Iron-mediated removal of ammonium from strong nitrogenous wastewater from food processing

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Abstract The combination of microbial reduction and further microbial oxidation of iron was applied to the treatment of food-processing wastewater and recovery of ammonium. Fe^{2+} ions were formed by iron-reducing bacteria under anaerobic conditions. Ammonium was recovered by co-precipitation with negatively charged iron hydroxides produced during oxidation of Fe^{2+} by iron-oxidizing bacteria under microaerophilic conditions. The value-added by-product of this process can be used as a slowly released ammonium fertilizer.

Keywords Ammonium; iron-oxidizing bacteria; iron-reducing bacteria; nitrogenous wastewater

Introduction

Sludge digester effluent and wastewaters from food processing, production of nitrogen fertilizers, cattle breading complexes, and aquaculture farms have high concentrations of ammonium. Ammonium, as well as its oxidation products, is toxic for humans, animals, and aquatic organisms. Therefore, it must be removed during wastewater treatment. Biological methods play an important role in ammonium removal from wastewater. The most common biotechnologies are microbiological nitrification, denitrification, and anammox processes, which sequentially convert ammonium to nitrite, nitrate, and finally to nitrogen gas or nitrous oxide (van Dongen *et al*., 2001; Fux *et al*., 2002; Green *et al*., 2002; Hibiya *et al*., 2003). These microbiological processes are slower and very sensitive to environmental conditions in comparison with chemical or physical removal of ammonium from wastewater. An additional disadvantage of these processes is that ammonium is not recovered. Recovery of ammonium can be performed by the formation of struvite, magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4\cdot 6H_2O$), but it requires addition of Mg salt and must be performed at pH higher than 9 (Altinbas *et al*., 2002). Theoretically, the recovery of ammonium can be performed by application of iron-reducing bacteria (IRB) and iron-oxidizing bacteria (IOB). Some engineering applications of iron-reducing bacteria in the remediation of groundwater and soil have been reported (Fredrickson and Gorby, 1996; Monserrate and Haggblom, 1997; Phels and Young, 1999). However, the reduction of iron has not yet been used in anaerobic treatment of wastewater.
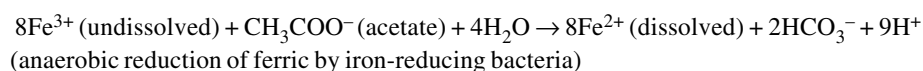
It has been shown that slow oxidation of Fe(II) in water and soil model systems prevents nitrification due to the binding of ammonium ions with negatively charged ferric hydroxide, which was formed under microaerophilic conditions (Ivanov *et al*., 1997). The existence of ferric hydroxide as anions $Fe(OH)_4^-$ at pH from 6–8 is well known (Cornell and Schwertmann, 2003). These anions could bind and precipitate positively charged ammonium ions. The removal of ammonium from solution occurred during chemical oxidation of Fe(II) (Ivanov *et al*., 1997). The molar ratio ammonium/iron in sediment was

0.77, while, theoretically, this ratio is 1 for $(NH_4)Fe(OH)_4$. The reason was probably the large size of the iron hydroxide particles.

Ammonium concentration in the effluent of anaerobic digester of food waste or activated sludge is generally from 500 to 2,000 mg/l. To recover this ammonium, co-precipitation with negatively charged Fe(III) hydroxides formed by iron-oxidizing bacteria in aerobic treatment of wastewater was proposed (Stabnikova *et al.*, 2000). Iron-oxidizing bacteria could be found at sites where groundwater, containing ferrous ions or chelates of Fe(II), contacts with oxygen (Emerson and Revsbech, 1994). An addition of ochreous sludge (containing iron-oxidizing bacteria) from blocked irrigation bores to a solution of soluble Fe(II) increased the oxidation rate at pH 5.8 (Ralph and Stevenson, 1995).

The salts of Fe(II) are expensive and not stable at neutral pH. The Fe^{2+} ions can be formed from Fe(III) in an anaerobic reactor due to the activity of iron-reducing bacteria (IRB). It is known that IRB are present in an anaerobic reactor (Nielsen et al., 2002). IRB reduce Fe(III) to Fe(II) coupled with oxidation of hydrogen or organic substances under anaerobic conditions (Lovely, 2000). Fe(III) added into an anaerobic reactor can be reduced to Fe(II) when the anaerobic sludge was previously adapted to the presence of Fe(III) (Ivanov *et al.*, 2002). Iron-reducing bacteria can release Fe(II) from undissolved Fe(III) minerals. The cheapest suitable sources of Fe(III) for reduction by iron-reducing bacteria are iron-containing clay, crushed iron ore, wetland iron ore, or industrial grade iron hydroxide (Ivanov *et al.*, 2002).

