Fe(II)EDTA^{2-} regeneration, biomass growth and EDTA degradation in continuous BioDeNOx reactors

Peter van der Maas, Paula van den Brink, Sudarno Utomo, Bram Klapwijk, and Piet Lens
Wageningen University, Dept. of Environmental Technology, P.O. Box 8129, 6700 EV, Wageningen, The Netherlands

ABSTRACT. BioDeNOx is a novel technique for NOx removal from industrial flue gases. In principle, BioDeNOx is based on NO absorption into an aqueous Fe(II)EDTA^{2-} solution combined with biological regeneration of that scrubber liquor in a bioreactor. The technical and economical feasibility of the BioDeNOx concept is strongly determined by high rate biological regeneration of the aqueous Fe(II)EDTA^{2-} scrubber liquor and by EDTA degradation. This investigation deals with the Fe(II)EDTA^{2-} regeneration capacity and EDTA degradation in a lab-scale BioDeNOx reactor (10-20 mM Fe(II)EDTA^{2-}, pH 7.2 ± 0.2, 55 ºC), treating an artificial flue gas (1.5 $m^3.h^{-1}$) containing 60 – 155 ppm NO and 3.5-3.9 % O_2.
The results obtained show a contradiction between the optimal redox state of the aqueous FeEDTA solution for NO absorption and the biological regeneration. A low redox potential (below – 150 mV vs Ag/Cl) is needed to obtain a maximal NO removal efficiency from the gas phase via Fe(II)EDTA^{2-} absorption. Fe(III)EDTA^{-} reduction was found to be too slow to keep all FeEDTA in the reduced state. Stimulation of Fe(III)EDTA^{-} reduction via periodical sulfide additions (2 mM spikes twice a week for the conditions applied in this study) were found to be necessary to regenerate the Fe(II)EDTA^{2-} scrubber liquor and to achieve stable operation at redox potentials below –150 mV (pH 7.2±0.2). However, redox potentials of below –200 mV should be avoided since sulfide accumulation is unwanted. Very low values for biomass growth rate and yield, respectively, 0.043 d^{-1} and 0.009 mg protein per mg ethanol, were observed. This might be due to substrate limitations, i.e. the electron acceptors NO and presumably polysulfide, or to physiological stress conditions induced by radical formation during Fe(II)EDTA^{2-} oxidation by oxygen present in the flue gas. Radicals possibly also induce EDTA degradation, which occur at a substantial rate: 2.1 (± 0.1) $mM.d^{-1}$ under the conditions investigated.

1 INTRODUCTION

Industrial flue gases are a major source of NOx emission to the atmosphere (Schnelle and Brown, 2002). At present selective catalytic reduction (SCR) is the most common technology applied for NOx abatement. High energy consumption and costs, as well as ammonia emissions are, however, major drawbacks of the SCR technology. Biological NOx removal techniques may become attractive alternatives, but the poor solubility of NO in water results into relatively high residence times of at least 1 minute (see Jin *et*

al., 2005), and, therefore, large reactor volumes. To enhance the NO transfer from the gas to the liquid phase, aqueous solutions of ferrous chelates, e.g. $Fe(II)EDTA^{2-}$, can be applied as scrubber liquor, because ferrous EDTA reactively absorbs NO according to:

$$NO\ (aq) + Fe(II)EDTA^{2-} \leftrightarrow Fe(II)EDTA\text{-}NO^{2-} \quad (1)$$

The BioDeNOx process (Buisman *et al*., 1999) utilises this principle of wet absorption of NO in an aqueous $Fe(II)EDTA^{2-}$ solution and combines it with biological reduction of the sorbed NO according to the overall reactions:

$$6\ Fe(II)EDTA\text{-}NO^{2-} + C_2H_5OH \rightarrow 6\ Fe(II)EDTA^{2-} + 3\ N_2 + 2\ CO_2 + 3\ H_2O \quad (2)$$

Since flue gases generally contain oxygen, part of the $Fe(II)EDTA^{2-}$ is oxidized to $Fe(III)EDTA^{-}$ (reaction 3). Therefore, reduction of EDTA chelated Fe(III) (overall reaction 4) is, besides NO reduction, a core reaction of the biological regeneration pathway in the BioDeNOx process.

$$4\ Fe(II)EDTA^{2-} + O_2 + 4\ H^+ \rightarrow 4Fe(III)EDTA^{-} + 2\ H_2O \quad (3)$$
$$12\ Fe(III)EDTA^{-} + C_2H_5OH + 3\ H_2O \rightarrow 12\ Fe(II)EDTA^{2-} + 2\ CO_2 + 12\ H^+ \quad (4)$$

Previous research demonstrated that the volumetric NO reduction rate in aqueous $Fe(II)EDTA^{2-}$ solutions is determined by the NO and $Fe(II)EDTA^{2-}$ concentration and by the amount of denitrifying biomass (Van der Maas *et al.,* 2005a). However, not the NO reduction but $Fe(III)EDTA^{-}$ reduction was found to be the process rate limiting factor of BioDeNOx reactors treating flue gas with more than 1 % O_2 oxygen (Van der Maas *et al*., 2005b). The volumetric $Fe(III)EDTA^{-}$ reduction rate can be enhanced by the presence of low concentrations of an electron mediating compound (EMC), presumably polysulfides, that are formed upon addition of sodium sulfide to the aqueous $Fe(III)EDTA^{-}$ solution (Van der Maas *et al.,* 2005c).
Except a high rate biological regeneration of the aqueous $Fe(II)EDTA^{2-}$ scrubber liquor, pilot scale trials using real flue gases indicated that the technical and economical feasibility of the BioDeNOx concept is strongly determined by EDTA degradation (Biostar, personal communication). Both chemical (Witschel and Egli, 2001) and biological (Nörtemann, 1999) degradation of EDTA is well documented, but it is unknown to what extend these processes contribute to EDTA degradation in BioDeNOx reactors.
The present study investigates the effect of sulfide addition and biomass concentration on the $Fe(II)EDTA^{2-}$ regeneration capacity of BioDeNOx reactors, i.e. the volumetric NO and $Fe(III)EDTA^{-}$ reduction rate. The investigations were conducted using a continuous operating laboratory scale BioDeNOx reactor (pH 7.2 ± 0.2, 55 ºC), treating an artificial flue gas (1.5 $m^3.h^{-1}$) containing 60 – 155 ppm NO and 3.5-3.9 % O_2. Furthermore, the biomass growth and EDTA degradation during BioDeNOx operation was determined.

