Bioengineering

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Graphical Abstract
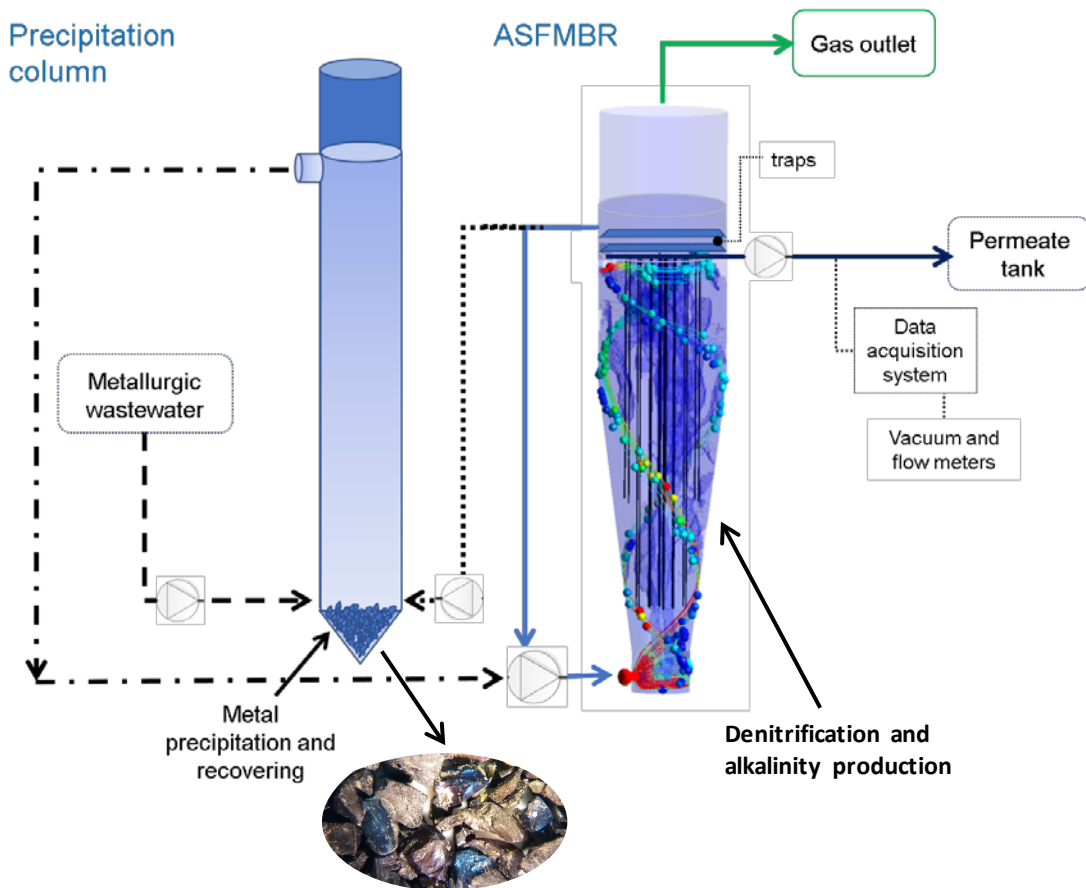
Bio-recovery of metals from a stainless-steel industrial effluent through denitrification performed in a novel anaerobic swirling fluidized membrane bioreactor (ASFMBR)

J. Ernesto Ramírez[1], Saúl Esquivel-González[1], J. René Rangel-Mendez[1], Sonia L. Arriaga[1], Marisol Gallegos-García[2], Germán Buitrón[3], Francisco J. Cervantes[3] *

[1]IPICYT/División de Ciencias Ambientales, Camino a la Presa San José 2055, Col. Lomas 4ª. Sección. San Luis Potosí, 78216 Mexico

[2]Facultad de Ingeniería, Universidad Autónoma de San Luis Potosí. Av. Dr. Manuel Nava No. 8, San Luis Potosí, SLP, 78290, Mexico

[3]Laboratory for Research on Advanced Processes for Water Treatment, Engineering Institute, Campus Juriquilla, Universidad Nacional Autónoma de México (UNAM), Blvd. Juriquilla 3001, 76230 Querétaro, Mexico

*Corresponding author. E-mail: FCervantesC@iingen.unam.mx

ASTRACT

A novel technology was developed for the treatment of a stainless-steal wastewater, which contains high nitrate concentration (6.8 g $N-NO_3^-$/L), acidic pH (pH=3.3) and high concentration of metals. Denitrification was performed in an Anaerobic Swirling Fluidized Bed Membrane Bioreactor (ASFMBR) with synthetic and real wastewater. The treatment concept was also integrated by a precipitation column in which high recirculation of the alkalinity produced from denitrification was introduced to drive the precipitation and recovery of metals present in the

1

wastewater. The novel reactor configuration achieved high nitrate removal (>94 %) and the acidic pH was efficiently neutralized in the reactor, by recycling the alkalinity produced from denitrification. More than 40% of the metals present in the industrial wastewater was recovered. Membrane fouling was avoided by the hydrodynamic regime established in the ASFMBR. The treatment concept is promising to achieve efficient removal of nitrate and recovery of metals from metallurgic wastewater.

1. INTRODUCTION

Biological treatment of industrial wastewaters is challenging due to their complex, and sometimes extreme, physicochemical conditions, and because of the presence of toxic components, which negatively affect consortia in bioreactors.[1] Metallurgic effluents are particularly defiant because they are highly acidic and contain large concentrations of heavy metals and nitrate. For food and pharmaceutical industries, citric acid is used to remove a high amount of metals due to its chelating capacity. The discharge of this type of effluents, without a previous treatment, causes several environmental risks, such as eutrophication of water bodies,[2] and toxicity to aquatic organisms due to the presence of heavy metals.[3-4]

Denitrification is a cost-effective process widely applied to remove nitrate from wastewaters.[5] During denitrification, nitrate (NO_3^-) is sequentially converted to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen gas (N_2).[6] Heavy metals present in metallurgic effluents could contribute as trace elements to carry out denitrification since denitrifying microorganisms require cofactors, such as Fe, Cu and Mo.[7] However, high concentrations of heavy metals can

2

inhibit denitrification, resulting in the accumulation of NO_2^- and N_2O.[8-12] NO_2^- causes eutrophication of water bodies and triggers the formation of carcinogenic amines,[2] while N_2O is a potent greenhouse gas.[13] Likewise, denitrification could also be affected by the acidic pH of metallurgic effluents.[14]

A strategy to decrease the negative effects of metals to microorganisms is their precipitation and recovery from the metallurgic wastewater. In the last years, biotechnologies have been applied as metals recovery techniques with high efficiency at low cost.[15] The alkalinity produced in denitrifying processes could be recycled to achieve neutralization of acidic metallurgic effluents, which could also promote the precipitation of heavy metals present in this type of wastewaters. Nevertheless, several mechanisms involved in metals removal in bioreactors should be considered. These mechanisms include physical retention of heavy metals precipitated in sludge flocs; integration, adsorption and complexation of metallic ions with soluble microbial products (SMP); adsorption and diffusivity of metallic ions in biomass particles; as well as precipitation triggered by the alkalinity[16] or by coprecipitation with other biogenic solids.[17]

Citrate plays an important role in denitrification as an electron donor. At the same time, its complexing capacity modifies the bioavailability of both metals and citrate to microorganisms, affecting the rate of the process. However, citrate biodegradation releases complexed metals, which could in turn be precipitated with denitrification by-products (OH^-, CO_3^{2-}). Previous studies elucidated the mechanisms involved in denitrification with citrate as electron donor in the presence of three of the main metals present in effluents from the stainless-steel industry (Fe(III)/Fe(II), Cr(III), Ni(II)).[9] However, further studies are demanded to understand the mechanisms involved during the treatment of metallurgic effluents.

