



# Kinetics and microbial ecology of batch sulfidogenic bioreactors for co-treatment of municipal wastewater and acid mine drainage



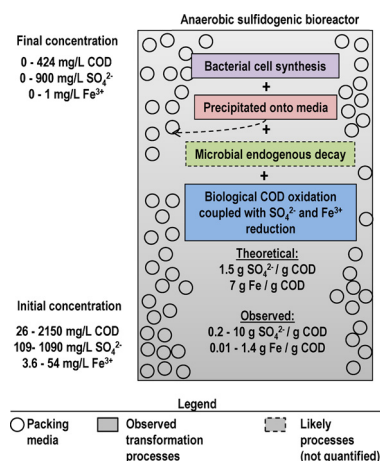
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## HIGHLIGHTS

- Innovative technology for co-treatment of municipal wastewater and acid mine drainage.
- Maximum COD oxidation rate can be reasonably achieved with the MWW/AMD mixtures.
- Ferric iron at 60 mg/L completely inhibit the sulfidogenic bioreactors.
- COD oxidation was mostly facilitated by SRB with a small contribution from IRB.
- COD oxidation had a strong positive correlation with SRB *dsrA* gene copies.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The kinetics and microbial ecology in sulfidogenic bioreactors used in a novel two-stage process for co-treatment of acid mine drainage (AMD) and municipal wastewater (MWW) were investigated. Michaelis–Menten modeling of COD oxidation by sulfate reducing bacteria (SRB) ( $V_{\max} = 0.33 \text{ mg L}^{-1} \text{ min}^{-1}$ ,  $K_m = 4.3 \text{ mg L}^{-1}$ ) suggested that the  $V_{\max}$  can be reasonably achieved given the typical COD values in MWW and anticipated mixing with AMD. Non-competitive inhibition modeling ( $K_i = 6.55 \text{ mg L}^{-1}$ ) indicated that excessive iron level should be avoided to limit its effects on SRB. The COD oxidation rate was positively correlated to COD/sulfate ratio and SRB population, as evidenced by *dsrA* gene copies. Phylogenetic analysis revealed diverse microbial communities dominated by sulfate reducing delta-proteobacteria. Microbial community and relative quantities of SRB showed significant differences under different COD/sulfate ratios (0.2, 1 and 2), and the highest *dsrA* gene concentration and most complex microbial diversity were observed under COD/sulfate ratio 2. Major species were associated with *Desulfovira*, *Desulfobulbus*, *Desulfovibrio*, and *Syntrophus* sp. The reported COD kinetics, SRB abundances and the phylogenetic profile provide insights into the co-treatment process and help identify the parameters of concerns for such technology development.

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## 1. Introduction

Co-treatment study of municipal wastewater (MWW) and acid mine drainage (AMD) can be traced back to 1900s when Roetman

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**Table 1**  
Clone library results of bioreactors B1, B2 and B3.

Clone	Sequence length	Closet species in GenBank [Accession no.]	Putative function	Identity (%)	Clone no.	Abundance (%)	Phyla
B1-1	781	<i>Desulfovibrio idahonensis</i> [NR114908.1]	Sulfate reduction	97%	4	33.3	Deltaproteobacteria
B1-3	812	<i>Desulfovira adipica</i> [NR36764.1]	Sulfate reduction	94%			Deltaproteobacteria
B1-5	826	<i>Desulfovulbus elongatus</i> [NR29305.1]	Sulfate reduction	93%			Deltaproteobacteria
B1-11	881	<i>Desulfatibacillum alkenivorans</i> [NR25795.1]	Sulfate reduction	92%			Deltaproteobacteria
B1-6	842	<i>Desulfomonile limimaris</i> [NR25079.1]	Dehalogenation	94%	1	8.3	Deltaproteobacteria
B1-4	792	<i>Mucilaginibacter polysacchareus</i> [KM19772.1]	Hydrolysis	86%	3	25.0	Bacteroidetes
B1-7	820	<i>Actinomyces bacterium</i> [DQ994722.1]	Hydrolysis	87%			Acidobacteria
B1-8	817	<i>Mucilaginibacter polysacchareus</i> [KM19772.1]	Hydrolysis	86%			Bacteroidetes
B1-2	795	<i>Clostridium</i> sp. CYP5 [DQ479415.1]	Fermentation	99%	4	33.3	Firmicutes
B1-9	786	<i>Acidaminobacter hydrogenoformans</i> [NR28683.1]	Fermentation	98%			Firmicutes
B1-10	821	<i>Prolixibacter bellariivorans</i> [NR43273.1]	Fermentation	86%			Bacteroidetes
B1-12	831	<i>Marinilabilia salmonicolor</i> [NR104682.1]	Fermentation	86%			Bacteroidetes
B2-1	824	<i>Desulfomicrobium escambiense</i> [42018.1]	Sulfate reduction	99%	2	22.2	Deltaproteobacteria
B2-9	1387	<i>Desulfocaldus</i> sp. <i>Hobo</i> [EF442977.1]	Sulfate reduction	85%			Deltaproteobacteria
B2-7	831	<i>Clostridium</i> sp. [AB596885.1]	Dehalogenation	96%	1	11.1	Bacteroidetes
B2-2	611	<i>Cloacibacillus porcorum</i> [NR109636.1]	Fermentation	90%	6	66.7	Synergistetes
B2-3	776	<i>Leptolinea tardivitalis</i> [NR40971.1]	Fermentation	89%			Chloroflexi
B2-4	782	<i>Gracilibacter thermotolerans</i> [NR115693.1]	Fermentation	85%			Firmicutes
B2-5	776	<i>Gracilibacter thermotolerans</i> [NR115693.1]	Fermentation	86%			Firmicutes
B2-6	824	<i>Gracilibacter thermotolerans</i> [NR115693.1]	Fermentation	86%			Firmicutes
B2-8	1388	<i>Ruminococcaceae bacterium</i> [LK391549.1]	Fermentation	91%			Firmicutes
B3-2	807	<i>Desulfovulbus elongatus</i> [NR29305.1]	Sulfate reduction	96%	2	16.7	Deltaproteobacteria
B3-7	1397	<i>Desulfovulbus elongatus</i> [NR29305.1]	Sulfate reduction	97%			Deltaproteobacteria
B3-3	771	<i>Bellilinea caldifistulae</i> [NR41354.1]	Methanogenesis	90%	4	33.3	Chloroflexi
B3-5	1414	<i>Syntrophus</i> sp. [AJ133796.1]	Methanogenesis	95%			Deltaproteobacteria
B3-6	1390	<i>Cloacimonetes bacterium</i> [KJ535434.1]	Methanogenesis	93%			Cloacimonetes
B3-11	840	<i>Longilinea arvoryzae</i> [NR41355.1]	Methanogenesis	90%			Chloroflexi
B3-8	790	<i>Thermophilic bacterium</i> [AJ242834.1]	Fermentation	84%	2	16.7	Firmicutes
B3-9	543	<i>Sedimentibacter</i> sp. [AY766466.1]	Fermentation, dehalogenation	96%			Firmicutes
B3-1	731	<i>Clostridium</i> sp. 6–44 [AB596885.1]	dehalogenation	94%	1	8.3	Bacteroidetes
B3-10	1023	<i>Prolixibacter bellariivorans</i> [LC015091.1]	Nitrate-reducing	87%	1	8.3	Bacteroidetes
B3-4	1394	<i>Smithella propionica</i> [NR24989.1]	Acetogenesis	96%	1	8.3	Deltaproteobacteria
B3-12	719	<i>Mycobacterium llatzerense</i> [AJ746071.2]	Hydrogen-oxidizing	99%	1	8.3	Actinobacteria