2 MATERIAL AND METHODS

2.1 Reactor configuration

The reactor configuration used in this study is schematically presented in Figure 1. The bioreactor consisted of a 5.5 dm^3 cylindrical double jacket glass column. Scrubbing of

the artificial flue gas occurred in a simple bubble column (wet volume 0.5 liter), mounted on top of the bioreactor (Figure 1). The artificial flue gas was composed of pure N_2 gas, pure NO gas and pressured air in adjusted amounts with mass flow controllers (Brooks, Veenendaal, The Netherlands). The gas was continuously recirculated over the gas scrubbing column with a compressor (KNF, Freiburg, Germany) at a fixed flow of 1.5 $m^3.h^{-1}$, resulting in a gas residence time of 1.2 seconds. The NO concentrations in both the influent and effluent gas were analyzed using an automatically operating gas selector (Wageningen University, The Netherlands).

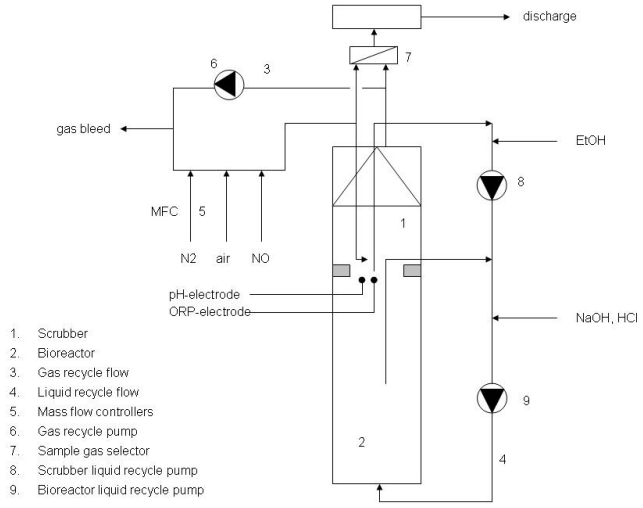

Figure 1. Schematic flow diagram of the continuous BioDeNOx reactor.

The scrubber liquor was continuously recirculated over the bioreactor with a flow of 17 $l.h^{-1}$, corresponding to a bioreactor residence time of 20 minutes. A second recirculation flow was applied to provide extra mixing of the bioreactor liquor and to increase the superficial liquid upflow velocity to 18 m/h. The temperature of the bioreactor was controlled at 55° C by means of a temperature controlled water jacket. The redox potential (ORP) and pH of the bioreactor were monitored continuously (WTW Sentix electrodes, Weilheim, Germany). The pH was controlled at 7.2 (± 0.2) by means of automatic HCl or NaOH addition. Ethanol was supplied as electron donor both manually and automatically. In the latter case, the ORP signal was used as steering parameter: the ethanol supply switched on when the ORP became higher than -175 mV. Ethanol supply stopped when the ORP of the bioreactor liquor dropped below -180 mV versus Ag/AgCl.

2.2 Medium composition

The medium used for all experiments contained 25 mM Na_2H_2EDTA (titriplex, Merck), 30 mM $FeCl_3$, 5 mM $MgSO_4$, 5 mM $CaCl_2$ and nutrients according to Van der Maas *et al.* (2003). The excess of di- and trivalent cations over Na_2H_2EDTA was applied to prevent cell lysis (Ayres, 1998).

2.3 Sources of biomass

The bioreactor was inoculated with two sources of biomass: denitrifying Veendam sludge and methanogenic Eerbeek sludge. The denitrifying 'Veendam' sludge originated from a methanol fed denitrifying fluidized bed reactor treating surface water (Veendam, The Netherlands). Veendam sludge has a high NO removal capacity (Van der Maas *et al.,* 2003). The methanogenic 'Eerbeek' granular sludge originated from a full scale UASB reactor treating pulp and paper mill wastewater (Eerbeek, The Netherlands). Eerbeek sludge has a high Fe(III)EDTA$^-$ reducing activity (Van der Maas *et al*. 2005d), but no NO reducing activity (Van der Maas *et al.,* 2003). Before use, the Eerbeek granules were mixed with the Fe(III)EDTA$^-$ medium (1/4 w/w) and crushed for 3 minutes with a kitchen blender.

2.4 Start up of the BioDeNOx reactor

The BioDeNOx reactor was started up with 5.4 liter of Fe(III)EDTA$^-$ medium inoculated with 600 ml Veendam sludge up to an initial VSS concentration of 60 mg/l (equal to 30 mg/l protein). To enhance the Fe(III)EDTA$^-$ reduction capacity, 2 mM Na_2S was added to the reactor mixed liquor from a concentrated stock solution. In aqueous Fe(III)EDTA$^-$ solutions, these sulfide additions generate polysulfides according to (Clarke *et al.,* 1994):

$$2n\ Fe(III)EDTA^- + (n+1)\ S^{2-} \rightarrow\ S_{n+1}^{2-} + 2n\ Fe(II)EDTA^{2-} \qquad (5)$$

The reactor mixed liquor was pre-cultivated for 79 days by means of discontinuous NO (100 ppm) and O_2 (3%) supply to the flue gas (1.5 $m^3.h^{-1}$). The NO and O_2 loads were switched off when the ORP of the bioreactor liquor became higher than -150 mV, meaning that the FeEDTA was partly (± 40%) in the oxidized state and that the NO and/or Fe(III)EDTA$^-$ reduction capacity was lower than the NO and O_2 load of the bioreactor (Van der Maas *et al.,* 2005b).

