Influence of pH shocks on trace metal dynamics and performance of methanol fed granular sludge bioreactors

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Abstract

The influence of pH shocks on the trace metal dynamics and performance of methanol fed upflow anaerobic granular sludge bed (UASB) reactors was investigated. For this purpose, two UASB reactors were operated with metal pre-loaded granular sludge (1 mM Co, Ni and Fe; 30 °C; 96 h) at an organic loading rate (OLR) of 5 g COD l reactor^{-1} d^{-1}. One UASB reactor (R1) was inoculated with sludge that originated from a full scale reactor treating alcohol distillery wastewater, while the other reactor (R2) was inoculated with sludge from a full scale reactor treating paper mill wastewater. A 30 h pH shock (pH 5) strongly affected the metal retention dynamics within the granular sludge bed in both reactors. Iron losses in soluble form with the effluent were considerable: 2.3 and 2.9% for R1 and R2, respectively, based on initial iron content in the reactors, while losses of cobalt and nickel in soluble form were limited. Sequential extraction of the metals from the sludge showed that cobalt, nickel, iron and sulfur were translocated from the residual to the organic/sulfide fraction during the pH shock in R2, increasing 34, 47, 109 and 41% in the organic/sulfide fraction, respectively. This is likely due to the modification of the iron sulfide precipitate stability, which influences the extractability of iron and trace metals. Such a translocation was not observed for the R1 sludge during the first 30 h pH shock, but a second 4 day pH shock induced significant losses of cobalt (18%), iron (29%) and sulfur (29%) from the organic/sulfide fraction, likely due to iron sulfide dissolution and concomitant release of cobalt. After the 30 h pH shock, VFA accumulated in the R2 effluent, whereas both VFA and methanol accumulated in R1 after the 4 day pH shock. The formed VFA, mainly acetate, were not converted to methane due to the loss of methanogenic activity of the sludge on acetate. The VFA accumulation gradually disappeared, which is likely to be related to out-competition of acetogens by methanogens. Zinc, copper and manganese supply did not have a clear effect on the acetate removal and methanol conversion, but zinc may have induced the onset of methanol degradation after day 152 in R1.

Introduction

In full scale anaerobic wastewater treatment, trace elements are generally supplied with the influent to ensure good bioreactor performance. These metals accumulate partly in the anaerobic granular sludge bed, thus yielding a stock of trace metals, which sustains the trace metal requirements of the microbial population that develops in the bioreactor. The metals are distributed over the different phases of the granular matrix: biomass, extracellular polymeric substances (EPS) and inorganic precipitates, e.g., sulfides, phosphates and carbonates (van Hullebusch et al. 2003).

Little is known about the dynamics, i.e. storage versus leaching, of various trace metals in anaerobic granular sludge and the effect of operational conditions and process disturbances on these

dynamics. Such knowledge is essential to gain a better understanding of metal retention and bio-availability, thus allowing more effective supplementation of trace elements to anaerobic bioreactors. Already small differences in reactor conditions can influence the retention and speciation of trace metals. For instance, the presence and/or type of sulfur source, even at concentrations as low as 13 mg l^{-1}, already influence the metal retention and speciation (Zandvoort et al. submitted). Also the pH can strongly influence the metal retention, as metal solubility increases at low pH values, independent of the mineralogical composition (Alloway 1990). Operational disturbances can result in severe pH drops of the reactor mixed liquor due to volatile fatty acid (VFA) accumulation when treating weakly buffered wastewaters (Lettinga et al. 1979). Moreover, transient pH gradients within a single aggregate might occur as well (Lens et al. 1993). These pH variations can therefore influence trace metal speciation in the granular matrix, resulting in e.g. translocation of metals in the sludge due to dissolution of metal precipitates, which can lead to metal losses with the effluent.

In this paper, methanol fed upflow anaerobic sludge bed (UASB) reactors inoculated with sludge pre-loaded with the methanogenic key-metals cobalt, nickel and iron were used to investigate the influence of short term (30 h and 4 days) pH drops from pH 7 to 5 on the metal retention dynamics and reactor performance. A pH of 5 was chosen as metals are significantly mobilised at that pH without inducing irreversible toxic effects on the sludge. The metal retention was assessed by monitoring the metal content of the effluent and sludge. The distribution of the metals over different operationally defined fractions of the sludges was also monitored as a function of time using a sequential extraction procedure. The metabolic properties of the sludge that developed in the UASB reactors were monitored as well by batch activity tests.

