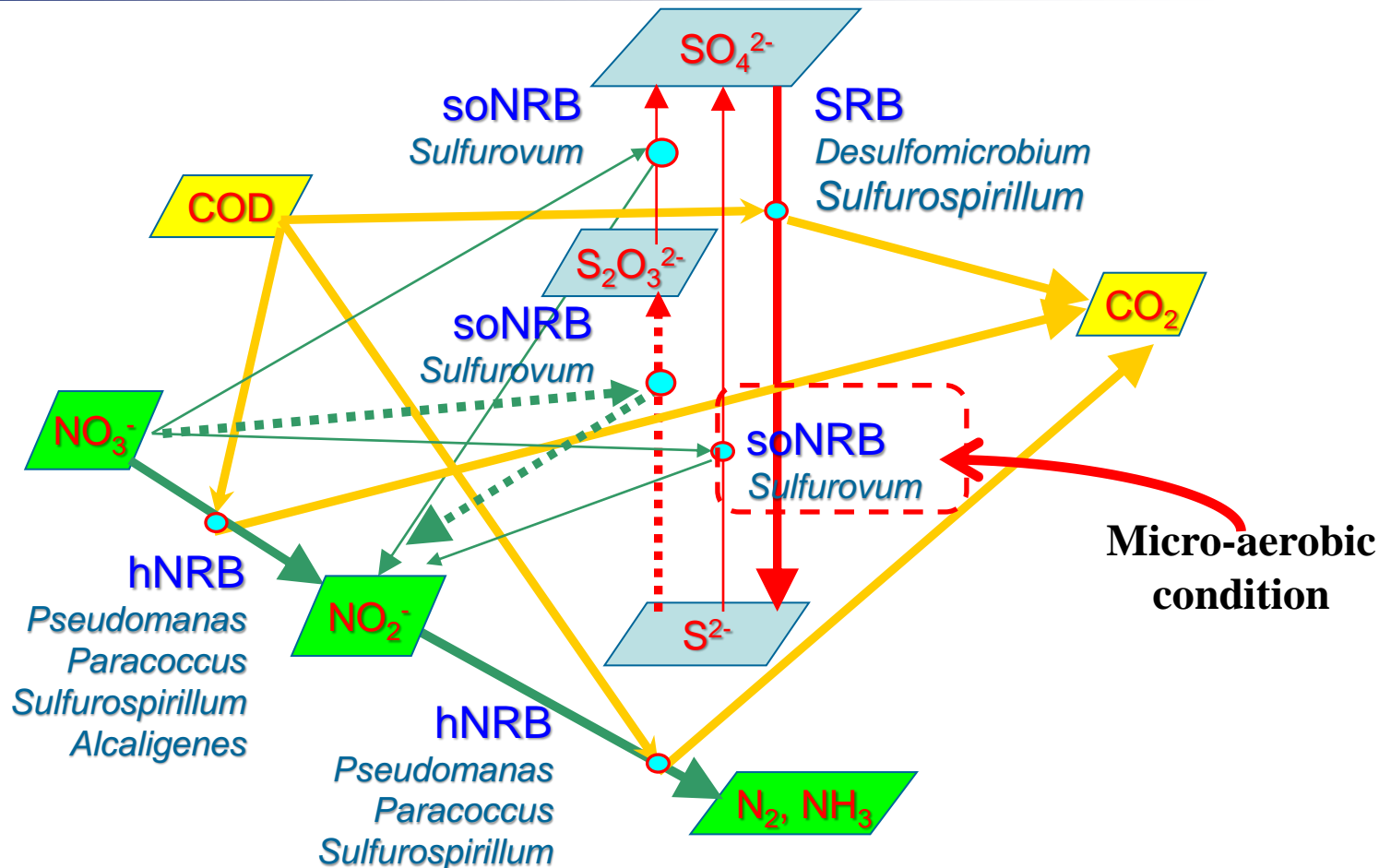
Hierarchical cluster analysis of sulfur oxidizing genes



The abundance of **SOB genes** under micro-aerobic condition was significantly higher than that under anaerobic condition. These indicated that micro-aerobic had higher effect on sulfide oxidation and sulfur recovery