2.5 Continuous operation and experimental design

After 79 days of discontinuous operation, 3 liter of reactor liquor was replaced with 2.8 liter of fresh Fe(III)EDTA$^-$ medium and 0.2 liter of Eerbeek sludge (± 20 g VSS). From the onwards, the reactor was continuously operated with 1.5 $m^3.h^{-1}$ flue gas containing NO (55-190 ppm) and O_2 (1.0-3.9 %). The NO and Fe(III)EDTA$^-$ load were gradually increased by means of increasing the NO and O_2 concentration of the flue gas, respectively. This load elevation was continued until the regeneration capacity, i.e. the volumetric NO and/or Fe(III)EDTA$^-$ reduction rate, became limiting. In that case, the ORP of the bioreactor exceeded the value of -150 mV at pH 7.2 (±0.2), which is equal to an ORP of -140 mV versus Ag/AgCl when corrected for pH 7.0 (Van der Maas *et al.,* 2005b). To supply nutrients and EDTA (to compensate degradation), 300 ml fresh Fe(III)EDTA$^-$ medium was added daily to the reactor with the simultaneous withdrawal of 300 ml reactor liquor, resulting a hydraulic retention time (HRT) of 20 days.

During the continuous operation period, the scrubber performance was characterized by determination of the relation between the ORP of the reactor mixed liquor and the NO removal efficiency from the gas phase. To investigate the effect of bio-augmentation on the NO reduction capacity, ± 5 g VSS Veendam sludge was added to the reactor at day 98. At the start of the continuous regeneration regime (day 80), the Fe(III)EDTA$^-$ reduction capacity was enhanced by the addition of 0.2 liter Eerbeek sludge to the bioreactor. To investigate the effect of sulfide supply on the Fe(III)EDTA$^-$ reduction

rate, Na_2S was added in shots of 2 mM at days 93, 98 and 111. The regeneration capacity, biomass growth and EDTA degradation were quantified as described below.

2.6 Quantification of the bioreactor regeneration capacity

To quantify the overall regeneration capacity of the bioreactor, the flue gas flow was switched off periodically, i.e. the NO and Fe(III)EDTA$^-$ load to the bioreactor were set at zero. The volumetric Fe(II)EDTA^{2-} production rate was measured by monitoring the redox potential of the reactor mixed liquor, because it is a proper indicator of the Fe(II)EDTA^{2-} concentration (Kolthoff and Auerbach, 1952; Van der Maas *et al*., 2005c). The specific NO and Fe(III)EDTA$^-$ reduction rate of the reactor mixed liquor was determined in batch experiments at 55 °C in at least duplicate. The NO reduction rate was quantified by measuring the N_2O and N_2 production as described previously (Van der Maas *et al*., 2005a). The Fe(III)EDTA$^-$ reduction rate was measured by means of the redox potential as described elsewhere (Van der Maas *et al*., 2005c).

2.7 Quantification of biomass growth and EDTA degradation

Biomass growth and EDTA degradation were quantified in daily samples by measuring the protein and EDTA concentration of the reactor mixed liquor, respectively. The ethanol consumption was quantified on the basis of a mass balance using the amount of ethanol supplied to the bioreactor and the ethanol concentration of the reactor mixed liquor. To investigate EDTA degradation in the absence of oxygen, bioreactor mixed liquor was incubated in 120 ml serum flasks in the dark at both 20 and 55 ºC.

2.8 Analyses and chemicals

The NO concentrations in the in- and effluent gas of the bench-scale installation was measured continuously by a chemolumisence NO analyzer (Beckman model 951). H_2, O_2, CH_4 in the gas phase were determined by gas chromatography as described by Weijma *et al.* (2000). The N_2 and N_2O concentration were also determined by gas chromatography as described elsewhere (Van der Maas *et al.,* 2005a). The Fe(II)EDTA^{2-} concentration was determined colorimetrically using 1,10-phenantroline (Akzo Nobel, 1996). Ethanol and acetate were determined by gas chromatography as described by Cervantes et al. (2000). EDTA was determined by High Performance Liquid Chromatography (HPLC) with UV spectrofotometric detection at 354 nm. The HPLC was equipped with a Vydac 302IC4.6 column (pressure 50 bar, 20°C) and a phosphate buffer (KH_2PO_4 13.6 g/l, pH 3) was used as eluent (flow rate 1.5 ml/min).

The biomass content of the reactor liquor was quantified via its protein concentration. Reactor samples were centrifuged (10 min, 10.000 rpm) and the supernatant was discharged. The pellet was dissolved in 1 N NaOH and placed in boiling water for 15 min. After boiling, the sample was neutralized with 1 N HCl and the protein concentration was determined according to Bradford (1976).

2.9 Load calculations

The bioreactor load, i.e. the amount of Fe(III)EDTA$^-$ and Fe(II)EDTA-NO^{2-} supplied to the bioreactor, was calculated using mass balances for O_2 and NO as described previously (Van der Maas *et al.,* 2005b). The load of the electron acceptors (O_2, Fe(III)EDTA$^-$ and NO) and the electron donor (ethanol) is expressed as molar electron equivalents (meq): 1 mol of ethanol, O_2, NO and Fe(III)EDTA$^-$ corresponds to 12, 4, 2 and 1 meq, respectively.

3 RESULTS

3.1 Effect of redox potential on NOx removal from the gas phase

The NOx removal efficiency from the gas phase showed to be greatly influenced by the redox potential of the bioreactor mixed liquor. Figure 2 shows that the redox potential should be maintained below -150 mV vs Ag/AgCl for a maximal NO removal efficiency. Under these conditions, the NO concentration in the flue gas decreased from 155 to 93 ppm, meaning that 39 % of the gaseous NO was sorbed into the aqueous $Fe(II)EDTA^{2-}$ solution. At redox potentials exceeding -150 mV, the NO removal efficiency strongly declined (Figure 2). It should be noted that the results of Figure 2 relate to the NO removal capacity of the scrubber, and not to the bio-regeneration capacity (see Discussion).

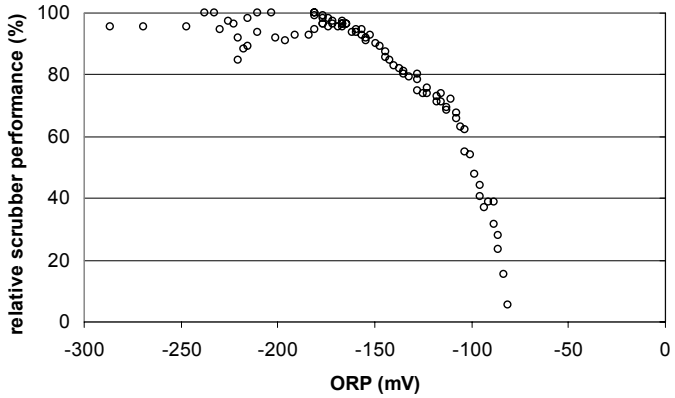

Figure 2. Relation between the redox potential (ORP) of the bioreactor mixed liquor and the relative scrubber performance for NO removal.