The sequence of iron transformations during anaerobic–aerobic treatment of strong nitrogenous wastewater may be shown by the following equations:

$$8Fe^{3+} \text{ (undissolved)} + CH_3COO^- \text{ (acetate)} + 4H_2O \rightarrow 8Fe^{2+} \text{ (dissolved)} + 2HCO_3^- + 9H^+$$
(anaerobic reduction of ferric by iron-reducing bacteria)

Fe(II), produced in an anaerobic digester, may be further oxidized chemically. However, if Fe(II) is chelated with organic acids, its oxidation is accelerated by so-called iron-oxidizing bacteria. These bacteria actually degrade the organic "envelope" of the iron atom, which is chemically oxidized:

$$4Fe^{2+} \text{ (dissolved)} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$$
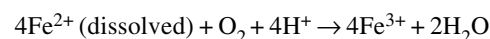
The main product of ferrous oxidation under neutral pH is iron hydroxide $Fe(OH)_4^-$ (Cornell and Schwertmann, 2003). If ammonium is present in the water, Fe(II) oxidation follows co-precipitation of $Fe(OH)_4^-$ with ammonium:

$$4Fe^{3+} + O_2 + 12H_2O + 4NH_4^+ \rightarrow 4Fe(NH_4)(OH)_4 + 12H^+$$
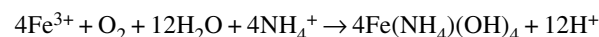
Theoretically, the ratio of consumed oxygen to removed ammonium is 0.57 g O_2/g NH_4^+-N and 4.57 g O_2/g NH_4^+-N for new process and for the conventional nitrification process, respectively. Therefore, the new process may be 8 times more effective in the consumption of oxygen than that of the nitrification process. However, the negative effect of these reactions on anaerobic treatment of wastewater may be caused by the decrease of pH due to the release of protons. Therefore, addition of iron in the process must be accompanied by pH control or by addition of the buffering substance.

The aim of this research was to develop effective technology for iron-mediated removal and recovery of ammonium from wastewater using microbiological reduction and oxidation of iron.

Materials and methods

The experiments were performed with wastewater simulating strong nitrogenous food processing wastewater with the following composition, g/l: potato starch, 20; peptone, 18;

KH_2PO_4, 1; K_2HPO_4, 0.1; NH_4Cl, 1.0; $CaCO_3$, 0.3; distilled water, 1 litre. Suspension of ferric hydroxide was added in the experiment to create a final concentration of Fe(III) of 12 g/l. The synthetic wastewater was treated anaerobically for 25 days. Effluent was separated from sludge by decanting for 0.5 h and used for aerobic treatment. Suspensions of activated sludge and IOB were added in the dosage of 10% (v/v) each. The pH was maintained at 6.5–7.5 during the aerobic stage. The intensity of aeration was 0.1 l/l·min. Activated sludge was collected from a municipal wastewater treatment plant. Enrichment culture of IOB was produced by cultivation of 10% of natural source of iron oxidizing bacteria in modified *Sphaerotilus – Leptothrix* medium from the list of media of Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany, www.dsmz.com.de) with the following composition, g/l: yeast extract, 1.0, peptone, 1.5, $MgSO_4·7H_2O$, 0.2; $CaCl_2$, 0.05; $MgSO_4·H_2O$, 0.05; $FeCl_3·6H_2O$, 0.01; ferric lactate, 2.42; tap water to 1 litre, pH 7.0. The enrichment culture of IOB was obtained after 7 days of cultivation at 30°C. Oxidation of ferrous lactate (0.5 g/l) was analyzed by addition of 10% (v/v) enrichment culture of IOB by the quantity of precipitated ferric hydroxide. Chemical oxidation of ferrous lactate by air was used as the control. Chemical oxygen demand (COD), concentrations of ammonium, nitrate, and nitrite were determined by Standard Methods (1998). A phenanthroline method was used to determine the concentrations of Fe(II) and Fe(III) (Standard Methods, 1998). To measure soluble Fe(II), a sample was acidified by 1N HCl (1:1) and filtrated through 0.2 mm membrane. To measure total Fe(II), 1N HCl was added to the sample (1:1) and incubated for 30 min to extract Fe(II).

Results and discussion

Enrichment culture of iron-oxidizing bacteria included mainly the representatives from genera *Leptothrix* and *Siderocapsa.* An addition of IOB to the medium with ferrous lactate increased the rate of ferrous oxidation three times (Figure 1). The average rate of biological oxidation was 4.5 mg/l·h, while the rate of chemical oxidation was 1.0 mg/l· h.

