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Effect of Ethylenediamine-N,N′-disuccinic acid (EDDS) on the speciation and bioavailability of Fe^{2+} in the presence of sulfide in anaerobic digestion

Pham Minh Thanh [a,b], Balachandran Ketheesan [b], Zhou Yan [b,c], David Stuckey [b,*]

[a] Interdisciplinary Graduate School, Nanyang Technological University, Singapore 639798, Singapore
[b] Nanyang Environment & Water Research Institute, Advanced Environmental Biotechnology Centre, Nanyang Technological University, Singapore 637141, Singapore
[c] School of Civil & Environmental Engineering, College of Engineering, Nanyang Technological University, Singapore 637141, Singapore

HIGHLIGHTS

- Speciation and bioavailability of Fe^{2+} was determined at steady state conditions.
- Sulfide precipitated Fe^{2+} as FeS and hence reduced the performance of the SAMBR.
- EDDS was able to compete with sulfide and improve Fe^{2+} bioavailability in a SAMBR.

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ABSTRACT

The effects of a biodegradable chelating agent, Ethylenediamine-N,N′-disuccinic acid (EDDS), on the speciation and bioavailability of iron (Fe^{2+}) in anaerobic digestion were examined. Fe^{2+} supplementation at 10 mg/L increased methane yield, but the presence of 8 mg/L sulfide led to the precipitation of Fe^{2+} as FeS which limited its bioavailability. The results confirmed that the EDDS could replace common chelating agents with low biodegradability (EDTA and NTA), and improve the bioavailability of Fe^{2+} by forming an Fe-EDDS complex, thereby protecting Fe^{2+} from sulfide precipitation. Experimental findings from sequential extraction using the Community Bureau of Reference (BCR) method, and quantification of free EDDS and Fe-EDDS complex using UHPLC, confirmed that 29.82% of Fe^{2+} was present in bioavailable forms, i.e. soluble and exchangeable, when EDDS was added at 1:1 molar ratio to Fe^{2+}. As a result, the methane production rate increased by 11.17%, and the methane yield increased by 13.25%.

1. Introduction

The role of trace metals in anaerobic digestion as essential components of cofactors and enzymes has been well reported in the literature (Oleszkiewicz and Sharma, 1990; Fermoso et al., 2009; Demirel and Scherer, 2011). In practice, trace metals are often dosed in excessive amounts (Gonzalez-Gil et al., 2003) because supplementing trace metals at their optimal concentrations does not ensure that they will be bioavailable for microbial uptake (Zitomer et al., 2008). Trace metal bioavailability relies on their speciation, which in turn is controlled by many factors including pH, temperature, redox potential, precipitation kinetics, adsorption, and complexation (Aquino and Stuckey, 2007; Schmidt et al., 2014). The need to understand the relationship between the speciation and bioavailability of trace metals in anaerobic digestion was emphasized in a recent comprehensive review by Thanh et al. (2016). Precipitation of the metal sulfide is one of the most important processes in anaerobic digestion as it can limit the trace metal bioavailability for microbial uptake, and hence trace metals need to be supplemented in higher quantities. Sulfide is often present in an anaerobic digester, and originates from sulfide, sulfate, and sulfur-containing organic compounds in the feed (Callander and Barford, 1983). Many studies have confirmed the influence of sulfide formation/dissolution on trace metal speciation in anaerobic digestion (Gonzalez-Gil et al., 1999; van der Veen et al., 2007; Gustavsson et al., 2013).

To prevent the precipitation of trace metals with sulfide in anaerobic bioreactors it is possible to add chelating agents, which will complex with the trace metals and keep them in a soluble form. Thanh et al. (2016) suggested that trace metal bioavailability

* Corresponding author at: Department of Chemical Engineering, Imperial College London, London SW7 2AZ, UK.
E-mail address: d.stuckey@imperial.ac.uk (D. Stuckey).

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in anaerobic digestion can be improved by the supplementation of natural chelating agents eg. soluble microbial products (SMPs) (Aquino and Stuckey, 2007) or synthetic chelating agents eg. Ethylene diaminetetraacetic acid (EDTA) and Nitrilotriacetic acid (NTA) (Fermoso et al., 2008; Hu et al., 2008; Bartacek et al., 2012; Vintiloiu et al., 2013). It has been reported that the EDTA and NTA have higher complexing capabilities with trace metals than SMPs, however, these materials are poorly biodegradable and could pose a threat to the environment when ultimately discharged in the effluent (Tandy et al., 2004). Alternatively, readily biodegradable chelating agents such as Ethylenediamine-N,N′-dis uccinic acid (EDDS), Imino disuccinic acid (IDS), and Glutamic acid diacetic acid (GLDA) can be used; however, there is limited research looking at the viability of using such chelating agents in anaerobic systems.

Due to its biodegradability, EDDS has been an alternative for traditional chelating agents in several commercial products e.g. industrial detergents. In environmental applications, EDDS has been used for induced phytoextraction and soil washing to remove heavy metals such as Pb, Zn, Cu, and Cd (Kos and Leštan, 2003; Zhao et al., 2010; Satyro et al., 2014; Ferraro et al., 2015; Beiyuan et al., 2016). However, to date, there is only one study that has demonstrated the use of EDDS to increase metal bioavailability in anaerobic digestion (Zhang et al., 2015). In this study, the optimum dosage of EDDS was not justified, and the effect of EDDS addition was only determined on a combination of trace metals i.e. Fe, Co, and Ni, rather than on any individual trace metal.