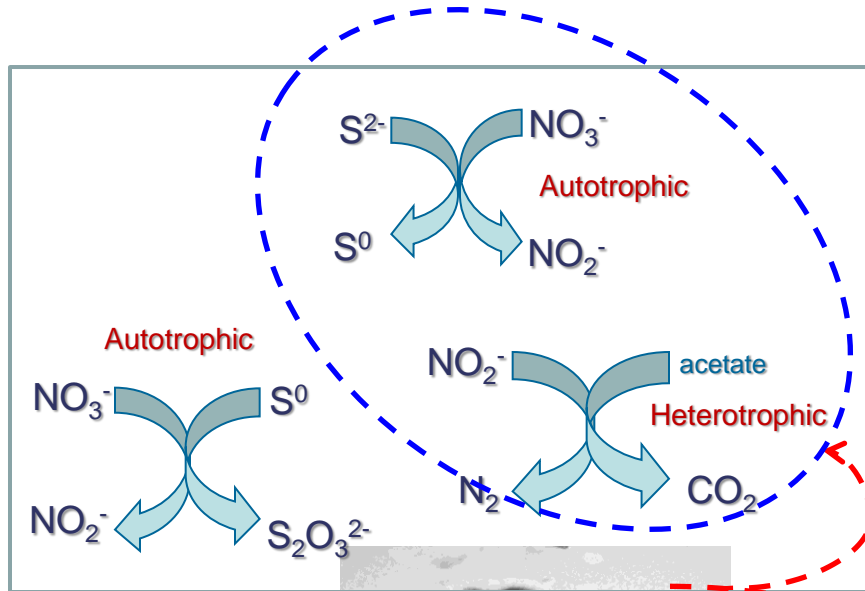
Proposed microbial relationships in micro-aerobic condition



- Micro-aerobic condition can enhance activity of **soNRB**
- Micro-aerobic condition impact little on oxygen-sensitive SRB

Micro-aerobic enhanced performance by pure culture

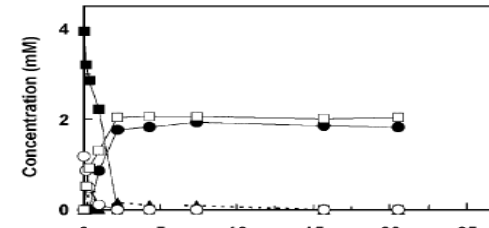
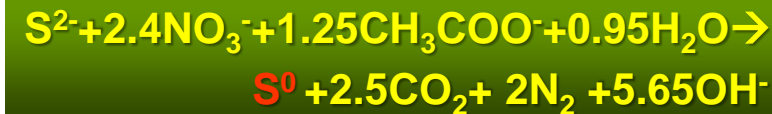
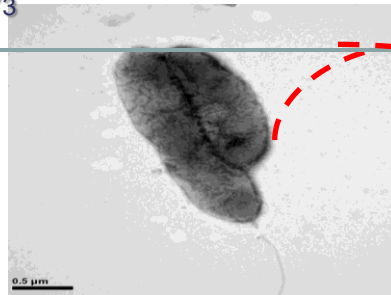
- **Breakthrough:** Heterotrophic bacteria function in sulfide oxidation $S^{2-} \rightarrow S^0$
- **Known theory:** Heterotrophic bacteria function in denitrification $NO_3^- \rightarrow N_2$



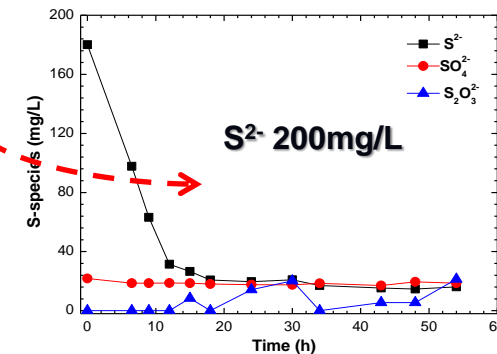
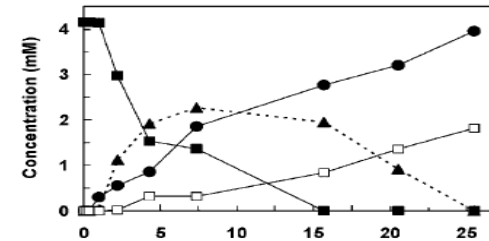
Obtained