3.2 Quantification of the bioreactor loading rate

The redox potential of the bioreactor mixed liquor, i.e. the oxidation state of the bioreactor mixed liquor, depended on two factors: (1) the bioreactor loading rate, i.e. the amount of $Fe(III)EDTA^-$ as well as NO that enters the bioreactor via the scrubber, and (2) the regeneration capacity of the bioreactor, i.e. the volumetric $Fe(III)EDTA^-$ and NO reduction rate. Figure 3 shows the relation between the NO and O_2 content of the flue gas and the calculated bioreactor load based on mass balances. During the continuous operation with 1.5 $m^3.h^{-1}$ flue gas containing 3.5-3.9 % O_2 and 155 ppm NO, the NO and O_2 removal from the gas phase amounted to, respectively, 65 and 405 $mmol.d^{-1}$. The NO removal corresponds to an electron acceptor flow of 0.043 $meq.l^{-1}.d^{-1}$, whereas the O_2 removal corresponds to a $Fe(III)EDTA^-$ loading rate of 11.3 $mM.h^{-1}$ or 0.27 $meq.l^{-1}.d^{-1}$, based on the stoichiometric 1:4 ratio for $Fe(II)EDTA^{2-}$ oxidation by oxygen (reaction 3). The volumetric ethanol consumption rate was fairly constant at 1.0 $mM.h^{-1}$.

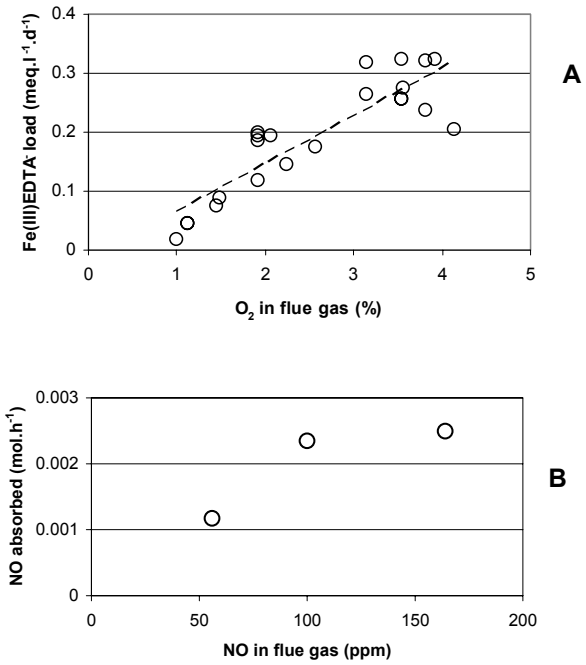

Figure 3. Relation between O_2 (A) and NO (B) concentration of flue gas and, respectively, the Fe(III)EDTA$^-$ and NO loading rate of the bioreactor.

3.3 Effect of sulfide additions on the Fe(III)EDTA$^-$ reduction capacity

In order to maintain the redox potential below -150 mV versus Ag/AgCl, i.e. to avoid Fe(III)EDTA$^-$ overloading, the Fe(III)EDTA$^-$ load of the bioreactor was controlled by the air supply, e.g. the O_2 concentration in the flue gas. The ethanol concentration in the bioreactor mixed liquor was kept above 5 mM, i.e. the electron donor was available in excess. Figure 4 shows that increasing the Fe(III)EDTA$^-$ load from 0.12 to 0.27 meq.l^{-1}.d^{-1} at day 108 resulted in an elevation of the redox potential from -480 to -120 mV vs Ag/AgCl, i.e. an increase of the Fe(III)EDTA$^-$ concentration, but the redox potential subsequently dropped to -480 mV within 1 day.

Repetition of this load increase did, however, not always lead to such a successful recovery of a low redox potential. Increase of the Fe(III)EDTA$^-$ load from 11 to 16 mM.h^{-1}, e.g. on day 110 resulted in an ORP elevation to above -80 mV (Figure 4), indicating that the Fe(III)EDTA$^-$ reduction capacity decreased in time. Figure 4 shows that a supply of 2 mM sulfide addition on day 111 induced an elevated Fe(III)EDTA$^-$ reduction capacity, i.e. the redox potential dropped rapidly to –400 mV (pH 7.2±0.2). These low redox potentials (below -200 mV) were maintained for approximately 4 days, after which the redox potential increased again (results not shown), indicating that the sulfide additions were only effective for limited periods of time.

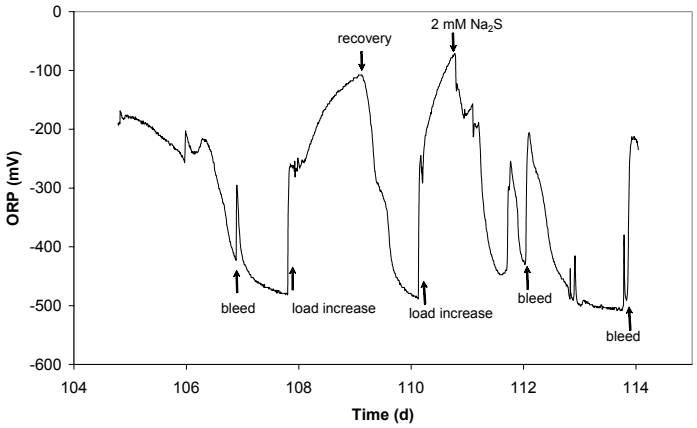

Figure 4. Effect of Fe(III)EDTA$^-$ load increase (day 108 and 110) and sulfide addition (day 111) on the redox potential (ORP) of the bioreactor mixed liquor (pH 7.2±0.2). The Fe(III)EDTA$^-$ load was increased due to elevation of the O_2 concentration of the flue gas.