Therefore, the objective of this study was to evaluate the effect of sulfide on the bioavailability of Fe^{2+}, and to determine how supplying the biodegradable chelating agent EDDS could improve Fe^{2+} bioavailability, and hence increase the methane yield in anaerobic digestion. The effect of EDDS on the Fe^{2+} bioavailability in the presence of sulfide was first investigated in a model media and then extended to an actual anaerobic digestion process. In order to give deeper insights into the relationship between metal speciation and metal complexation, the applicability of Community Bureau of Reference (BCR) extraction method was systematically assessed in dried/wet sludge conditions, and a new UHPLC method was developed for the determination of free-EDDS and Fe-EDDS complex.

2. Material and methods

2.1. Experimental plan

2.1.1. Seed and anaerobic growth media

Seed sludge was obtained from municipal wastewater treatment plant digesters (Ulu Pandan Water Reclamation Plant, Singapore), and grown in 5 L continuously mixed laboratory reactors for more than 3 months. The seed reactors were fed once a week in fill-and-draw mode (7 d HRT), and the feed consisted of glucose (333 mg COD/L), peptone (99 mg COD/L), meat extract (33 mg COD/L), sodium bicarbonate (812.5 mg/L), K_2HPO_4 (10 mg/L), and essential metal nutrients including $CoCl_2{\cdot}6H_2O$ (0.29 mg/L), $FeCl_2{\cdot}4H_2O$ (1.96 mg/L), $MnCl_2{\cdot}4H_2O$ (0.09 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (0.04 mg/L), and $NiCl_2{\cdot}6H_2O$ (0.05 mg/L).

2.1.2. Metal extraction of dried and wet samples with the BCR extraction method

This experiment was aimed at comparing the speciation and recovery of metals from dried and wet anaerobic sludge samples with the BCR method. For dried samples, the sludge was centrifuged at 4000 rpm for 15 min and then the pellets were dried in aluminium pans at 105 °C overnight. Dried pellets were then ground in a porcelain mortar to reduce particle size, and then stored in a desiccator at room temperature. During the extraction step, 0.6 g of dried sample was placed into a 50 mL centrifuge tube. For wet samples, a volume of anaerobic sludge equivalent to 0.5 gTSS was centrifuged at 4000 rpm for 15 min in a 50 mL centrifuge tube. The sequential extraction for both dried and wet sludge was carried out in quadruplicate, and the concentration of metals from each extraction step was measured using MP-AES.

2.1.3. Effects of metal nutrient supplementation on anaerobic digestion

This experiment was aimed at comparing the effects of selected metal nutrients on methane production with anaerobic sludge. The "Control" set did not have substrate added or metal nutrients, while the "No Metal" set contained only substrate, but the test reactors were supplemented with a group of metal nutrients that were characterized by similar chemical properties i.e. set 1: Fe + Co + Ni (transitional metals), set 2: Cu + Mn + Zn (other trace metals), and set 3: Ca + Mg (macronutrients). The substrate was glucose, the F/M ratio was fixed at 1:1 (2 gCOD as glucose to 2 gVSS), and $NaHCO_3$ was added as a buffer.

The experiment was carried out in a batch automatic methane potential test system (AMPTS II, Bioprocess Control AB, Sweden) in triplicate. The tests were performed in standard 500 mL bottles, and the working volume of each test reactor was 400 mL. The reactors were flushed with nitrogen gas passing through tubing to ensure anaerobic conditions prior to start-up, and the temperature was controlled at 35 °C. Stirrers in all the reactors were set at 60 s on and 30 s off at 46 rpm during the whole experiment. Metal speciation was assessed using the BCR method with wet samples.

The overall batch tests were carried out in phases. During Phase 1, the metal nutrients were added at the concentrations as in the BMP recipe (Table 3a) which was used by Hu (2004) and Souto et al. (2010). During Phases 2 and 3, the same dose of substrate was injected again into the test reactors, while the metal concentrations were doubled and trebled from the initial concentrations, respectively, in order to magnify the effects of metal supplementation on methane production.

2.1.4. Determination of optimal concentration for Fe^{2+} in anaerobic digestion

The aim of this study was to evaluate the speciation and bioavailability of Fe^{2+} in anaerobic digestion. The experiment was performed in batch reactors with 60 mL serum bottles (45 mL

Table 1
Operating conditions in the revised BCR sequential extraction method (van Hullebusch et al., 2005).

Fraction	Extracting agent	Extraction conditions	
		Shaking time[a]	Temperature
F1. Exchangeable + water and acid soluble	40 mL CH_3COOH (0.11 M, pH = 7)	16 h	20 °C
F2. Iron and manganese oxides	40 mL NH_2OH–HCl (0.5 M, pH = 1.5)	16 h	20 °C
F3. Organic matter and sulfides	20 mL H_2O_2 (30%, pH = 2), 50 mL CH_3COONH_4 (1 M, pH = 2)	1, 2, 16 h	20, 85, 20 °C
F4. Residual	10 mL demineralised water, 10 mL aqua regia (HCl:HNO_3, 3:1)	26 min	Microwave-oven[b]

[a] Shaking was applied at 30 rpm.
[b] Extraction of the residual fraction in the microwave was equal to the pseudo-total extraction method.