Pseudomonas sp. C27

Gene bank registration number : GQ241351



Autotrophic bacteria (*T. denitrificans*):
72 hours



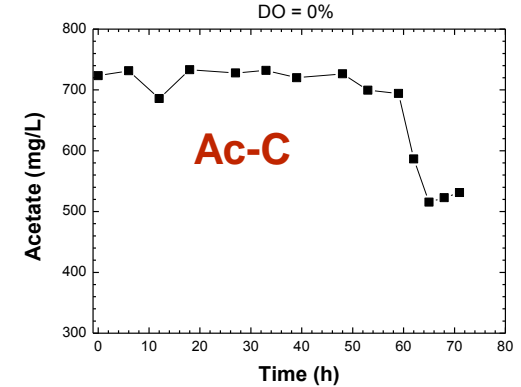
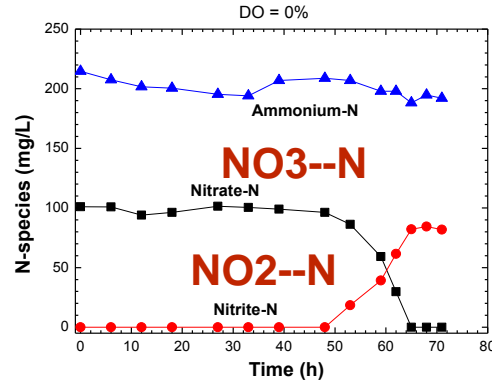
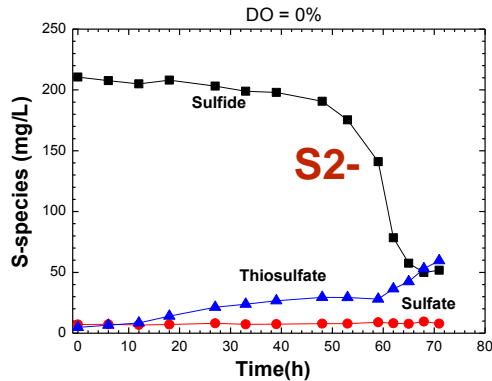
Heterotrophic bacteria (strain C27):
15 hours

- Heterotrophic bacteria increase desulfurization and denitrification, sulfur transformation rate increase more than twice times.

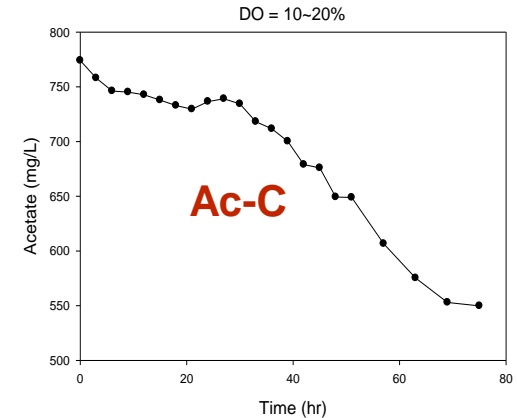
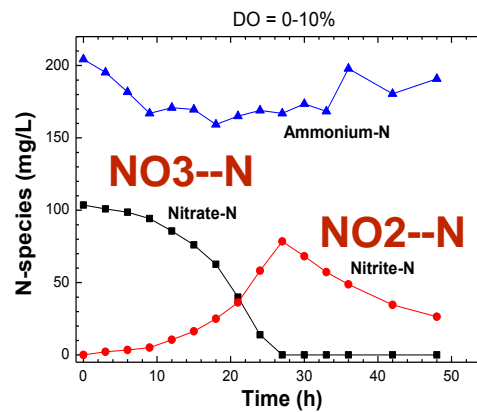
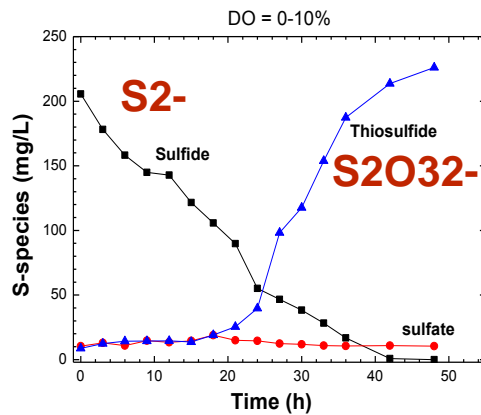
Micro-aerobic enhanced performance by pure culture

