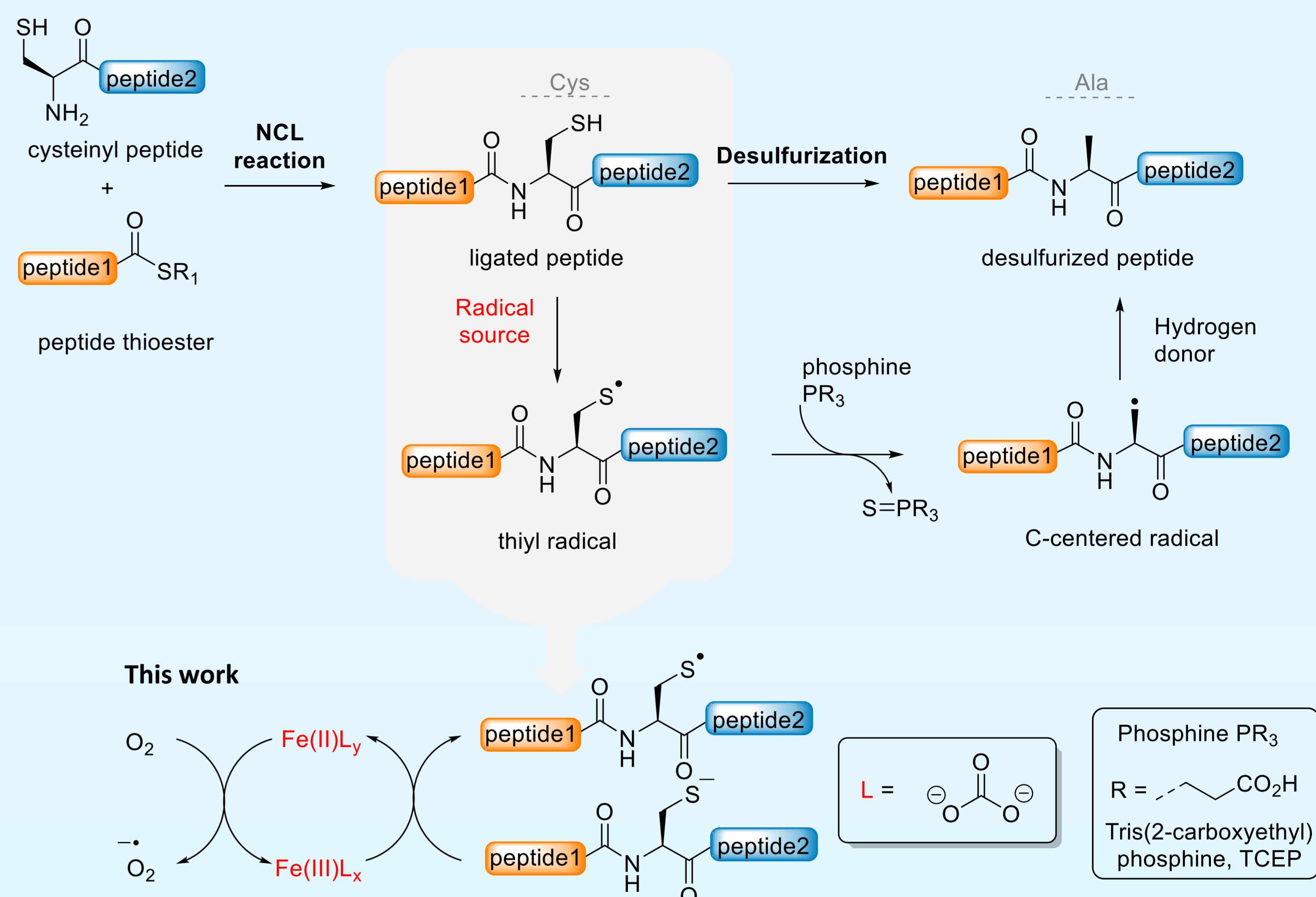


# An Iron-Catalyzed Protein Desulfurization Method Reminiscent of Aquatic Chemistry<sup>1</sup>

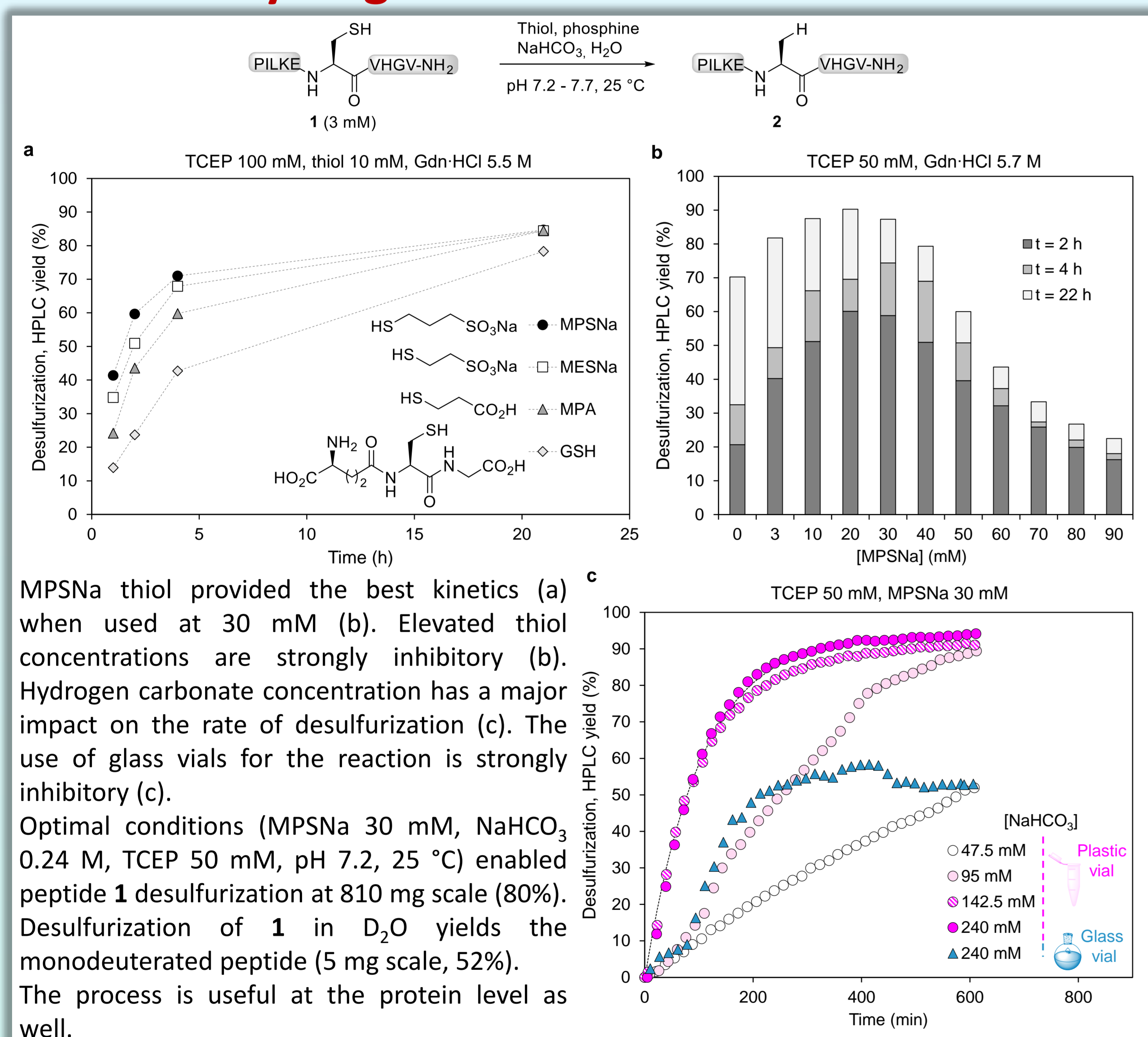
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**1. Introduction.** One pillar of protein chemical synthesis based on the application of ligation chemistries to cysteine is the group of reactions enabling the selective desulfurization of cysteine residues into alanines.<sup>2</sup> Modern desulfurization reactions use a phosphine as a sink for sulfur under activation conditions involving the generation of sulfur-centered radicals. Here we show that cysteine desulfurization by a phosphine such as *tris*(2-carboxyethyl)phosphine TCEP can be effected efficiently by micromolar concentrations of iron under aerobic conditions in hydrogen carbonate buffer, that is using conditions that are reminiscent of iron-catalyzed oxidation phenomena occurring in natural waters.