[1] first proposed mixing the two to reduce pathogens in sewage. In more recent years, technical feasibility of the co-treatment was investigated by several research groups [2–11]. Overall, these studies showed significant water quality improvements through removal of metals, nutrients, and organics along with increases in pH and alkalinity.

From a wastewater treatment perspective, incorporation of AMD in MWW treatment can provide significant environmental benefits over the widely adopted activated sludge processes, which were made possible by the complementary water chemistry of the two wastes. For examples, metals in AMD (e.g., iron and aluminum) can form chemical precipitation of low-solubility salts (i.e., iron phosphate) and help remove both dissolved and particulate materials. High levels of sulfate can be used as an electron acceptor by sulfate reducing bacteria (SRB) for oxidation of organic compounds under anaerobic conditions. This eliminates the need for aeration, which is the most energy-intensive operation in wastewater treatment facilities [12,13]. The SRB-facilitated sulfate reduction to (bi) sulfide produces alkalinity and promotes metal sulfide precipitation. An additional benefit with the anaerobic treatment is the significant reduction in biological sludge production [14].

A range of factors are critical for the co-treatment system, including COD/sulfate ratios, mixed water chemistry, microbiological diversity, and reactor configuration [15]. Although deemed to play an important role in the treatment efficacy, there is scarce information about microbial ecology and its relationships with the co-treatment kinetics. Schmidtova and Baldwin [16] studied a bioreactor used to treat a landfill leachate and found a positive correlation between sulfate reduction rate and SRB abundance. Dann et al. [17] investigated microbial profiles in a passive compost-based system used for remediating acidic, high iron and sulfate industrial wastewater, and concluded that compost/straw decom-

position and associated sulfate and iron reductions were facilitated by a complex mix of aerobic and anaerobic bacteria. Sánchez-Andrea et al. [18] recently reviewed and discussed important factors for utilizing SRB in sulfidogenic reactors used to treat AMD, as well as microbial communities in the bioreactors.

Metal toxicity needs to be taken into consideration in order to maintain active and diverse sulfate reducing microbial communities. Iron (Fe), one of the most prevalent metals in AMD, was reported to inhibit SRB and lower sulfate reduction by 39–100% in two ways: deposit of FeS causing the inhibition of the cells activity [19–21], and the competition of Fe<sup>3+</sup>-reducing bacteria for electron donors [22–24]. Metals such as Zn, Cd, Cu, Ni, Pb and Mn often remain at significant concentrations in acid mine drainage even after the pretreatment process such as alkaline chemical additions [25]. Sulfate reduction by SRB was found to be completely inhibited at 2–50 mg Cu/L, 13–40 mg Zn/L, 75–125 mg Pb/L, 4–54 mg Cd/L, and 10–20 mg Ni/L [21]. However, Castillo et al. [26] evaluated the tolerance of SRB to Zn up to 260 mg/L and found SRB activities reduced Zn concentration almost completely by forming ZnS precipitation. These metal inhibitive effects are expected to vary depending on the reactor configuration, SRB species, metals concentration, pH, and Eh conditions [27–29].

A previously reported two-stage process for co-treatment of field-collected AMD and MWW (i.e., mixing of the two wastes followed by sulfidogenic treatment of the mixture) has demonstrated effective removal of metals, COD, sulfate and acidity [30]. This study focuses on the kinetics, iron inhibitive effects, and microbial ecology in the sulfidogenic bioreactors. Specifically, COD degradation kinetics and inhibition by Fe were modeled to characterize the biological treatment. Bacterial 16S rRNA gene clone libraries were analyzed to describe microbial ecology and its relationship with the treatment kinetics. In addition, quantitative polymerase chain reac-

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