The volumetric Fe(III)EDTA$^-$ reduction rate in the reactor mixed liquor at day 116, i.e. when the total FeEDTA concentration was 15 mM and 5 days after the final sulfide shot, amounted 3.3 mM.h^{-1}, corresponding to a specific reduction rate of 0.06 mmol.mg prot.$^{-1}$.h^{-1}. Batch experiments showed, however, that the specific Fe(III)EDTA$^-$ reduction rate depended on the biomass (protein) concentration in case a fixed amount of sulfide (0.5 mM) was added to the assays (Figure 5A). The specific Fe(III)EDTA$^-$ reduction rate is linearly related to the amount of sulfide added per gram of protein (Figure 5B).

3.4 Effect of bio-augmentation on NO reduction capacity

Table 1 shows the volumetric NO reduction rate observed at various protein concentrations. From these data, a specific NO reduction rate of 1.2 µmol. $mg_{prot}^{-1}.h^{-1}$ can be extracted. This means that, with a NO flue gas concentration of 155 ppm, i.e. an NO loading rate of 0.45 mM.h^{-1}, complete Fe(II)EDTA^{2-} regeneration from Fe(II)EDTA-NO^{2-} was only possible when the reactor mixed liquor had a sufficiently high denitrification capacity, i.e. when the reactor mixed liquor contained protein concentrations higher than 350 mg/l. Bio-augmentation of the reactor with denitrifying Veendam sludge on day 98 to a biomass (protein) concentration higher than 350 mg/l (Figure 6A) indeed induced an ORP drop of the reactor mixed liquor to below -200 mV (results not shown), indicating that the total NO load of the bioreactor was reduced.

Table 1. Volumetric NO reduction rates of the BioDeNOx reactor mixed liquor at various protein concentrations.

Protein concentration (mg.l^{-1})	NO reduction rate (mM.h^{-1})
100	0.14
180	0.25
540	0.64

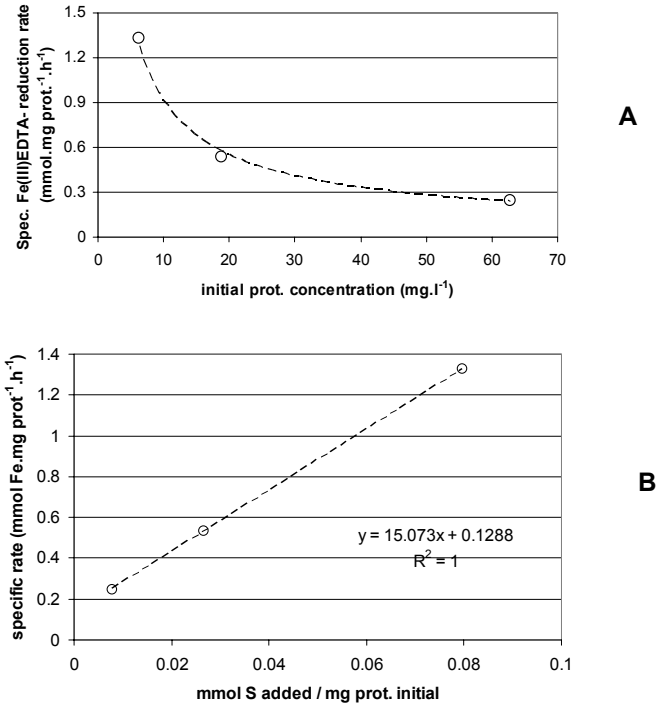

Figure 5. Effect of the protein concentration (B) and the relative amount of sulfide added (B) on the specific Fe(III)EDTA$^-$ reduction rate.

3.5 Biomass growth and yield

Figure 6A shows that the protein concentration of the BioDeNOx reactor amounted circa 180 mg.l^{-1} during the continuous operation period (starting at day 80), in which the reactor was operated at an HRT of 20 days. High protein concentrations were detected only after bio-augmentation with methanogenic Eerbeek sludge (day 80) and denitrifying Veendam sludge (day 98), but these concentrations stabilized again around the average value of 150 mg.l^{-1} within 2 weeks (Figure 6A). When the data of Figure 6A are corrected for dilution due to medium supply to create a bleed stream, the protein concentration follows an exponential curve with time (Figure 6B), from which an observed growth rate (μ_{obs}) of 0.043 d^{-1} can be estimated. The observed biomass yield (Y_{obs}) amounted only 0.009 mg protein per mg ethanol, based on the average protein increase (45 mg.d^{-1}) and the average ethanol consumption rate (0.8 mM.h^{-1}) during the continuous operation.