3

Anaerobic Fluidized Bed Reactors (FBR) with a denitrifying biofilm supported on granular activated carbon (GAC) have been used as a promising strategy to decrease the inhibitory effects of heavy metals by high dilution rates imposed inside of the reactors and by pH neutralization of the acidic feed with denitrification byproducts. Additionally, precipitation of heavy metals by products derived from denitrification has also been observed.[8,12] However, despite high nitrate removal and pH neutralization obtained, heavy metals precipitation remains low, yielding an effluent with high metals content.[18] Membrane bioreactors have been used to increase the removal of inorganic components in anaerobic membrane bioreactors (MBR),[19] to comply with environmental regulations. Heavy metals in MBR can be joined to sludge components and be rejected by the membranes.[20] However, heavy metals in MBR have shown negative effects on the biological process, as well as on membrane fouling.[16,21]

In the present work, the capacity of a novel Anaerobic Swirling Fluidized Membrane Bed Bioreactor (ASFMBR) to achieve denitrification of synthetic and real metallurgic wastewater was tested. A precipitation column was integrated to the ASFMBR in order to neutralize the metallurgic effluent and to promote metals recovery.

2. MATERIALS AND METHODS

2.1. Biofilm formation. A coconut-shell based granular biochar (GC) with a size between 0.4 and 2.38 mm was used as supporting material for the biofilm, which was formed by mixing clean GC and disintegrated anaerobic granular sludge previously acclimated under denitrifying conditions. The anaerobic granular sludge originated from a wastewater treatment plant treating effluents from a brewery (Cd. Obregón, Mexico). Biofilm formation was performed in two up-flow

4

anaerobic sludge blanket (UASB) reactors with a working volume of 1.1 L. Both reactors were fed

with 500 mg N-NO$_3^-$/L and were supplied with stoichiometric amounts of either ethanol or citrate

as electron donors. The columns were operated almost for three months until reaching steady

state conditions. The basal medium had the following composition (mg/L): KH$_2$PO$_4$, 300;

MgSO$_4$·7H$_2$O, 61.3; FeSO$_4$·7H$_2$O, 17.2; CaCl$_2$·2H$_2$O, 75 and 1 mL/L of trace elements.[9]

2.2. Operational conditions. The ASFMBR was operated both with synthetic and real metallurgic

wastewater. Synthetic wastewater contained the main metals present in the real wastewater

(Fe(II)/Fe(III), Cr(III), Ni(II)). These metals were tested in individual form and jointly using citric

acid as electron donor. The real wastewater originated from a stainless-steel company

(Querétaro, Mexico, Table 1). Citric acid and ethanol were tested as electron donors under the

conditions described in Table 2. The treatment concept was integrated by a precipitation column

(Fig. 1). The precipitation column was of 1.1 L, from which the flow was pumped to the ASFMBR

by a centrifugal pump through a tangential inlet located in the lower part of the conical section

(see Fig 1), which together with the geometry, creates a swirling fluidization inside the reactor.

The tangential inlet serves as diffuser of the scoring media. The recirculation flow rate was

adjusted to 3.8 L/min to expand the GC particles in the entire reactor covering the membranes.

A portion of 150 mL of GC from the column of biofilm formation was added to the ASFMBR, which

corresponds to 0.56 g of volatile suspended solids (VSS)/L. The microfiltration module with

porous size of 0.1 µm was submerged in the reactor and was composed of 18 hollow fiber

membranes of polyvinylidene fluoride (PVDF) with an external diameter of 1.2 mm. The outlet

(permeate) was equipped with pressure and flow sensors to monitor membrane fouling. Every

testing period was conducted as a new experiment, with a preliminary operational stage in the

5

absence of metals to reach denitrifying steady state conditions before every test. The hydraulic

retention time (HRT) during the treatment of real wastewater was adjusted to obtain a similar

nitrate load as compared to the experiments performed with synthetic wastewater. The

concentration of NO_3^-, NO_2^-, NH_4^+, metals in solution, as well as pH and redox potential (ORP)

were measured in the sampling points shown in Fig. 1. Additionally, total suspended solids (TSS),

and SMP in terms of carbohydrates (SMPc) and proteins (SMPp) were monitored in the reactor

and permeate line. At the upper part of the reactor, gas samples were taken for N_2O

measurements.

Table 1. Characterization of wastewater generated from a stainless-steel industry.*

	mg/L	Element	mg/L	Element	mg/L	Element	mg/L
$N-NO_3^-$	6864.8 ± 100.2	Fe	12505.7 ± 110.1	K	48.3 ± 3.0	Pb	13.5 ± 2.3
$N-NO_2^-$	48.15 ± 4.9	Cr	2912.3 ± 111.5	B	45.6 ± 8.8	Cu	12.6 ± 1.0
$N-NH_4^+$	93.4 ± 6.2	Ni	2211.5 ± 53.7	Co	37.5 ± 0.7	V	12.5 ± 0.4
COD	0.0	Sn	836.5 ± 14.2	Na	33.7 ± 2.0	Mg	5.4 ± 0.1
		Mn	579.5 ± 32.3	P	28.6 ± 1.6		
pH, pH units	3.3	Si	243.7 ± 5.4	Ca	26.5 ± 4.4		
ORP, mV	170.0	Mo	87.3 ± 3.7	Al	13.8 ± 0.6		

*Reported data represent average from triplicate measurements ± standard deviation.

6

Table 2. Experimental conditions for the coupled system precipitation column-ASFMBR.

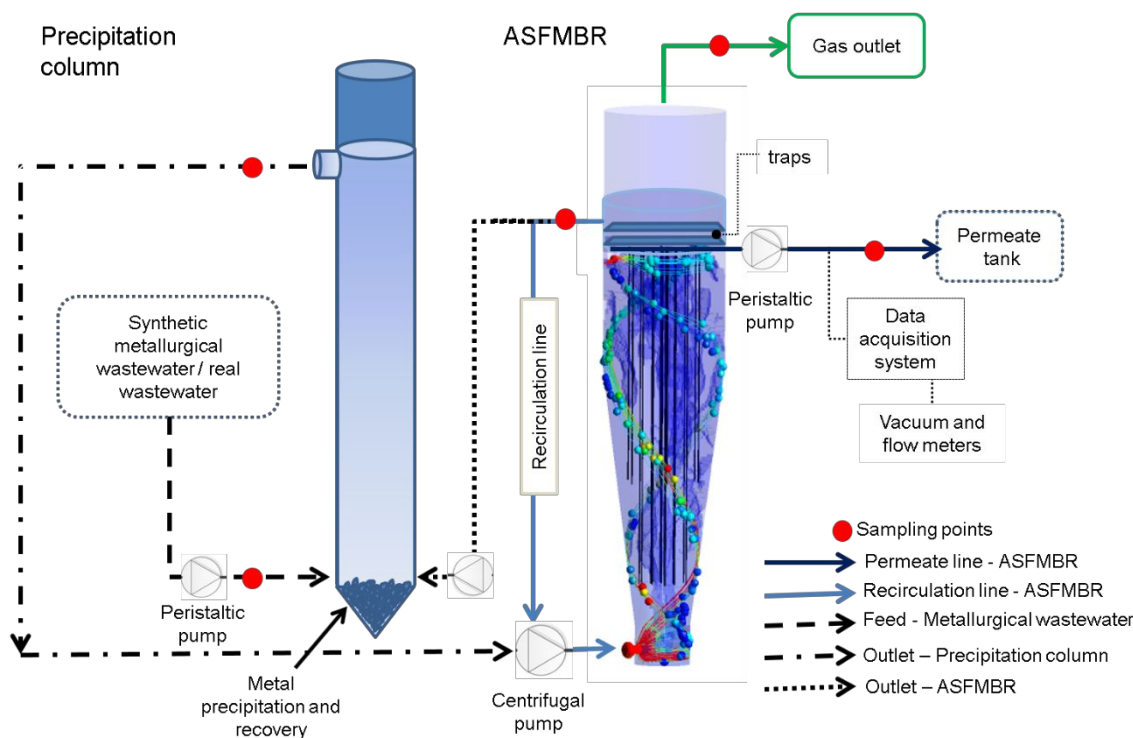| Test	Fe	Cr	Ni	Fe-Cr-Ni	Real effluent	Real effluent
electron donor, g C/g N	citrate, 2.26					ethanol, 1.43
$N-NO_3^-$, mg/d	793.7	756.6	762.6	765.6	854.5	849.2
$N-NO_2^-$, mg/d	0	0	0	0	4.1	3.1
$N-NH_4^+$, mg/d	0	0	0	0	33.1	54.9
Fe, mg/d	217.6	0	0	186.2	1755.8	
Cr, mg/d	0	122.5	0	126.9	387.2	
Ni, mg/d	0	0	45.1	44.2	299.0	
pH, pH units	2.02	2.10	2.27	2.04	3.16	2.21
HRT, h - precipitation column	8.3				91.7	
HRT, h – ASFMBR	26				287.8	
Membrane flux, $L/m^2/h$	3.4				0.31	

7

Figure 1. Experimental set-up of the coupled system precipitation column-ASFMBR.