## 2. Influence of thiol type, thiol concentration and hydrogen carbonate concentration



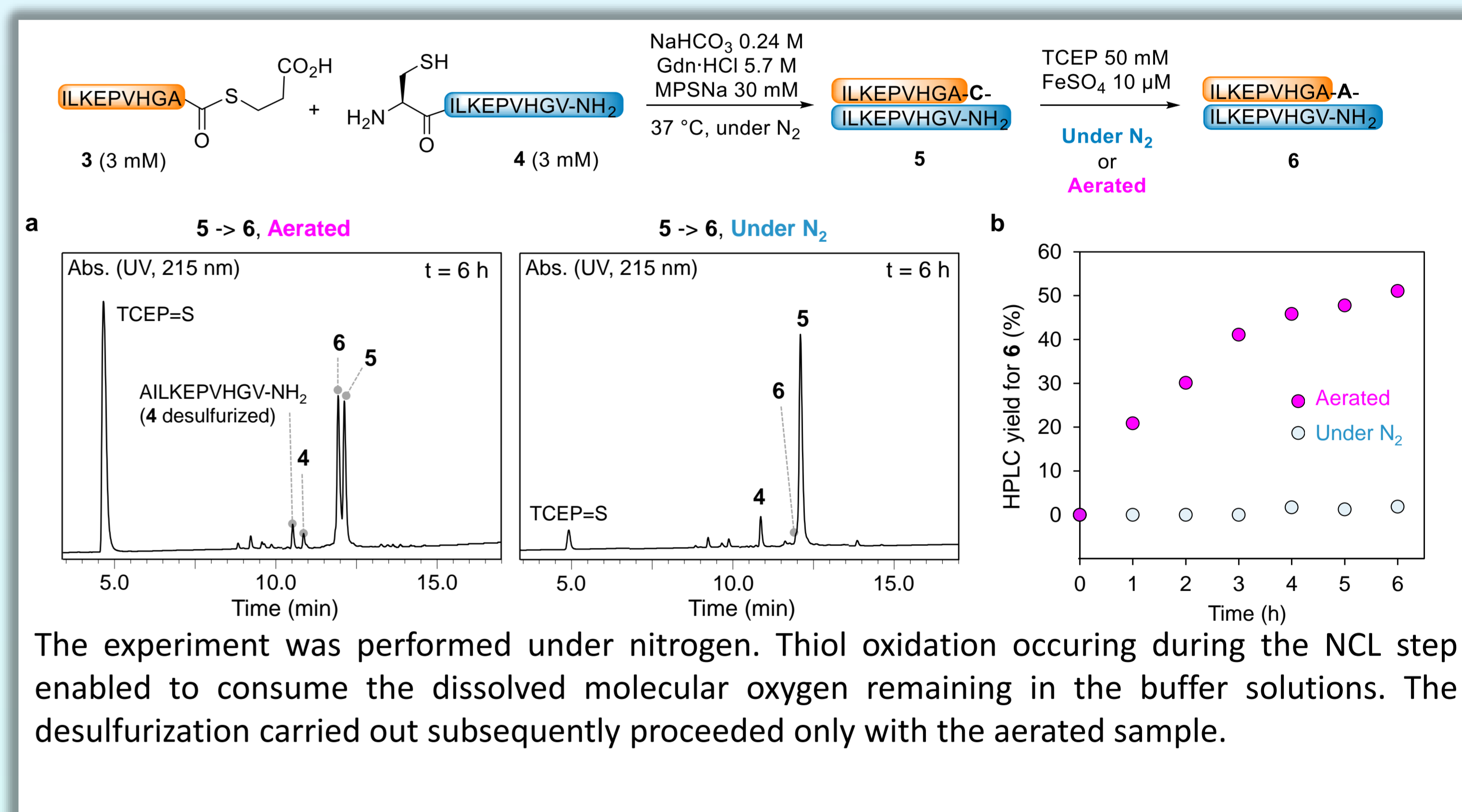
## 5. Discussion & conclusion

In our experiments, the concentration of iron species is three orders of magnitude less ( $\mu\text{M}$ ) than the peptide concentration (mM). In such conditions, Fe(II) species must be recycled into Fe(III) to achieve useful desulfurization yields.