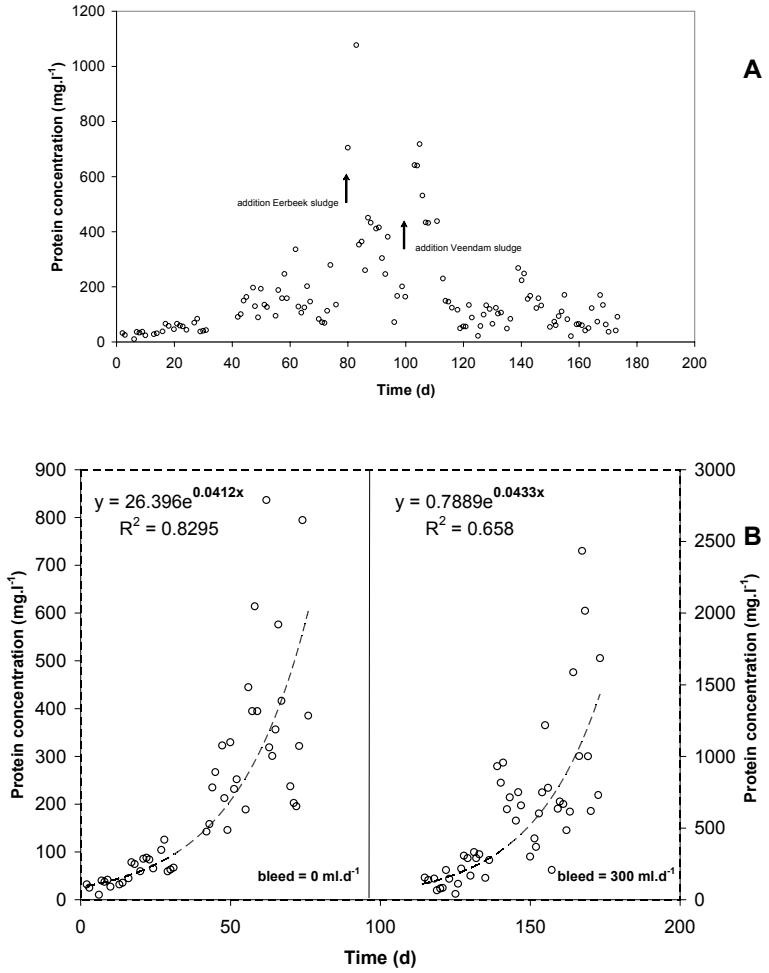

Figure 6. Protein concentration of the bioreactor mixed liquor (A) and accumulation of the protein concentration during the start up period (no bleed stream applied) and during the continuous operation period (measured concentration corrected for the bleed stream) (B).

3.6 EDTA degradation

Figure 7A shows the course of the EDTA concentration of the bioreactor mixed liquor during the continuous operation with 1.5 $m^3.h^{-1}$ flue gas containing 3.5-3.9 % O_2 and 55-155 ppm NO. When corrected for the EDTA that was supplied by daily addition of fresh medium, a degradation rate of 2.1 (± 0.1) $mM.d^{-1}$ was observed. Taking the O_2 load of 0.27 $meq.l^{-1}.d^{-1}$ into account (Figure 3), the relative EDTA degradation amounted 0.9 % (mol EDTA degraded per mol $Fe(II)EDTA^{2-}$ oxidized). No EDTA degradation was observed in anaerobic batch incubations of the reactor liquor in the dark, both at 20 and 55 ºC, during 35 days (Figure 7B).

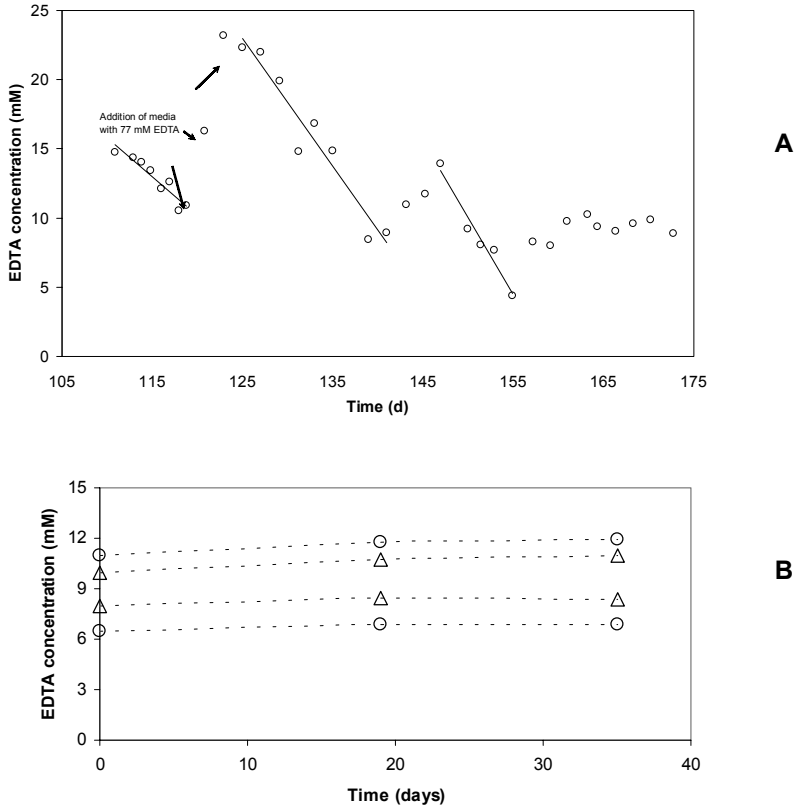

Figure 7. EDTA concentration of the bioreactor mixed liquor during continuous operation with artificial flue gas containing 3.5-3.9 % O2 and 55-155 ppm NO (A) and during anaerobic batch incubations at 20 ºC (△) and 55 ºC (○).

4 DISCUSSION

4.1 NO removal from the gas phase

The present study shows that the redox potential of the bioreactor mixed liquor needs to be maintained below -150 mV versus Ag/AgCl (pH 7.2 ± 0.2) for achieving the maximal NO removal efficiency from the flue gas under the conditions tested (155 ppm NO, 3.5-3.9 % O_2) (Figure 2). This can be explained by the relation between the redox potential and the Fe(II)EDTA^{2-} concentration, which is described by the Nernst equation (Kolthoff and Auerbach, 1952; Van der Maas *et al.,* 2005c). High Fe(II)EDTA^{2-} concentrations result in fast NO absorption into aqueous Fe(II)EDTA^{2-} solutions (reaction 1), since the kinetics are first order for both NO and Fe(II)EDTA^{2-} (Demmink *et al.*, 1997). A sufficiently fast NO absorption obviously is crucial for the economic feasibility of the BioDeNOx concept, since it directly determines the gas residence time (GRT) of the scrubber needed for a given application. In case of a fixed Fe(II)EDTA^{2-} and NO concentration, the NOx removal from the gas phase is limited by the scrubber configuration, i.e. contact between scrubber liquor and flue gas. In this study, the gas

residence time in the wet scrubber volume amounted to only 1.2 seconds, which gave a NO removal efficiency of 40 % at an $Fe(II)EDTA^{2-}$ and influent NO concentration of 10-15 mM and 155 ppm, respectively. Earlier studies with the same bioreactor set-up gave 80 % removal at a GRT of 11 seconds (Van der Maas *et a*l., 2005b), which is still low compared to the empty bed retention times (EBRT) of 1-2 minutes needed for more conventional biological NO removal techniques which do not utilize $Fe(II)EDTA^{2-}$ (Jin *et al.,* 2005).