2.3. Analysis. NO_3^-, NO_2^-, NH_4^+, TSS and VSS were measured according to APHA methods.[22] N_2O

and citrate were measured as described before.[9] VSS measurement on GC particles was

conducted by detachment of the biofilm of 1 g of GC in 10 mL of distilled water by ultra-sound

treatment for 30 min. The soluble concentration of proteins and carbohydrates were determined

by the colorimetric methods of Bradford[23] and Dubois,[24] respectively. At the end of the

experimentation, samples of GC circulating from the conical and cylindrical sections of the

reactor were taken to measure the amount of adsorbed metals. A section of 1 cm of height of

the membrane was cut at different heights of the reactor to determine metal deposition. Metal

extraction from GC particles and membranes was accomplished by organic matter oxidation with

H_2O_2 and acidic digestion. Samples for heavy metals measurements were filtered through 0.45 μm and analyzed by ICP-OES. Ferrous iron was determined by the ferrozine method.[25] Minerals recovered in the precipitation column and in the ASFMBR were analyzed by XRD.

3. RESULTS AND DISCUSSION

3.1. Performance of the ASFMBR. Denitrification was initially evaluated in the ASFMBR with citrate as electron donor. The effects of the three main heavy metals present in metallurgic wastewater on denitrification were studied. Denitrification with citrate proceeds as follows:

$$C_6H_5O_7^{3-} + 3.6NO_3^- + 0.6H^+ + 0.2H_2O \rightarrow 6HCO_3^- + 1.8N_2 \ (4)$$
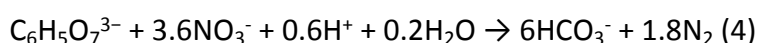
$$\Delta G° = -1881.0 \ kJ \ mol^{-1}$$

Effects of Fe(III)/Fe(II). Iron addition to the ASFMBR resulted in an increase on nitrate and citrate removal efficiencies (Fig. 2). This stimulating effect to the denitrification process was also observed in previous reports.[8] According to the operational conditions prevailing in the ASFMBR, the main iron-citrate species present were [Fe(II)-citrate]⁻ and [Fe(III)-citrate]. Previous experiments[9] showed that [Fe(II)-citrate]⁻ is a very stable complex inhibiting the uptake of citrate for nitrate reduction. The structural characteristics of the metal-citrate complex has a large influence on its biodegradability. Ferric iron has been reported to form a bidentate citrate complex, which is more easily degraded than the tridentate ferrous iron-citrate complex.[26] Thus, degradation of the ferrous iron-citrate complex depends on its oxidation and hydrolysis to form the ferric iron-citrate complex. During the operation of the ASFMBR, the Fe(II) concentration supplied from the precipitation column was removed in the reactor so that its concentration remained below 3 mg/L in the outlet (Fig. 3). This could be due to the precipitation of minerals

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containing Fe(II), or by Fe(II) oxidation linked to denitrification, with a subsequent precipitation

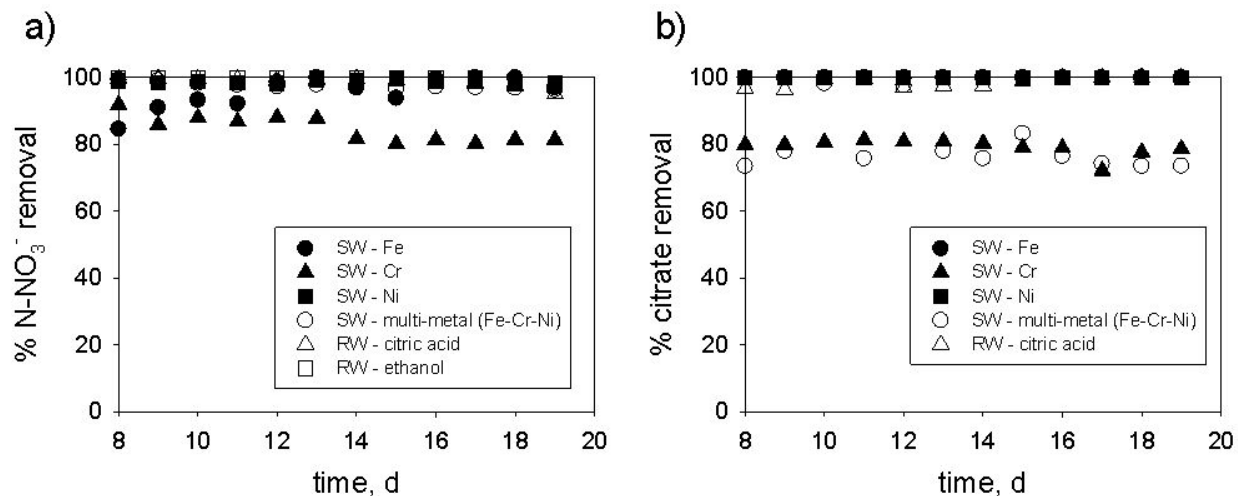of solids including both Fe(II) and Fe(III).

Figure 2. (a) Nitrate and (b) citrate removal during the treatment of synthetic and real

wastewater. SW and RW mean synthetic and real wastewater, respectively.

Autotrophic denitrification can be improved by ferrous iron oxidation.[27] In the present work,

Fe(II) could have improved nitrate removal, promoting the precipitation of produced Fe(III).

Moreover, precipitation of Fe(II) also occurred as $FeCO_3$ in the traps of the reactor, which was

evidenced by XRD analysis (see Fig. S1 in Supplementary material (SM)). Moreover, solid species