✓ DO=0%

Initial $S^{2-}=200\text{mg/L}$ S/N=5:6 C/N=3:1



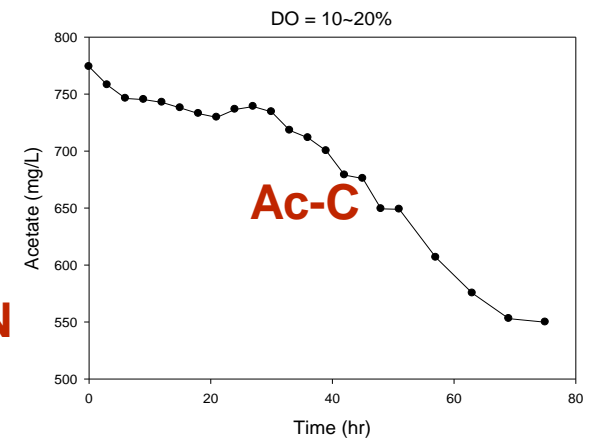
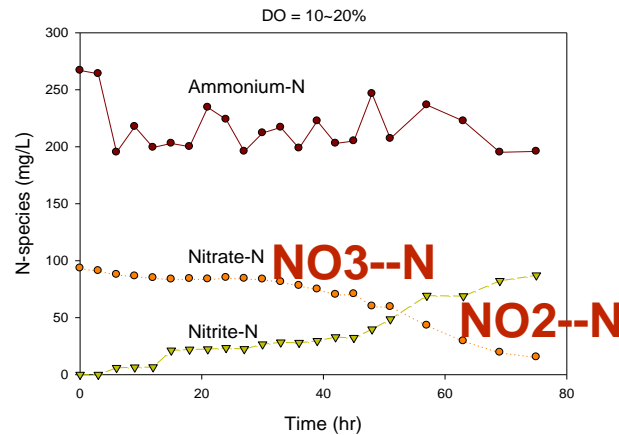
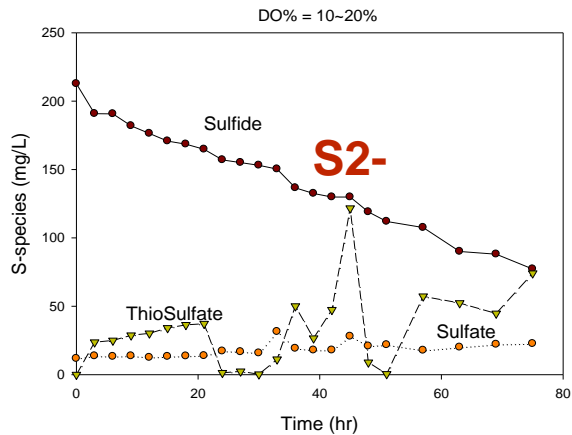
✓ DO=0-10%



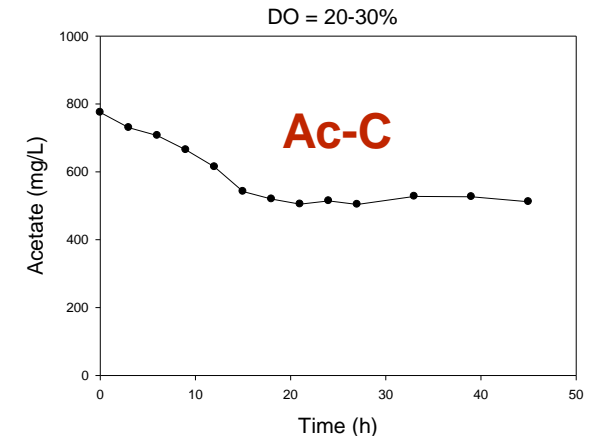
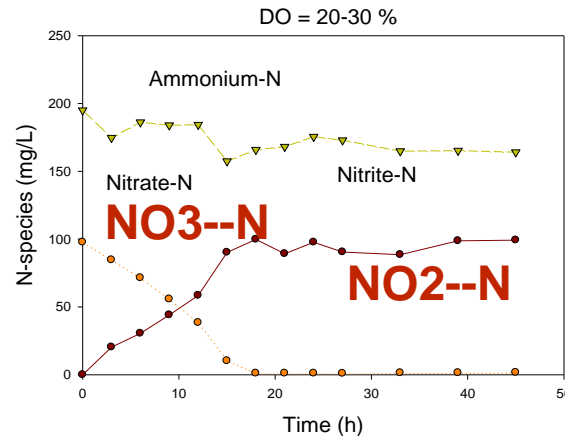
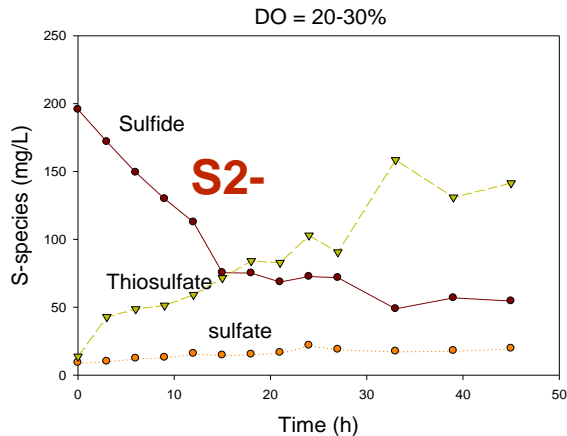
🌍 The micro-aerobic condition (DO=0-10%) can enhance the performance of sulfide and nitrite removal

Micro-aerobic enhanced performance by pure culture

✓ DO=10-20%



✓ DO=20-30%

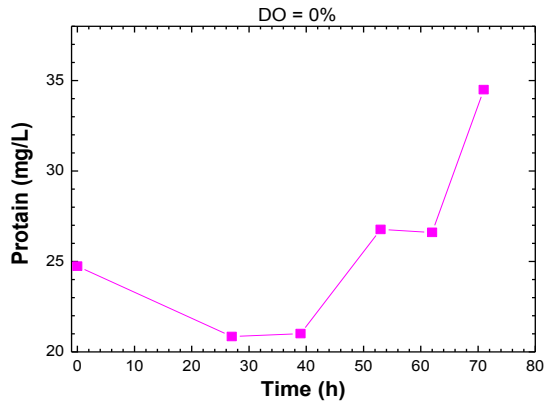


🌍 The high DO (>10%) inhibited the denitrification of NO₂- to NO_x and acetate removal.