Materials and methods

UASB reactor operation

Two UASB reactors (working volume 0.75l; inner diameter 5 cm) were operated at a hydraulic retention time (HRT) of 8 h and at a superficial upflow velocity of 1 m h^{-1} in a temperature-controlled (30 $\pm$ 2 °C) room as described in Zandvoort et al. (2004). A pH controller maintained the pH at 7.0 ($\pm$0.2) by the addition of NaOH in the effluent recycle tube. The produced biogas was led through a water lock filled with a NaOH (15%) solution in order to remove CO_2 and H_2S. The produced methane volume was measured by water displacement from mariot-flasks using an on-line balance system as described by Gonzalez et al. (1999). Due to a problem with the data collection system, no gas production data are available from day 41 until day 49 and from day 163 onwards.

Basal medium

The reactors were fed a synthetic wastewater containing methanol and macro nutrients as described previously (Zandvoort et al. 2002). The influent contained 0.41 mM sulfate (13 mg S l^{-1}) as sulfur source and no trace elements. Individual metals were added to the influent in pulse or continuous mode to one or both reactors from day 70 onwards, the conditions and time of dosage are presented in Table 1. To avoid precipitation in the storage vessels, the influent consisted of three different streams; viz. basal medium without K_2HPO_4; methanol with K_2HPO_4 and dilution water. The medium was prepared with demineralised water.

Source of biomass

The anaerobic granular sludge used to inoculate R1 originated from a full scale UASB reactor treating alcohol distillery wastewater (Nedalco, Bergen op Zoom, the Netherlands) and the sludge from R2 originated from a full scale UASB reactor treating paper mill wastewater (Industriewater Eerbeek B.V., Eerbeek, the Netherlands). The sludge was elutriated to remove the fines. The total suspended solids (TSS) and volatile suspended solids (VSS) content were, respectively, 8.3 ($\pm$0.2)% and 7.6 ($\pm$0.2)% for the R1 sludge and 22.6 ($\pm$0.2)% and 16.7 ($\pm$0.2)% for the R2 sludge. The sludge concentration after inoculation was 14 and 27 g VSS l^{-1} in R1 and R2, respectively. This resulted in sludge beds of similar

Table 1. Times and conditions of metal additions to the UASB reactors

Time Day	Code[a]	Metal	Amount μmol d^{-1}	Concentr. mg l^{-1}	Mode	Reactor
70	a	Cu	11.25	96	Pulse[2b]	R2
73	b	Zn	11.25	96	Pulse	R2
75	c	Cu, Zn, Ni, Mn	11.25	96	Pulse	R1 and R2
84	d	Zn	11.25	96	Pulse	R1 and R2
96	e	Ni, Mn	22.50	192	Pulse	R1 and R2
102	f	Zn	33.75	288	Pulse	R2
123	g	Zn	33.75	288	Pulse	R1 and R2
130–151	h	Zn	1.12	0.5	Continuous	R1 and R2
152–162	i	Zn	11.25	5	Continuous	R1 and R2
163–end	j	Cu, Zn, Ni, Mn, W, Mo, Se	11.25	5	Continuous	R1 and R2

[a]Code used in the figures to express time of metal dosage
[b]Pulses were dosed in a 1.25 h periods

heights, which allowed frequent sludge sampling for metal analyses.

In order to study the metal retention dynamics in the two different sludges, the sludges of both reactors were pre-loaded with cobalt (CoCl$_2$·6H$_2$O), nickel (NiCl$_2$·6H$_2$O) and iron (FeCl$_2$·2H$_2$O) by contacting 400 g of sludge (wet weight) with 1600 ml of a solution containing 1 mM of each of these metals for 96 h at 30 °C in a 2 l serum bottle in the absence of substrate. The characteristics and the metal content of the initial sludges before loading are presented in Table 2. The cobalt, nickel and iron

content of the sludges after loading is presented in Table 6.

Experimental design

Four operational periods were defined (Table 3). The organic loading rate (OLR) of both reactors was maintained at 5 g COD l reactor^{-1} d^{-1} during the entire experiment, corresponding to a sludge loading rate (SLR) of 0.27 and 0.14 g COD g VSS^{-1} d^{-1}, for R1 and R2, respectively,

Table 2. Initial characteristics and total metal content of the two anaerobic granular sludges