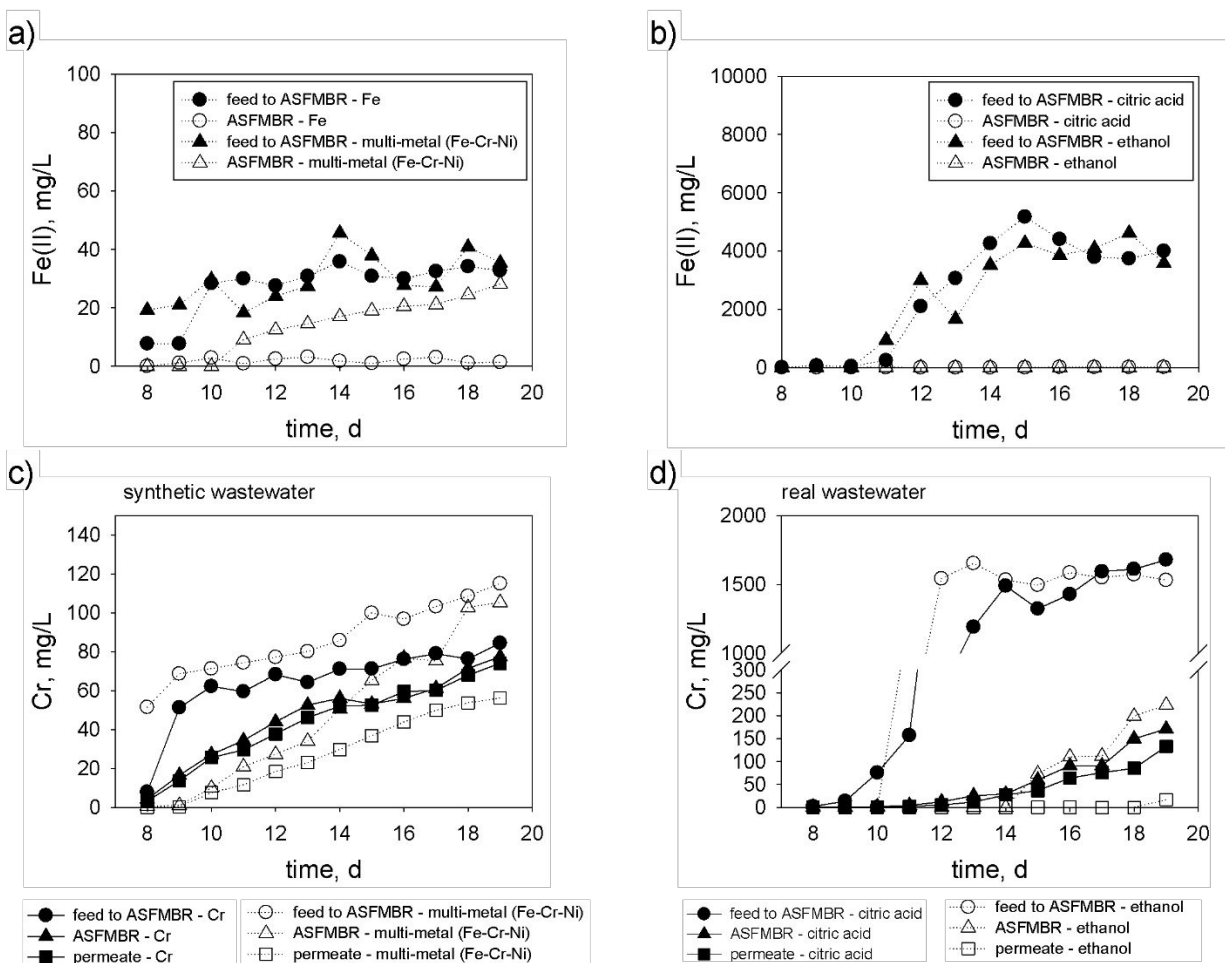containing Fe(II) can also serve as electron donors[28] contributing to denitrification.[27]

10

Figure 3. Fe(II) and Cr concentrations during the treatment of (a,c) synthetic and (b,d) real wastewater in the ASFMBR.

NO_2^- accumulation decreased from 5.2 ± 0.4 to 1.4 ± 0.3 mg $N\text{-}NO_2^-$/d with iron addition. Moreover, N_2O was produced at a lower concentration of 20.2 ± 2.6 mg $N\text{-}N_2O$/d (only 2 days) as compared to the reactor performance in the absence of iron. Accumulated NO_2^- and N_2O represent less than 2% of the nitrogen feed. On the other hand, biogas emission decreased with iron addition (data not shown). This could be explained by the formation of insoluble carbonates that decreased CO_2

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emissions from the ASFMBR. Decrease on gas emissions was also observed in experiments with

real wastewater (see below).

Effects of Cr(III). Cr(III) addition to the ASFMBR yielded mild inhibition to denitrification. The

amount of nitrate and citrate remaining in the treated effluent was ~20% with respect to the

added concentrations (Fig. 2). Similar results were obtained in batch experiments, in which the

inhibition on nitrate removal was attributed to the stable Cr(III)-citrate complexes formed.[9] This

stability hinders biodegradation of citrate while linked to Cr(III) (Fig. 3). However, a recent study

showed the difficulty to precipitate the complex Cr(III)-citrate under alkaline conditions,[29] which

was possibly associated with a very stable molecular structure formed, which prevailed

solubilized even under alkaline conditions. Hydroxides could be incorporated into the structure

of Cr(III)-bearing complexes and form polymers thereof. The formation of these complexes could

affect Cr(III) recovery and also the bioavailability of citrate for denitrification. Certainly, even

though citrate remains solubilized, microorganisms could not take it, affecting denitrification.

Nitrite reduction was not affected by Cr(III), while N_2O was produced just during two operational

days at low levels. Thus, nitrate reduction was the only denitrifying step affected by Cr(III), which

is in agreement with results obtained in batch assays.[9]

Effects of Ni(II). Nitrate removal efficiency was not affected by Ni(II) addition. These findings

contrast with those obtained in batch experiments,[9] which showed inhibition of denitrification

and accumulation of intermediates (NO_2^- and N_2O). Very low NO_2^- concentration and absence of

N_2O were observed in the ASFMBR outlet during the operation with Ni(II). Further studies report

negligible accumulation of nitrate and nitrite by the addition of Ni (5.5 mg Ni/L) to a denitrifying

FBR.[12] However, additional works revealed inhibition in nitrate and nitrite removal at a

12

concentration of 60 mg Ni/L,[18] although no accumulation of denitrification intermediates was

observed at a high Ni concentration (500 mg/L). This suggests that the hydrodynamic conditions

prevailing in the FBR and the low HRT imposed (low contact time of Ni(II) with microorganisms),

together with the insoluble Ni(II) species formed, could have alleviated the inhibitory effects of

Ni(II). In the present work, the ASFMBR was operated at a high HRT (26 h) without an apparent

inhibition of the denitrification process. In fact, high Ni(II) precipitation was observed during the

operation of the ASFMBR in the presence of this metal (Fig. 4), which could have prevented the

inhibitory effects of Ni(II) to denitrifying microorganisms. Likewise, Ni(II) toxicity could have been

mitigated by the formation of Ni complexes with dissolved organic matter. The production of

SMPp increased with Ni addition after several days of operation (Fig. S2 in SM). This result could

be due to cellular damage and release of proteins[30] as a mechanism of cellular protection.[31] The

release of SMP has been reported under anaerobic conditions and these compounds showed the

capacity to form stable complexes with Ni.[32] The formation of these complexes could explain the

absent of negative effects by Ni in the ASFMBR. Moreover, the hydrodynamic conditions

established in the reactor by swirling fluidization could also have played an important role on
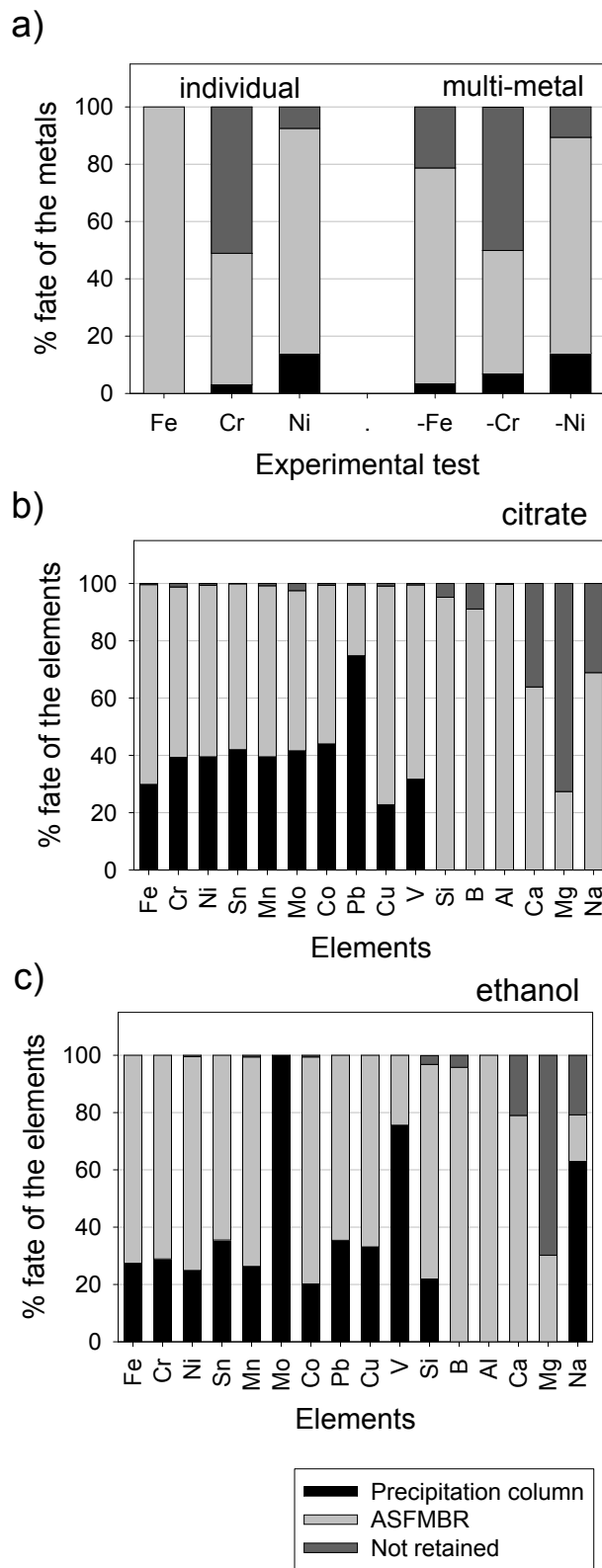
mitigating the effects of metals.