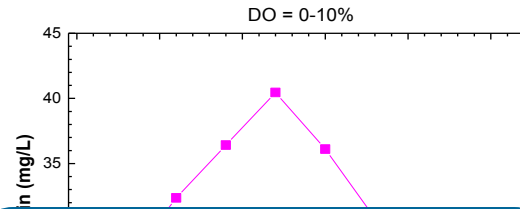
Micro-aerobic enhanced performance by pure culture

✓ Protein concentrations at different DO conditions

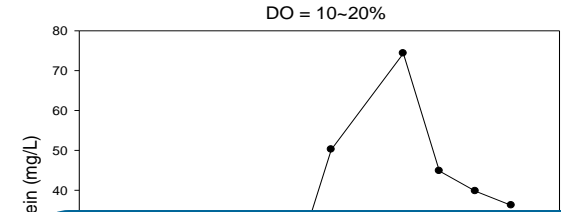
DO=0%



DO=0-10%



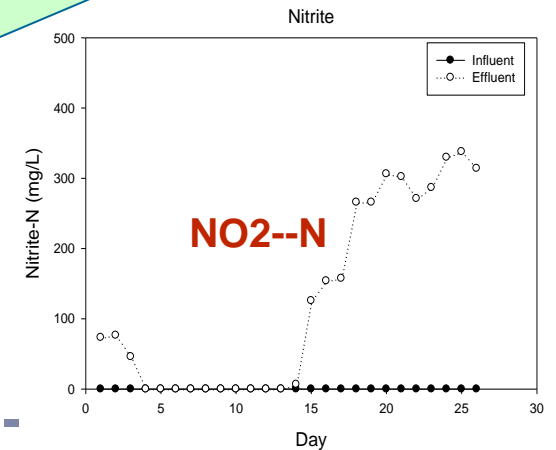
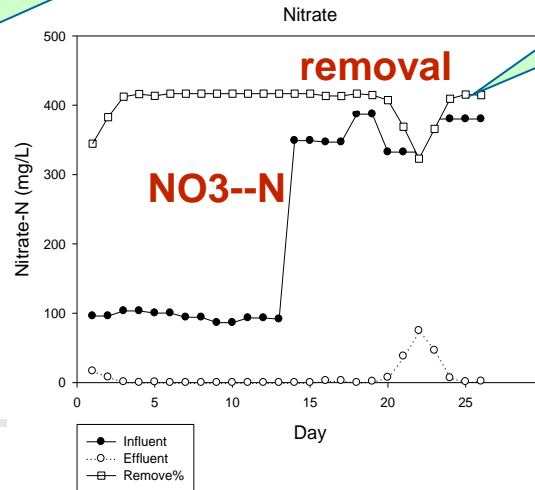
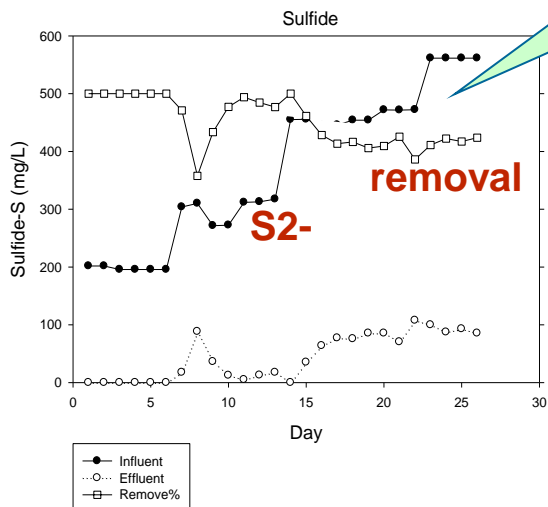
DO=10-20%



More than 500ppm sulfide, removal >80%

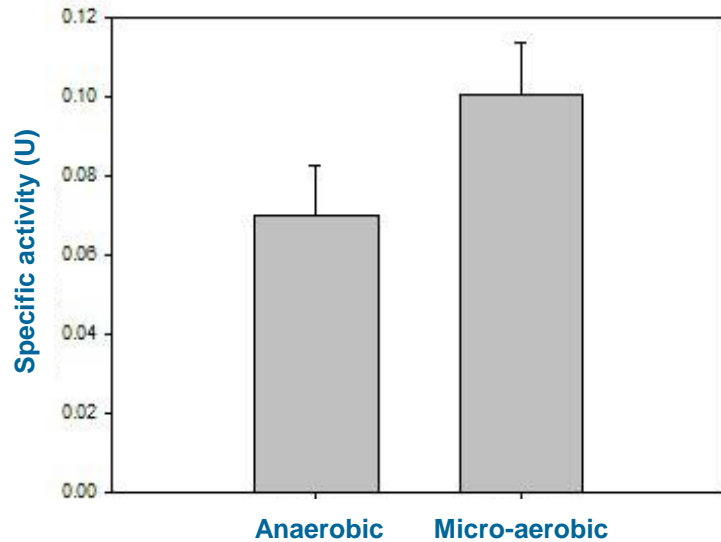
More than 500ppm sulfide, nitrate-N removal >98%