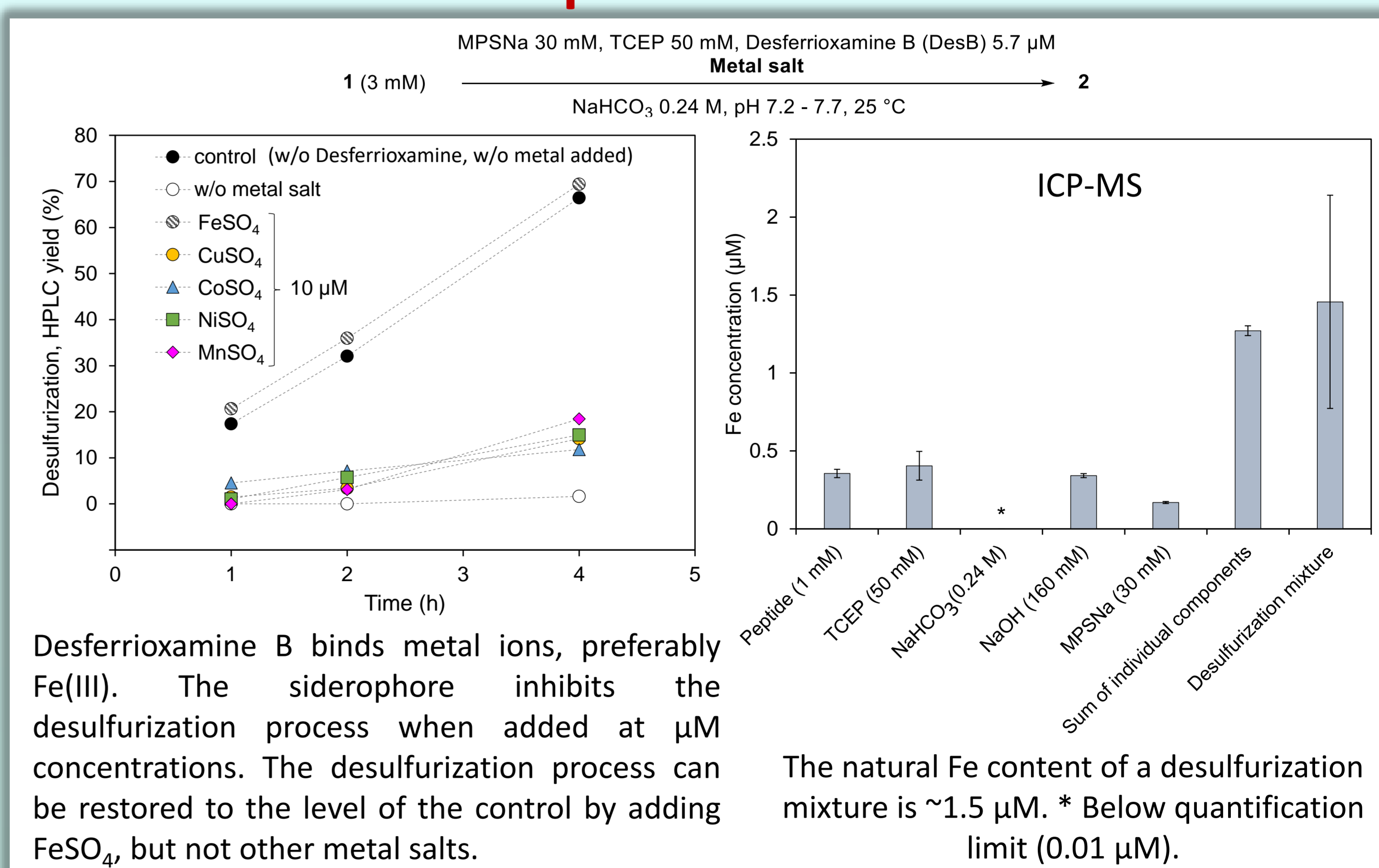
Several studies established that the rate of oxidation of Fe(II) into Fe(III) by molecular oxygen is critically dependent on iron speciation. In particular, King and coworkers showed that in natural waters containing more than 1 mM of carbonate ions, the ferrous carbonate complex  $\text{Fe}(\text{CO}_3)_2^{2-}$  is the most kinetically active species at neutral pH for reacting with molecular oxygen, and hence plays a major role in the degradation of organic matter in many aquatic systems.<sup>3</sup>

Our calculations of iron speciation support the presence of  $\text{Fe}(\text{CO}_3)_2^{2-}$  in the desulfurization mixture.<sup>1</sup> Therefore, we think that part of the iron(II)-catalyzed peptide desulfurization process reported here is likely reminiscent of the iron-catalyzed oxidation phenomena occurring naturally in aquatic systems.

## 3. Implication of molecular oxygen



## 4. Implication of iron



## References

- Desmet, R.; Boidin-Wichlacz, C.; Mhida, R.; Tasiemski, A.; Agouridas, V.; Melnyk, O. An iron-catalyzed protein desulfurization method reminiscent of aquatic chemistry. *Angew. Chem. Int. Ed.* 2023, 62 (18), e202302648, DOI.
- a) Diemer, V.; Melnyk, O. Protein desulfurization: Sodium tetraethylborate makes it fast. *Chem* 2022, 8, 2571-2573, DOI. b) Diemer, V.; Roy, E.; Agouridas, V.; Melnyk, O. *Chem. Soc. Rev.*, DOI.
- King, D. W. Role of carbonate speciation on the oxidation rate of Fe(II) in aquatic systems. *Environmental Science & Technology* 1998, 32, 2997-3003.