13

Figure 4. Fate of metals during the treatment of synthetic (a) and real metallurgic (b-c) wastewater. "Not retained" refers to elements not removed in the system and discharged with the permeate.

14

Joined effects of Fe, Cr and Ni. Addition of the three studied heavy metals altogether to the ASFMBR resulted in some differences with respect to the individual tests. During these experiments, iron precipitated to a lower extent than the experiments exploring its individual addition. Meanwhile, Cr(III) and Ni(II) showed partial and high precipitation, respectively, during the joined addition assays. The amount of Fe(II)/Fe(III) and Cr(III) remaining in solution (Fig. 4) limited citrate bioavailability (Fig. 2). Nevertheless, nitrate removal efficiency was maintained (Fig. 2), suggesting that the negative impact of Cr(III) was counteracted by the positive effects driven by Fe and Ni addition. Autotrophic denitrification promoted by Fe(II) could have also contributed to the denitrification performance observed. Unlike experiments conducted with Fe alone, the concentration of Fe(II) decreased to a lower extent in experiments performed with the three metals. In fact, Fe(II) was almost completely removed in the individual test (Fig. 3). This difference could be due to inhibition of autotrophic denitrification caused by the presence of Cr(III) and Ni(II), which prevented Fe(II) oxidation with a subsequent precipitation. Kiskira et al.[33] reported certain effect of Ni on Fe(II)-driven autotrophic denitrification. However, further research is necessary to clarify the effect of Cr(III) on this process. Similar to experiments conducted with individual addition of Fe, it was observed precipitation of solids containing Fe(II) during the operation of the reactor with all metals evaluated. Certainly, minerals containing both Fe(II) and Fe(III) and recovered from the treatment system include $FeCO_3$, Fe_3O_4, hematite and ferrihydrite (Fig. S3 in SM). Production of ferrihydrite in autotrophic denitrification has been observed.[27] Thus, Fe(II) oxidation could have contributed to sustain high nitrate removal efficiency, even when citrate was less bioavailable due to prevalence of metal-complexes, mainly including Fe(II) (Fig. 3) and Cr(III) (Fig. 3).

15

Accumulation of intermediates (NO_2^- and N_2O) was lower than that observed in individual tests. Similar results were observed in batch assays previously conducted.[9] In these tests, iron addition stimulated the denitrification process alleviating the inhibitory effects of Ni(II) and Cr(III). However, in batch assays, N_2O was only detected in incubations with Ni(II), while it was produced in the Fe and Cr tests in the present study. This discrepancy could be due to the hydrodynamic conditions prevailing in the ASFMBR.

3.2. Performance of the ASFMBR treating wastewater from a stainless-steel industry. Denitrification of wastewater originated from a stainless-steel industry was evaluated in the ASFMBR with both citrate and ethanol as electron donors. Denitrification with ethanol proceeds as follows:

$$C_2H_5OH + 2.4NO_3^- \rightarrow 2HCO_3^- + 1.2N_2 + 0.4OH^- + 1.8H_2O \qquad (5)$$
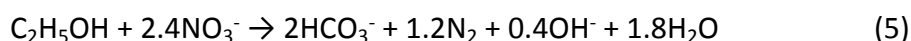
$$\Delta G° = -1230.7 \text{ kJ mol}^{-1}$$

High nitrate removal was achieved in the ASFMBR with both electron donors (Fig. 2(a)). Considering the high iron concentration present (Table 1), Fe(II)-driven denitrification could have contributed to nitrogen removal, as observed with synthetic wastewater. In fact, Fe(II) concentration fed to the ASFMBR reached ~4 g/L, which drastically decreased to values lower than 20 and 11 mg/L for the tests with citrate and ethanol, respectively (Fig. 3). During the experiments with citrate, it was difficult to detect Fe-bearing minerals by XRD analysis. However, for experiments conducted with ethanol, the main solids recovered include akaganeite β-FeOOH, Fe_3O_4 and $Fe(OH)_2$ (Fig. S4 in SM). Akaganeite is a product of Fe(III) precipitation, while magnetite could be formed via Fe(II) oxidation.[34]

NO_2^- was produced in a range of 12.4 to 37.4 mg/d with citrate, while experiments performed with ethanol did not show accumulation of this intermediate. Furthermore, N_2O was not detected during the treatment of the industrial effluent regardless the type of substrate supplied. Ethanol was a better electron donor than citrate to achieve denitrification of the stainless-steel industry wastewater, while a higher denitrification efficiency was obtained with citrate in previous batch assays.[9] This discrepancy could be due to the hydrodynamic conditions established in the ASFMBR, but further research must be conducted to clarify these issues. Ammonium was detected in very low concentrations in the reactor and permeate line for both electron donors tested, suggesting that it was consumed to support microbial growth in the ASFMBR.

Previous studies have reported inhibitory effects to denitrification by different metals. Cu has been reported to affect nitrate removal rate and promote NO_2^- accumulation, while a deficient concentration could increase N_2O production.[35] Nevertheless, long term exposure to Cu in bioreactors can decrease its inhibitory effects as compared to short-term tests.[36] Co has also been shown to cause nitrite accumulation,[12] while high Ca concentrations decreased denitrification rate.[37] In the present work, a fraction of Cu was recovered in the precipitation column, while a higher fraction was retained in the ASFMBR, maintaining a low concentration in solution (Fig. 4). Thus, no inhibitory effects were expected for this metal. Similar results were obtained with Co. Meanwhile, Ca was hardly removed in the precipitation column, but it was significantly retained in the ASFMBR (64% and 79% removal for citrate and ethanol experiments (Fig. 4), respectively). The large fraction of metals, which precipitated in the combined treatment system (precipitation column/ASFMBR) might have decreased the inhibitory effects.

17

The high denitrifying efficiency achieved during the treatment of stainless-steel industry wastewater was partly due to the effective neutralization of the acidic pH (Table 1), which was accomplished (Fig. S5 in SM) through high recirculation of the alkalinity produced from the denitrifying process with both electron donors tested. According to speciation analysis, the predominant (>90% of abundance) alkalinity species formed was HCO_3^-, which reached 0.77 and 0.82 mol HCO_3^-/L when citrate and ethanol were tested as electron donors, respectively.