✓ Continuous flow using C27

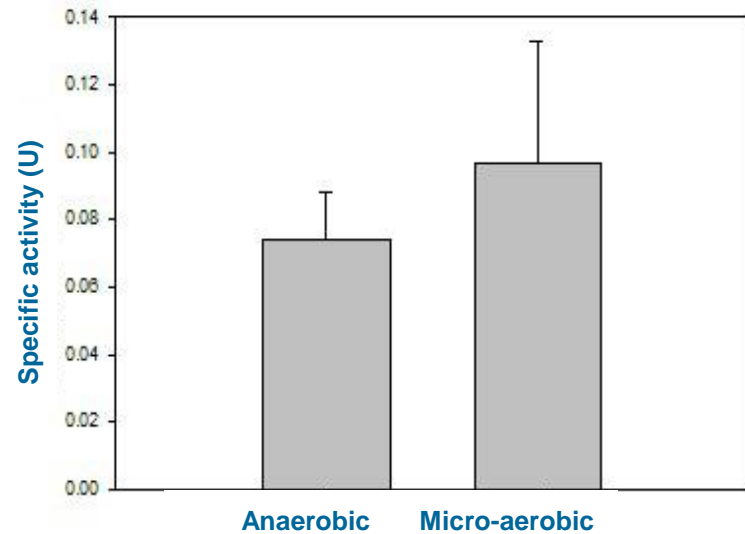


Functional enzyme activity in Micro-aerobic condition

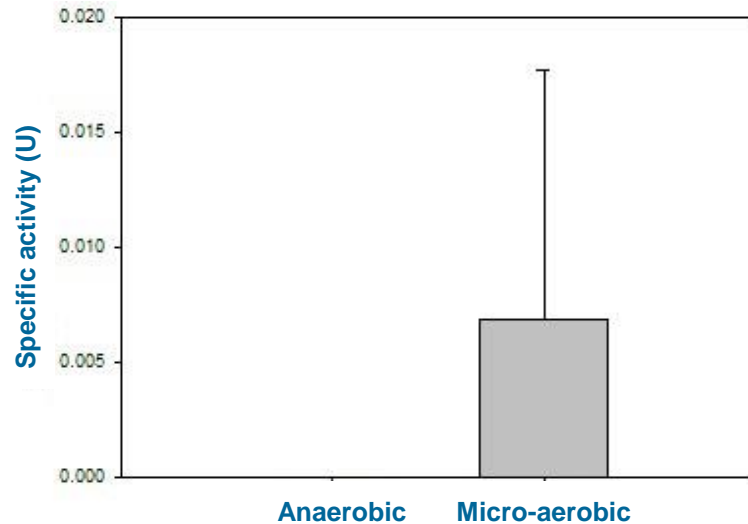
Sulfide oxidase



Nitrate reductase



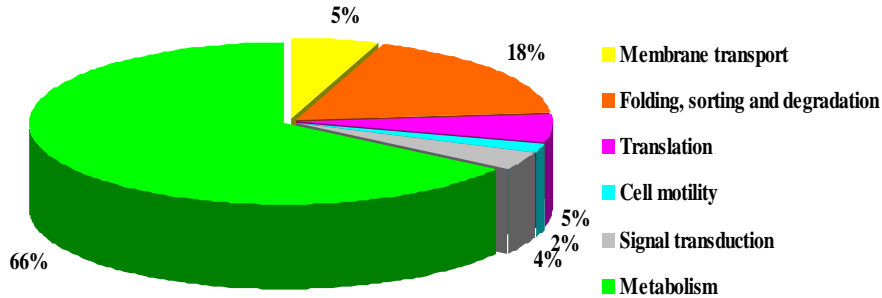
Nitrite reductase



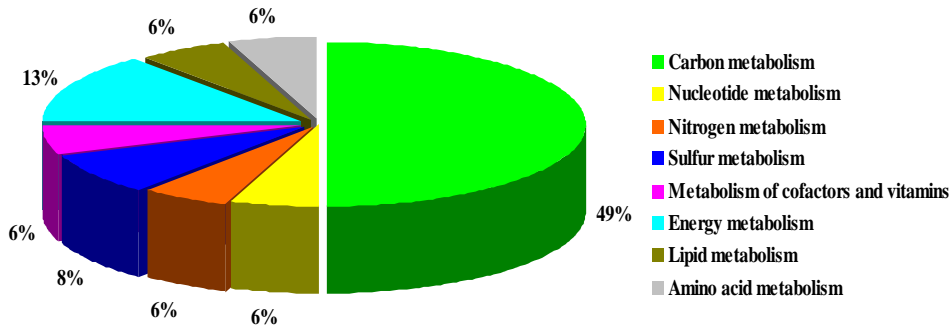
- Nitrite reductase activity inhibited by 200mg/L S^{2-}
- Enzyme activity were enhanced by micro-aerobic condition