Removal of ammonium during oxidation of ferrous lactate was proportional to the added quantity of ferrous lactate (Table 1). Additions of ferrous lactate were 2.42 g/l for control and experiment 1, 4.82g/l for experiment 2, and 9.64 g/l for experiment 3.
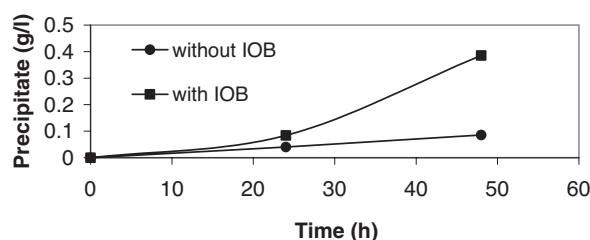

Figure 1 Formation of $Fe(OH)_3$ precipitate during chemical oxidation (without IOB) or chemical and biological oxidation (with IOB) of ferrous lactate

Table 1 Ammonium removal from wastewater after 7 days of ferrous lactate oxidation

Process	Ammonium concentration, mg/l		Ammonium removal, mg/l	Ammonium removal, % from initial
	initial	after 7 days		
Control (without IOB)	544±28	497±17	47±5	8.6
Experiment 1 (with IOB)	544±28	425±21	119±6	21.9
Experiment 2 (with IOB)	544±28	294±12	250±15	46.0
Experiment 3 (with IOB)	544±28	8±1	536±22	98.5

Table 2 Effect of Fe(II) and iron-oxidizing bacteria on aerobic treatment of wastewater with high COD content

Process	Concentration, mg/l	Time, days			
		0	4	8	10
Control (activated sludge without addition of Fe(II) and iron-oxidizing bacteria)	COD	3500	2400	1300	950
	NH_4^+	850	680	460	380
	NO_2^-	50	0	0	0
	NO_3^-	20	481	0	969
	Fe^{2+}	0	0	0	0
	Fe^{3+}	0	0	0	0
Experiment 1 (activated sludge with addition of iron)	COD	3500	2100	1900	800
	NH_4^+	750	545	320	115
	NO_2^-	38	0	0	0
	NO_3^-	10	314	138	119
	Fe^{2+}	2560	224	120	50
	Fe^{3+}	120	560	860	900
Experiment 2 (activated sludge with addition of iron and iron-oxidizing bacteria)	COD	3500	2200	1000	650
	NH_4^+	625	155	6	6
	NO_2^-	36	0	0	0
	NO_3^-	8	179	106	30
	Fe^{2+}	3010	356	110	110
	Fe^{3+}	210	580	120	120

Effect of iron-oxidizing bacteria and ferrous oxidation on aerobic treatment of model strengthen nitrogenous wastewater was studied. Nitrification was observed in the control (when ferrous was absent in wastewater) but bacterial nitrification was prevented when water contained ferrous ions was formed by iron (Table 2). Negative effect of Fe(II) on nitrification was caused by its oxidation and the binding of ammonium with the anionic iron hydroxides. Ammonium was removed from the solution as iron-ammonium precipitated during chemical oxidation of Fe(II) in the solution of ammonium under controlled pH 8. Molar ratio of ammonium and iron in the formed precipitate was close to 1:1. The precipitate formed during the aerobic stage of strong nitrogenous wastewater treatment could be expressed with an formula $(NH_4)Fe(OH)_4$. The precipitate, being suspended in water, released ammonium with average rate of 1.6% of N per day. Additional advantage of precipitation of negatively charged iron hydroxides was removal/recovery of such nutrients from wastewater as phosphate and potassium.

Plant growth experiments showed that this precipitate could be used as effective fertilizer. Test objects were the haricot beans grown for 15 days in sandy soil (control 1) with addition of 140 mg of ammonium sulphate/kg of soil (control 2) or equivalent by nitrogen quantity of iron-ammonium precipitate (experiment). Dry matter of plant was 0.04±0.01 g, 0.36±0.04 g, and 0.47±0.02 in control 1, control 2, and experiment, respectively. Nitrogen content in dry biomass was 0.26±0.02%, 3.64±0.08%, and 4.63±0.17% in control 1, control 2, and experiment, respectively.

Conclusions

Microbial reduction of Fe(III) following microbial oxidation of Fe(II) can be used to remove and recover ammonium from wastewater. It is eight times more efficient process using oxygen consumption than using nitrification.

Ammonium was removed/recovered by co-precipitation with negatively charged iron hydroxides produced during oxidation of Fe(II) by iron-oxidizing bacteria. By-product of this wastewater treatment was the slow-releasing ammonium fertilizer. However, process of ammonium recovery and properties of this fertilizer must be studied deeper before practical application.

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