4.2 Regeneration of the scrubber liquor – Fe(III)EDTA⁻ reduction

A really stable operation of BioDeNOx reactors can only be achieved in case the regeneration capacity of the bioreactor meets at least its $Fe(III)EDTA^-$ and $Fe(II)EDTA\text{-}NO^{2-}$ load. In this study, the theoretical $Fe(III)EDTA^-$ load was circa 11 $mM.h^{-1}$ or 0.27 $meq.l^{-1}.d^{-1}$ at a flue gas O_2 concentration of 3.5-3.9 % (Figure 3), based on the mass balance for O_2 and a stoichiometric ratio of 1:4 for the reaction between O_2 and $Fe(II)EDTA^{2-}$ (reaction 3). The volumetric $Fe(III)EDTA^-$ reduction rate was sometimes lower, resulting in elevated redox potentials (> -150 mV versus Ag/AgCl) of the bioreactor mixed liquor (Figure 4) and, subsequently, unstable operation.

The biological reduction of EDTA chelated Fe(III) was found to be catalyzed by an electron mediating compound (EM), presumably polysulfide, which is generated upon the injection of sulfide into an aqueous $Fe(III)EDTA^-$ solution (Van der Maas *et al.,* 2005c). The linear dependence between the relative EM concentration (amount of EM available per bacterium) and the specific $Fe(III)EDTA^-$ reduction rate (Figure 5B) suggests that a sulfur compound is used as electron acceptor for microbial respiration and that the volumetric conversion rate is limited by the concentration of that electron acceptor. To achieve high volumetric $Fe(III)EDTA^-$ reduction rates it is, therefore, of great importance that this electron mediating compound is available in BioDeNOx reactors at sufficiently high concentrations. In this study, sulfide additions induced an elevated $Fe(III)EDTA^-$ reduction capacity only for limited periods of time (approximately 4 days). Ideally, oxidation and reduction of the electron mediating compound is a cyclic process in which the total electron mediator concentration (oxidized plus reduced) stays constant. Both low and high redox conditions will, however, result in unbalance of the EM cycling, and thus in unstable BioDeNOx reactor operation.

At low redox potentials below -250 mV versus Ag/AgCl, i.e. when all $Fe(III)EDTA^-$ is reduced, and in the presence of ethanol, the polysulfide will be completely reduced to sulfide (reaction 6). The latter compound can precipitate with Fe^{2+}, which is the predominant valence of iron at these low redox potentials (Fe is present in excess over EDTA). In this way, the polysulfides are ultimately transformed to insoluble FeS according to reaction 7, meaning that the electron mediator has changed to a non or slowly reactive sulfur species.

$$S_n^{2-} + 2\ e^- + H^+ \rightarrow S_{n-1}^{2-} + HS^- \quad (6)$$

$$2\ Fe^{2+} + 2\ HS^- \rightarrow 2\ FeS\ (s) \quad (7)$$

High redox reactor conditions, i.e. low $Fe(II)EDTA^{2-}$ concentrations may also threaten the presence of electron mediating polysulfides. In that case, the scrubber liquor has a relatively low capacity for oxygen scavenging and thus, soluble oxygen may be present in the scrubber liquor. This might induce oxidation of polysulfides to sulfur and thiosulfate according to reactions 8 and 9, which lowers the concentration of the reactive, catalytic sulfur species.

$S_n^{2-} + ½\ O_2 + 2\ H^+ \rightarrow n\ S^0$ (8)

$2\ HS^- + 2\ O_2 \rightarrow\ S_2O_3^{2-} + H_2O$ (9)

4.3 Regeneration of the scrubber liquor – NO reduction

Besides Fe(III)EDTA$^-$ reduction, biological reduction of Fe(II)EDTA^{2-} complexed NO is a key reaction within the Fe(II)EDTA^{2-} regeneration. Batch studies showed that NO reduction is coupled to Fe(II)EDTA^{2-} oxidation, i.e., except absorbent, Fe(II)EDTA^{2-} is also an electron donor in BioDeNOx reactors (Van der Maas *et al*., 2005a). The volumetric NO reduction rate of the bioreactor liquor depended linearly on the biomass (protein) concentration (Table 1). This explains why stable operation of the BioDeNOx reactor at higher NO loadings (> 0.3 mM/h) was only possible after bio-augmentation with denitrifying biomass (at day 98). It is assumable that the biomass (protein) concentration is directly related to the concentration of Nitric Oxide Reductase, the key enzyme in bacterial NO reduction (Wasser et al., 2002). The observed specific NO reduction rate of 1.2 µmol NO.mg_{prot}^{-1}.h^{-1}. (0.34 nmol NO.mg_{prot}^{-1}.s^{-1}) is based on the total protein concentration of the bioreactor mixed liquor. It may be assumed that only part of the biomass contributes to NO reduction, since that conversion covers an electron flow of only 1.8 mmol e^-.l^{-1}.h^{-1} (14 %), against 11 mmol e^-.l^{-1}.h^{-1} (86 %) for Fe(III)EDTA$^-$ reduction. Also with respect to NO reduction, sulfidogenic reactor conditions, i.e. low redox potentials (<-250 mV versus Ag/AgCl) and thus the absence of Fe(III)EDTA$^-$, should be avoided, since already very low sulfide concentrations (0.1 µM) inhibit the second step of NO reduction, i.e. N_2O reduction to N_2 (Manconi *et al*., 2005; Sörensen *et al.,* 1980).

4.4 Biomass growth rate and yield

The assessed values for the growth rate (μ_{obs}) and biomass yield (Y_{obs}), respectively, 0.043 d^{-1} and 0.009 mg protein per mg ethanol are very low. The low net growth rate is presumably due to limitation of the substrates that are used as electron acceptor: NO (aq) and an oxidized electron mediating compound (EM), presumably polysulfides (for Fe(III)EDTA$^-$ reduction). Batch experiments indicated that the K_m value for NO is below 10 nM (Van der Maas *et al*., 2005a), meaning that the specific NO reduction rate is substrate limited at that NO concentration range. It is not clear, however, whether NO reduction is directly coupled to microbial growth, since NO reduction in aqueous Fe(II)EDTA^{2-} solutions might not generate a proton motive force (PMF) (Van der Maas *et al.,* 2005a). Possibly, only Fe(III)EDTA$^-$ serves as electron acceptor for the proton translocating enzyme systems (Rosen *et al*., 1981) which support the growth of the denitrifying biomass.