3.3. Recovery of minerals in the treatment system and their characterization. *Synthetic wastewater tests.* Before metals addition, precipitation column was maintained at a pH close to 6 (Fig. S5 in SM). Addition of Fe(II)/Fe(III) and Cr(III) decreased the pH just after the first days of metals addition, but increased to 6 afterwards. For the tests performed with nickel and the three metals together, the pH of the column was controlled without important affectation in a range between 7 and 8. According to the CO_2 equilibrium, neutralization of citric acid occurs by the formation of bicarbonate or carbonic acid species. Poor iron retention was achieved in this column, while only 3% and 14% of Cr(III) and Ni(II) fed, respectively, was retained. However, metals retention increased in the multi-metals experiments to 3.6%, 6.9% and 14.1% for Fe, Cr and Ni, respectively. This could be caused by the high metals concentrations that could have decreased the metal-citrate stability complex, and also due to metals co-precipitation. The strong stability of citrate-metal complexes, even when the pH was neutralized, might have affected metals recovery. According to chemical speciation analysis obtained in previous assays,[9] the [Fe(II)-citrate]$^-$ complex is maintained at alkaline pH, while the concentration of the [Fe(III)-citrate] complex decreased. The same pattern occurred for the complexes [Ni(II)-citrate$_2$]$^{4-}$ and [Cr-citrate$_2$]$^{4-}$. Iron-bearing minerals were difficult to identify by XRD, while Cr was found as

18

Cr(OH)$_3$.3H$_2$O and chromium oxides, such as CrO$_2$ and Cr$_2$O$_3$. Ni was recovered as NiCO$_3$ and Ni$_3$(PO$_4$)$_2$ (Fig. S6 in SM). Thus, acidic metallurgic effluents can be neutralized with denitrifying by-products promoting precipitation and recovery of metals; however, the stability of metals-citrate complexes challenges metals recovery efficiency.

Recovery in the ASFMBR. Metals bio-recovery from complexed species was accomplished after destroying ligands and forming insoluble compounds, which depended on metals-complexes stability. Citrate degradation conducted by denitrifying microorganisms in the ASFMBR released metals promoting their precipitation with denitrification by-products. The pH was maintained above pH 8 in the ASFMBR during the whole operational period with synthetic wastewater (Fig. S5). Iron was almost completely precipitated during the experiments with this metal. Nevertheless, its precipitation decreased 24.9% for the tests assessing the effects of the three metals together (Fig. 4). Individual addition of iron resulted in precipitation and/or oxidation of Fe(II), as previously discussed in Section 3.1.1. Citrate degradation together with Fe(II) oxidation triggered iron precipitation in the individual metal addition tests. However, for experiments conducted with the three metals together, residual Fe(II) was obtained in the reactor (Fig. 4(a)). Thus, as mentioned in Section 3.1.4, Fe(II) oxidation and its subsequent precipitation could be affected by the presence of the other metals studied (Cr and Ni). Another explanation is that the three metals added competed for the denitrifying by-products available to become precipitated. Thus, the stability of the Fe(II)-citrate complex was less affected so that Fe(II) was maintained in solution. FeCO$_3$ was detected during the individual addition of Fe, and additional iron-bearing minerals were recovered during the multi-metals experiments, such as Fe$_3$O$_4$, hematite and ferrihydrite. Thus, Fe(II) could have been oxidized followed by its subsequent precipitation in

19

these minerals. Regarding Cr(III) and Ni(II), their retention occurred at the same level both in individual and multi-metals experiments. Cr(III) was poorly removed (≤50%) because hydroxides produced from denitrification would have been incorporated into the structure of Cr(III)-bearing complexes, which are difficult to precipitate.[29] This limited Cr(III) bio-recovery and led to a large amount of residual Cr(III) in the outlet. Cr(III) could be recovered as $Cr(OH)_3 \cdot 3H_2O$ as evidenced by XRD analysis (Fig. S7 in SM) during Cr(III) individual supply, while it was recovered as CrOOH when the three metals were jointly tested (Fig. S3). For experiments conducted with Ni(II), a recovery of up to 90% of this metal was accomplished. The predominant species in solution of Ni(II) at the pH of the reactor operation were [Ni-citrate]$^-$ (pK~5.19)[38] and $[Ni(citrate)_2]^{4-}$ (pK~2.1).[39] In previous batch experiments, Ni(II) was complexed and maintained in solution.[9] The higher Ni(II) bio-recovery achieved in the present work in comparison with the batch assays could be caused by the higher dilution and alkalinity present in the reactor that saturated the insoluble Ni species and promoted their precipitation. In the present work, Ni was recovered as $Ni_3(PO)_2$ and $NiCO_3$ in individual studies (Fig. S7 in SM), while it precipitated as $Ni(OH)_2$ when the three metals were simultaneously added to the ASFMBR (Fig. S3 in SM). Additionally, microorganisms can reduce Ni(II) to Ni(0) under denitrifying conditions.[40] During the operational tests, the redox potential was maintained at values of up to -0.17 V (Fig. S8 in SM), which could have promoted the reduction of Ni(II) to Ni(0). However, Ni(0) could not be detected by XRD analysis.

A fraction of metals precipitated on GC particles circulating at the conical and cylindrical-traps sections of the ASFMBR (Table S1 in SM), which could be promoted by the activity of denitrifying microorganisms. VSS content in GC particles circulating in the upper part of the reactor was higher than that found in solids circulating in the conical section of the ASFMBR (Fig. S9 in SM).

Likewise, metals concentration on GC particles collected from the cylindrical-traps section was higher than in solids circulating at the conical part of the reactor. The hydrodynamic conditions of the reactor could have driven the higher metals concentration obtained in the upper part of the reactor by sending the lower particles sizes to this zone. However, it is also interesting to note metals deposition on GC particles circulating in the conical section of the ASFMBR. This zone of the reactor showed a high flow velocity, thus the contact time between the flow dragging (containing metals and denitrifying by-products) with GC particles is low. On the other hand, the amount of heavy metals precipitated on GC particles agreed with the amount of metals precipitated in the ASFMBR, which is related to the heavy metal-citrate complex stability, following the trend Fe>Ni>Cr. For the multi-metals experiments, minerals recovery was generally lower than the individual experiments.

Real wastewater tests. Precipitation column showed the capacity to retain significant amounts of Fe, Cr, Ni, Sn, Mn, Mo, Co, Pb, Cu and V for experiments performed with both carbon sources during the treatment of real stainless-steel industry wastewater (Fig. 4). However, B, Al, Ca and Mg were poorly recovered here. Additional elements were obtained during the operation with ethanol, such as Si and Na, while Mo was completely recovered. The higher metal removal achieved with ethanol as compared to citrate was probably due to the absence of metals-complexes formation, which allowed high metals precipitation. For both carbon sources, less than 40% of metals present in the industrial effluent was retained in the precipitation column. The acidic feed was neutralized in this column after a few days of operation. Nevertheless, the pH was drastically decreased to values around 3 and 2 for ethanol and citrate experiments, respectively. This may be explained by high metals precipitation occurring and deficient

21

recirculation of the alkalinity from the ASFMBR to the column. However, a higher amount of

metals was retained in the column during the treatment of real wastewater as compared to

experiments performed with synthetic medium. This could be explained by the higher HRT

applied during the treatment of real wastewater, which allowed saturation and precipitation of

a larger amount of insoluble species. Thus, further work should be conducted to optimize the

precipitation column to increase metals recovery.

Some studies have reported that citrate and heavy metals can be jointly precipitated both at

acidic and alkaline pH values.[41-43] Nevertheless, crystalline solids were observed with both

electron donors under acidic pH (prior the precipitation column), while citrate was not detected

by XRD analysis. Minerals detected by XRD under acidic pH included $Cr(OH)_3 \cdot 3H_2O$, $Fe(0)$, $K(OH)$

and $Ni(OH)_2$ for both electron donors tested (Fig. S10 in SM). Additional picks related to

phosphates of Fe, Cu and Ni were also detected by XRD for the ethanol test. The same solids

obtained under acidic pH were settled in the precipitation column for the citric acid test (Fig. S11

in SM). Additional species were recovered for the ethanol experiment, such as lepidocrocite (γ-

FeOOH), which could be formed by the oxidation of green rust.[34] Other precipitated minerals

included Fe_3O_4, ferrihydrite, $Fe(0)$, $Ni(0)$, CrO_2 and Cr_2O_3 (Fig. S11 in SM).