Proteomic analysis of *Pseudomonas* sp.C27

Functional categorization of all identified proteins

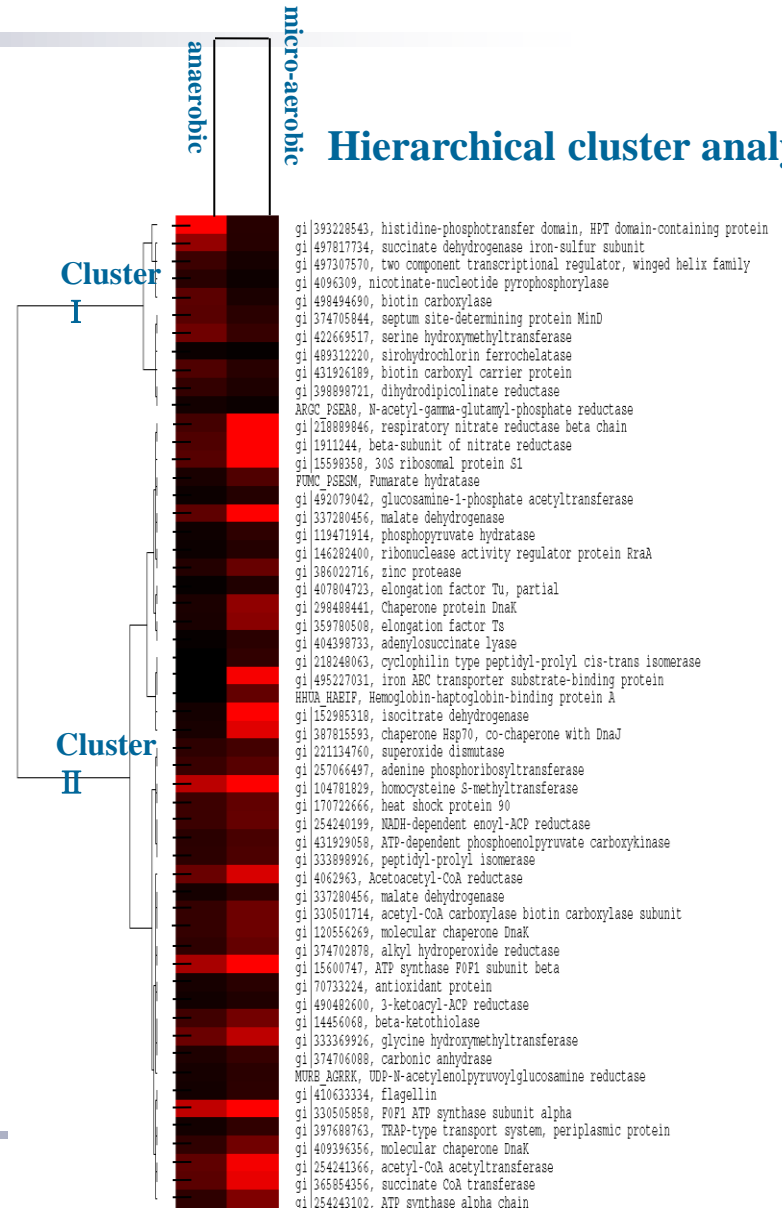


Functional categorization of the proteins involved in metabolism

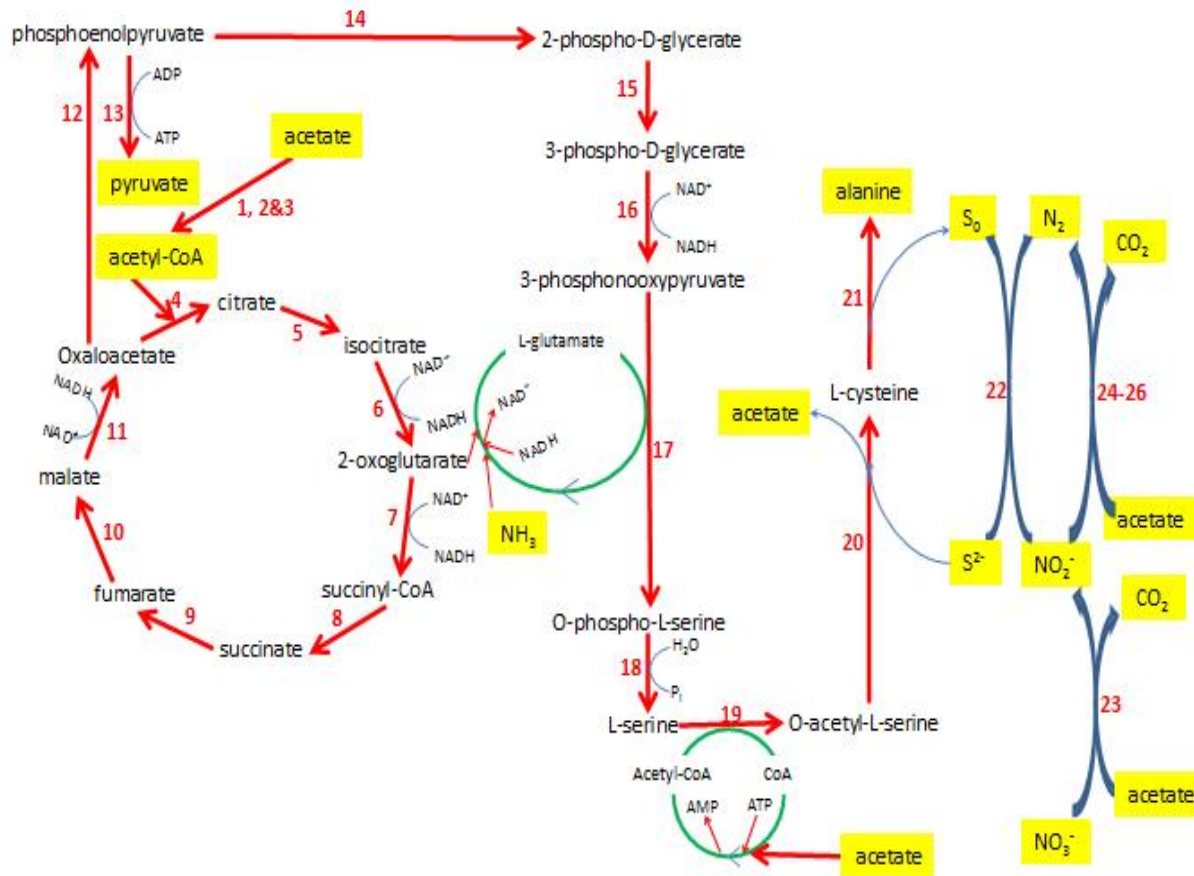


● **totally 41** related proteins went up in micro-aerobic condition, compared with anaerobic condition

Hierarchical cluster analysis



Proteomic analysis of *Pseudomonas* sp.C27



1. gi|104782577
2. gi|472327168
3. gi|333901560
4. gi|146282233
5. gi|339486839
6. gi|146282649
7. gi|104782661
8. gi|397687799
9. gi|397687793
10. gi|28871476
11. gi|70733947
12. gi|431929058
13. gi|146308281
14. gi|472324702
15. gi|512688177
16. gi|146280793
17. gi|512619571
18. gi|346642691
19. gi|28868628
20. gi|28871054
21. gi|104780229
22. gi|431926008
23. gi|152987611