Fe(III)EDTA$^-$ reduction is already substrate limited at low EM concentrations, formed by the supply of 0.08 mM Na_2S per mg protein (Figure 5B). This means that in a bioreactor with a biomass (protein) concentration of 180 mg/l (Figure 6A), maximal volumetric Fe(III)EDTA$^-$ reduction rates would be achieved at an EM concentration generated by a sulfide injection of at least 14 mM. In this study, the bioreactor was supplemented four times with 2 mM of sulfide to generate polysulfide according to reaction 5. However, that the EM (polysulfides) concentration most likely depleted in time either due to FeS precipitation or due to oxidation to sulfur and thiosulfate (see above). Therefore, it may be expected that also the growth of the microbial population responsible for Fe(III)EDTA$^-$ reduction was limited due to the low substrate concentrations.

The low biomass yield found in the present study correspond well with observations during BioDeNOx pilot studies at full scale applications (Biostar, personal communication). Electron balances of previous batch experiments (Van der Maas *et al.,* 2005d) also indicated a low biomass yield during Fe(III)EDTA$^-$ reduction by the methanogenic Eerbeek sludge, i.e. the inoculum used to start up the bioreactor in this study.

4.5 EDTA degradation

The observation that EDTA degradation (± 2 mM.d^{-1}) only occurred during the continuous operation with flue gas containing 3.5-3.9 % O_2 (Figure 7A), in contrast to the anaerobic batch incubations (Figure 7B), suggests that EDTA degradation is induced by the chemical oxidation of Fe(II)EDTA^{2-} by oxygen during gas scrubbing. Batch research indeed showed chemical EDTA degradation during the oxidation of Fe(II)EDTA^{2-} (Gambardella, 2005).

Although the (reaction) mechanism of Fe(II)EDTA^{2-} oxidation and EDTA degradation is beyond the scope of this study, it should be noted that radical formation may occur during flue gas scrubbing via the well known Fe(II)EDTA^{2-} catalysed Haber-Weiss reaction (Buettner *et al.,* 1983; Sutton, 1995; Welch *et al.,* 2002):

$$2\ Fe(II)EDTA^{2-} + 2\ O_2 \rightarrow 2\ Fe(III)EDTA- + 2\ O_2^{\cdot -} \quad (9)$$

$$2\ O_2^{\cdot -} + 2\ H^+ \rightarrow H_2O_2 + O_2 \quad (10)$$

$$H_2O_2 + Fe(II)EDTA^{2-} \rightarrow Fe(III)EDTA- + OH^- + HO^{\cdot} \quad (11)$$

$$3\ Fe(II)EDTA^{2-} + O_2 + 2H^+ \rightarrow 3\ Fe(III)EDTA^- + OH^- + OH^{\cdot} \quad (12)$$

Reaction 12 shows a Fe(II)/O_2 ratio of 3 instead of 4 (reaction 3). The hydroxyl radical formed is very reactive and bactericidal (Wolcott *et al*., 1994) and may induce a-specific EDTA degradation (Mochidzuki and Takeuchi, 1999). Further research is needed to illuminate the occurrence and role of radicals in the BioDeNOx process, e.g. their contribution to EDTA degradation as well as to biological stress, i.e. their effect on biomass yield and growth / decay.

EDTA can also be consumed via biological EDTA oxidation, i.e. via aerobic respiration (Witschel and Egli, 2001; Van Ginkel et al., 1997). It is, however, unlikely that biological EDTA oxidation plays a major role when the BioDeNOx process is running at redox potentials below -150 mV, since no molecular oxygen is present at these low redox conditions. Based on the kinetics of Fe(II)EDTA^{2-} oxidation (reaction 3) (Wubs and Beenackers, 1993), O_2 (aq) can only be expected at the gas-liquid interface, i.e. in the scrubber and not in the bioreactor.

4.6 Optimization of BioDeNOx reactor operation and design

This study reveals that there exists a contradiction between the optimal redox state of the aqueous FeEDTA solution required for NO absorption and biological regeneration. The NO absorption, i.e. NO removal from the gas phase, requires a low redox potential (Figure 2), thus the Fe(III)EDTA$^-$ and Fe(II)EDTA-NO^{2-} concentration should as low as possible. On the other hand, the latter compounds are required in the bioreactor to maintain biological activity and biomass growth (at least to compensate decay), as well as to prevent depletion of the EM compound (polysulfides) and inhibition of NO reduction by S^{2-}. Therefore, some Fe(III)EDTA$^-$ (10-20 %) should always be present in the bioreactor mixed liquor, which corresponds with an ORP of between –180 mV and

–200 mV versus Ag/AgCl at pH 7.2 (±0.2). This can be achieved when the ethanol supply is properly controlled with the redox potential as steering parameter (Van der Maas *et al.,* 2005b). In that way, ethanol (electron donor) is the substrate that limits the biological activity instead of NO and/or Fe(III)EDTA$^-$ (electron acceptors).
The availability of polysulfide as electron mediating compound during Fe(III)EDTA$^-$ reduction seems to be crucial for the stable operation of BioDeNOx reactors. Next to implementation of a proper ethanol supply, it is therefore also advisable to dose minor amounts sulfide or elemental sulfur to the reactor continuously. The amount required depends on the bioreactor Fe(III)EDTA$^-$ load and should be monitored carefully, e.g. with a pS electrode, to prevent overdosing.
With respect to the bioreactor design, well mixed systems as completely stirred tank reactors (CSTR) and gaslift reactors are preferable over plug-flow like systems, e.g. UASB reactors, because of the superior properties for substrate control. By intensive stirring, complete anaerobic zones, e.g. absence of Fe(III)EDTA$^-$, can be avoided. Another advantage of a CSTR over a plug-flow system is an equal distribution of the aqueous NO, which prevents the existence of local zones completely depleted of NO, i.e. substrate for the denitrifying biomass.
The scrubber design should be focused on minimization of Fe(II)EDTA^{2-} oxidation by oxygen, since that reaction causes a high Fe(III)EDTA$^-$ load and, therefore, an inefficient ethanol consumption (in this study only 14% of the ethanol consumption was linked to NO reduction). Furthermore, minimization of Fe(II)EDTA^{2-} oxidation may lead to less radical formation (via Haber-Weiss) and therefore to lower EDTA degradation rates.

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