Recovery in the ASFMBR. The pH in the ASFMBR gradually decreased from 9 to 7.5 for

experiments with citrate, while it remained above 8 with ethanol; although pH dropped to 5.5

for the last days of operation with the latter substrate (Fig. S5 in SM). The better control of pH

achieved with citrate (Eq. 4) could be due to higher alkalinity produced as compared with ethanol

(Eq. 5). pH decrease could be related to the amount of metals forming species with denitrifying

by-products. All elements were removed (>90%) in the ASFMBR with both electron donors,

22

except for Na and Ca with a retention <80%, and Mg with precipitation <30%. For the remaining

elements, the amount of metals retained in the reactor for citrate tests (≥90%) was lower than

that obtained in ethanol experiments (≥99%) (Fig. 4). Ethanol can be considered as a suitable

electron donor to achieve denitrification, but the pH can also decreased affecting the process,

which is also reflected in deficient alkalinity production, thus affecting metal precipitation. On

the other hand, citrate maintains an adequate pH, but the produced metals-citrate complexes

are difficult to be removed. Ethanol does not form any complex with the metals, but a low

fraction of Ni, Mn, Si B and Co crossed the permeate line, and thus were detected in the effluent.

The main minerals recovered in the ASFMBR during its operation with ethanol were $Fe(OH)_2$,

akageneite (β-FeOOH), Fe_3O_4, CrOOH, $Ni(OH)_2$, $NiCO_3$ and possible co-precipitation as Cr_2O_3NiO,

Cr_2FeO_4 and Cr_2O_3 (Fig. S4 in SM). For experiments performed with citric acid, it was difficult to

identify the precipitated species.

Similar to the results obtained with synthetic wastewater, the amount of elements precipitated

on GC particles circulating at the superior traps of the reactor was higher than on those prevailing

in the conical section, except for Si. The formation of metals-citrate complexes decreases metal

precipitation on GC particles, which can be observed with the higher concentration of metals for

ethanol experiments (Table S1 in SM). The amount of metals precipitated on GC particles showed

the following trend Fe>Si>K>Cr>Ni>P>Ca>Mn for elements with ≥ 0.1 mg/g-GC. Interestingly, Si

was present at high concentration on GC particles and characterized as SiO_2 by XRD analysis (Fig.

S12 in SM). However, it was hardly found in other solids analyzed. Dai and Hu[44] reported that a

surface of quartz particles can be affected by the presence of ions, such as Cr(III), which change

their charge, consequently inhibiting the precipitation of iron hydroxide on quartz. However,

23

further investigation must be performed to clarify these aspects. Additional elements, such as

Mn, Al, Mg, Mo, Zn, Co, Na, V, Cu, Li, Pb and Sn were detected at trace concentrations.

Precipitation of metals in a FBR under denitrifying and sulfate-reducing conditions has also been

reported using GAC as supporting material for biomass, obtaining the precipitation of Ni, Fe, Ca,

Mg, Mn, S and P, being Ni the principal metal of study.[18]

3.4. Filtration performance in the ASFMBR. Degradation of citrate and ethanol through

denitrification implies the production of SMP linked to microbial growth, cells decay and lysis.

Additionally, SMP production could also be associated to protection of microorganisms against

toxic effects of some metals.[31] This could contribute to the organic-inorganic membrane fouling

by metal deposition.[21] Besides, metal precipitation with by-products or dissolved organic matter

increases the content of TSS, which can also contribute to membrane fouling. During the reactor

operation, SMPp gradually increased for the tests with individual Cr(III) addition and with the

supply of the three metals together. For experiments run with iron addition, SMPp concentration

decreased, while the opposite happened during Ni(II) addition (Fig. S2 in SM). Despite this

considerable increase, the suction pressure of the permeate line remained unaltered (Fig. 5).

Nevertheless, a report indicates a link between SMP and Fe(III)/Fe(II)[45] and the co-precipitation

between SMP and Fe(III).[46] This co-precipitation could prevent membrane fouling. The addition

of the three metals together resulted in a gradual and slow increase on the SMPp in the reactor.

The negative effect of Ni(II) on high SMPp production could have been decreased by the positive

effect of iron on SMPp production. An important decrease on SMPp was observed during the

treatment of real metallurgic wastewater, perhaps due to the high Fe concentration fed, which

precipitated with proteins. This might have had an effect on the production of SMPp.

24

Despite the decrease observed on SMPp with the addition of iron, an increase in the suction

pressure was detected during this experiment (Fig. 5), indicating some membrane fouling.

According to the SMPc concentration in the reactor (Fig. S2 in SM), addition of metals resulted in

a gradual increase, except for the iron tests, where a high production of SMPc was obtained.

Likewise, the TSS concentration was higher with iron addition than with the other metals, which

may be related to the high iron precipitation obtained (Fig. 6). Therefore, membrane fouling

could be explained by the higher SMPc and TSS concentrations reached in the ASFMBR. The

higher attribution of SMPc with respect to SMPp on membrane fouling has previously been

reported[30] and the insertion of heavy metals resulted in an increase on SMP, concomitant to

membrane fouling, which was attributed to the attachment of SMPc to the membrane surface

during the treatment of domestic wastewater.[21]
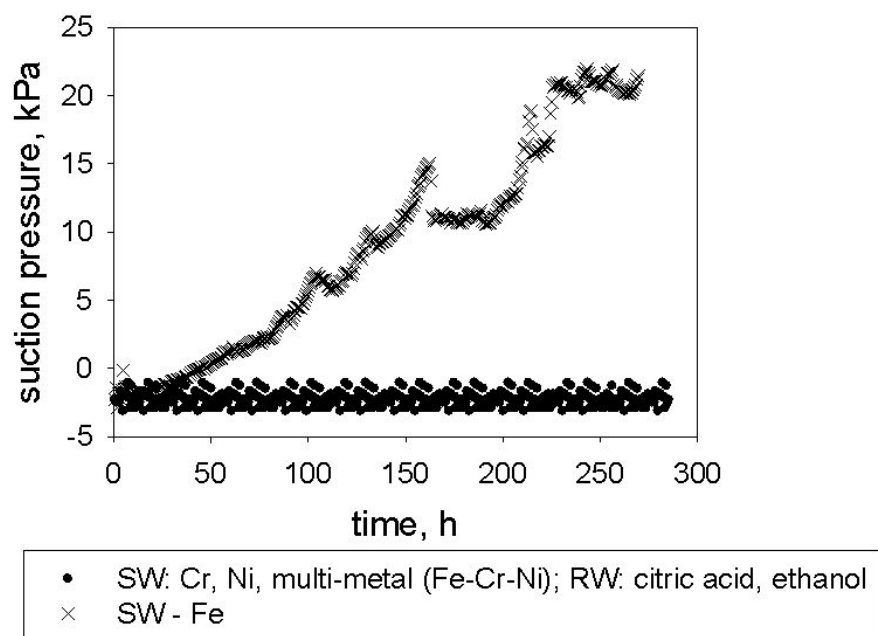
Figure 5. Suction pressure at the permeate line during the treatment of synthetic and real