24. gi|397685765
25. gi|146283850
26. gi|116051410

● **The metabolic pathways of acetate degradation, sulfide oxidation and nitrate reduction for C27 in high C/N ratios. Nos. 27 and 28 are newly identified enzymes. Bioresource Technology, 2014, 171: 120-126.**

◆ **Summary and Future challenges**

- **Feasible alternatives for DSR process under high C/N ratios in lab-scale mode.**
 - **Further parameters optimization and mechanisms exploration.**
 - **Verification with real wastewaters**
-

HIT— Where we are



**Harbin
Campu
s**

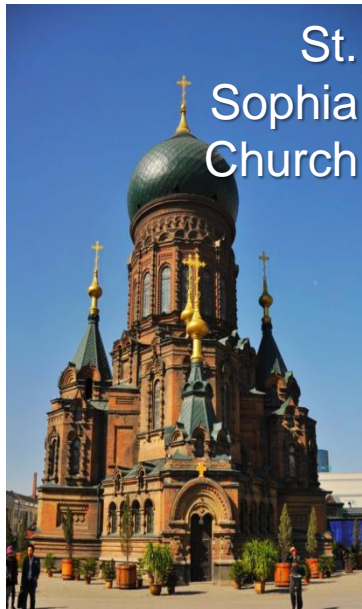
**Weihai
Campus**

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State Key Lab of Urban Water Resource & Environment

Thanks!

Any comments or questions?



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功夫到家