wastewater. SW and RW mean synthetic and real wastewater, respectively.
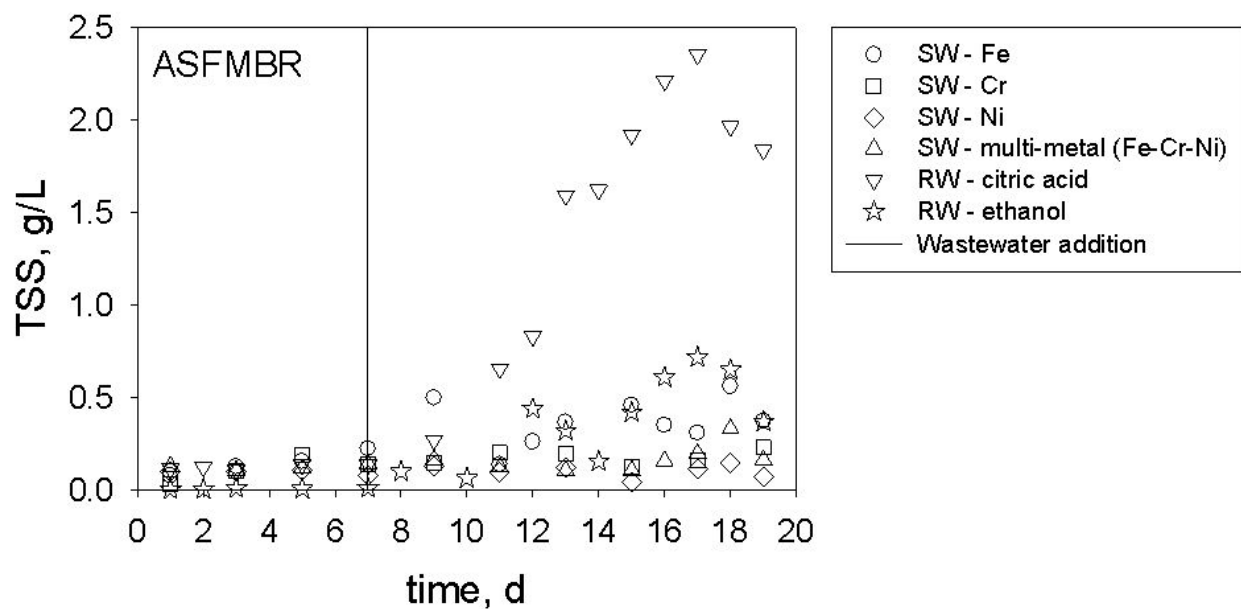
25

Figure 6. Total suspended solids (TSS) during the treatment of synthetic and real wastewater in the ASFMBR.

Metal precipitation could have also caused membrane fouling. Concentrations of Cr and Ni on the membranes were low for the experiments with citric acid (Fig. 7). This happened even at the higher metal rejection obtained with real wastewater compared with synthetic experiments. Meanwhile, for Fe-addition experiments, the Fe concentration on membranes was in agreement with the concentration of Fe added. A previous hydrodynamic study of the reactor revealed that higher shear stress and particle momentum was obtained at the lower part of the reactor (data not shown). Low shear stress and particle momentum on the membrane are related to higher SMP deposition. Similar metals concentration on the membrane was observed for the experiments with synthetic wastewater. Metals concentration on the membrane increased with the height of the reactor where the shear stress and particle momentum are low, excepting for

26

Fe experiments. However, lower amount of metals was detected at the highest part of the reactor. This could be explained because, at this height of the reactor, the adherence of SMP was higher due to deficient GC particles fluidization and low shear stress obtained in this zone of the reactor. SMP covered membrane surface, thus avoiding metals deposition thereafter. The presence of heavy metals, such as Zn(II), Cu(II), Pb(II) and Cd(II), in cake layer has been reported in MBR.[21] Another aspect is that, due to the accumulation of SMP on the membrane at the upper part of the reactor, porous were covered and thus it is expected that filtration was mainly carried out in membranes located in the lower part of the ASFMBR, where low concentration of SMP were found.
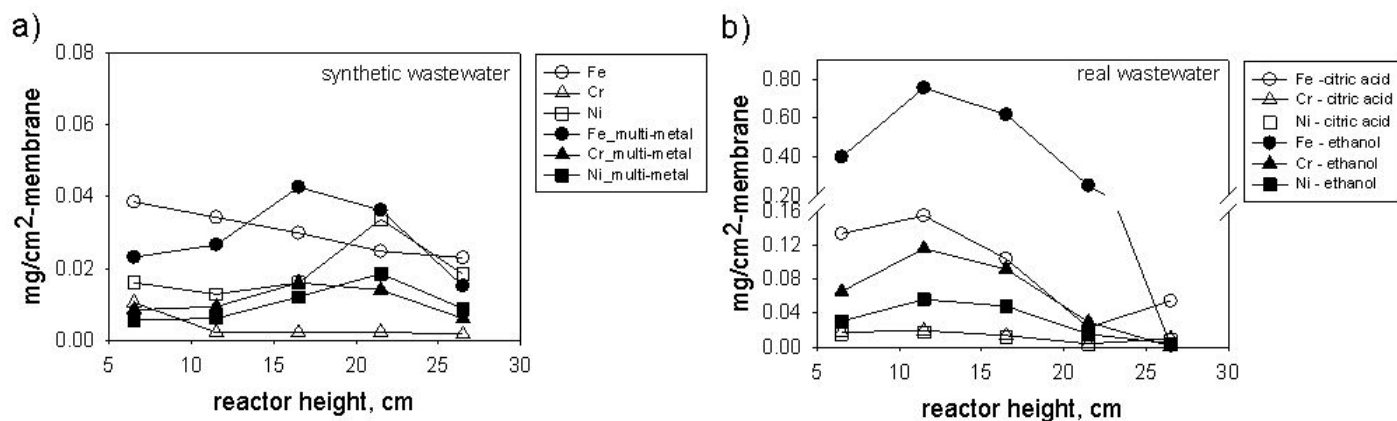

Figure 7. Principal metals on membrane surface at different heights of the reactor during the treatment of (a) synthetic and (b) real wastewater.

The high HRT applied during the treatment of real wastewater promoted large solids precipitation and, as a consequence, high metals rejection by the membrane. The Fe concentration on the membrane for the citrate experiments was higher than that obtained in

synthetic tests. However, the concentration greatly increased to 0.76 mg/cm^2-membrane for the experiment with ethanol. Therefore, the Fe-citrate complex moved the iron through the membrane and decreased its precipitation thereafter, obtaining iron in the permeate line. Despite the high precipitation of metals, especially Fe, on the membrane during the treatment of real wastewater, suction pressure remained unaltered (Fig. 5). A remarkable difference in concentration among the metals studied occurred at different reactor heights (Fig. 7). The lowest concentrations were detected in the upper part of the reactor, which could be related to the high amount of SMP deposited on this zone of the ASFMBR preventing metals deposition on the membrane. The concentration of TSS in the reactor was lower when ethanol was supplied as electron donor than that produced with citrate. This could be explained by the high metals concentration maintained in solution due to the metals-citrate complexes formed, which avoided metals precipitation. These solids are suspended and rejected by the membrane, which can be observed by the amount of TSS measured in the permeate line, reaching values ≤0.10 g/L for all the experiments.

The swirling fluidization of GC particles can decrease cake layer growth on the membrane surface; however, internal fouling of the membrane can occur. The presence of iron in MBR has resulted in amorphous ferric oxyhydroxides particles and gelatinous assemblages containing Fe(III) bound to polysaccharides responsible for irreversible membrane fouling.[47] For the present study, difference iron-bearing minerals were identified during the operation of the ASFMBR. Siderite (green color) was deposited in different zones of the reactor during the operation with iron, while additional iron-containing solids (brown color, characteristic for ferric oxides and hydroxides) were also detected in the remaining experiments. This was confirmed by XRD

28

analysis. Similar colored minerals were observed on the membranes at the end of the experimentation. Siderite precipitation could have caused membrane fouling in experiments performed with iron, while deposition of iron oxides and hydroxides in the other experiments could have occurred without affecting membrane filtration. Thus, the prevalence of different iron minerals may affect the half-life of the membrane.

4. CONCLUSIONS

The ASFMBR introduced in the present study showed high nitrate removal efficiency during the treatment of synthetic and real metallurgic wastewater with negligible production of NO_2^- and N_2O. The acidic pH was efficiently neutralized by high recirculation of denitrifying by-products. This strategy also allowed precipitation and recovery of a large amount of metals present in the metallurgic effluent, although further studies should be conducted to optimize the recovery of metals. The treatment concept is promising to achieve efficient removal of nitrate and recovery of metals, while preserving the membrane module by the hydrodynamic conditions prevailing inside the ASFMBR.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at:

Details about experimental conditions, recovery and characterization of minerals, as well as performance of the ASFMBR.

29

AUTHOR INFORMATION

Corresponding author

*E-mail: fcervantesc@iingen.unam.mx. Tel.: (+52) 4421926169.

ORCID

Francisco J. Cervantes: 0000-0002-8382-1757.

Germán Buitrón: 0000-0003-3975-7644.

Conflict of Interest Disclosure

A patent application has recently been deposited in Mexico.

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