Parameters	Nedalco sludge	Eerbeek sludge
Total suspended solids (TSS)	8.3 ($\pm$0.2) %	22.6 ($\pm$0.2) %
Volatile suspended solids (VSS)	7.6 ($\pm$0.2) %	16.7 ($\pm$0.2) %
Carbonates (% of TSS)	0.8 ($\pm$0.2) %	0.4 ($\pm$0.2) %
Total sulfur	22.7 ($\pm$0.1) mg g^{-1} TSS	41.8 ($\pm$1.0) mg g^{-1} TSS
Total phosphorus	3.3 ($\pm$0.1) mg g^{-1} TSS	6.6 ($\pm$0.1) mg g^{-1} TSS
Total metal content		
Cobalt	18.7 ($\pm$0.6) μg g^{-1} TSS	59.4 ($\pm$2.0) μg g^{-1} TSS
Nickel	130.3 ($\pm$3.6) μg g^{-1} TSS	38.3 ($\pm$0.9) μg g^{-1} TSS
Copper	690 ($\pm$10) μg g^{-1} TSS	124.6 ($\pm$5.6) μg g^{-1} TSS
Zinc	760 ($\pm$20) μg g^{-1} TSS	195.7 ($\pm$7.0) μg g^{-1} TSS
Manganese	54.8 ($\pm$0.6) μg g^{-1} TSS	275 ($\pm$8.0) μg g^{-1} TSS
Iron[a]	20.8 ($\pm$0.2) mg g^{-1} TSS	39.8 ($\pm$0.8) μg g^{-1} TSS

[a]Note that iron content is expressed as mg g^{-1} TSS.

Table 3. Operational periods defined for R1 and R2

Period	R1 (days)	R2 (days)	Description
I	0–36	0–36	Reactor start-up
II	37–41	37–41	Imposed 30 h pH shock (pH 5); R1 at day 37 and R2 at day 39
II	42–69	42–69	Recovery from 30 h pH shock
IV	70–194	70–178	Supply of trace elements with the influent (see Table 1)
IVa	105–109	–	Imposed 4 day pH shock (pH 5)
IVb	110–194	–	Recovery from 4 day pH shock

based on the initial amount of VSS added to the reactors at the start of the experiment.

Maximum specific methanogenic activity assays

Maximum specific methanogenic activity (SMA) of the sludge was assessed on methanol, acetate and H_2/CO_2 always in duplicate and at a temperature of 30 (± 2) °C using on-line gas production measurements (Zandvoort et al. 2002). The SMA of the sludge on these substrates was assessed at day 0, 34, 37/39 (R1 and R2 respectively), 45 and at termination of the experiment (day 194/178 for R1 and R2, respectively). The SMA on acetate of the R1 sludge was also measured on day 84 in order to assess whether the sludge was still capable to use this substrate. The SMA on H_2/CO_2 was not determined at termination of the experiment.

Metal analyses

The total metal content of the granular sludge was determined according to the method described previously (Zandvoort et al. 2002). In order to assess the metal speciation in the granular sludge matrix, a four-step sequential extraction procedure was used (Table 4), in which each next extraction step is more stringent. The nomenclature applied for the subsequent extraction steps is as follows, viz. the exchangeable fraction (1M NH_4CH_3COO), the carbonate fraction (1M CH_3COOH), organic/sulfide fraction (30% H_2O_2) and the residual fraction (3:1 HCl/HNO_3).

Chemical analyses

The metal concentrations in the effluent and the metal content of the sludge were determined

Table 4. Operating conditions of the modified Tessier sequential extraction procedure

Fraction	Extracting agent[a]	Extraction conditions	
		Shaking Time[b]	Temp
1. Exchangeable	10 ml NH_4CH_3COO, (1 M, pH = 7)	1 h	20 °C
2. Carbonates	10 ml CH_3COOH, (1 M, pH = 5.5)	1 h	20 °C
3. Organic/sulfide	5 ml H_2O_2, (30% pH = 2)	3 h	35 °C
4. Residual	10 ml demineralised water and 10 ml aqua regia (HCl/HNO_3, 3:1)	26 min	Microwave-oven[c]

[a]References to the applied methods: 1. Veeken (1998); 2. Tessier et al. (1979); 3. Modak et al. (1992).
[b]Shaking was applied at 100 rpm.
[c]Extraction of the residual fraction in the microwave was equal with the total extraction methods.

using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Varian Vista MPX, Palo Alto, USA) as described previously (Zandvoort et al. 2002). The concentration of methanol and VFA were determined using gas liquid chromatography as described by Weijma et al. (2000). The total sulfide concentration of the effluent was determined colourimetrically (Dr. Lange, LYW653, Düsseldorf, Germany). TSS and VSS concentrations were determined according to Standard Methods (American Public Health Association 1985). All chemicals were of analytical or biological grade and purchased from E. Merck AG (Darmstadt, Germany).

Results

Start up and response to short-term pH shock (30 h)

Reactor performance

Methanol removal started only after day 26 in R1 and day 14 in R2 and a complete conversion of methanol to methane was only attained on day 35 in R1 and day 28 in R2. During the first imposed pH shock at the start of period II, on day 37 and 39 in R1 and R2, respectively, the pH was lowered from 7 to 5 for 30 h (Figure 1A). This resulted in a fairly similar response of the reactors, viz. in both reactors the effluent methanol concentration instantly increased to 1418 mg COD l^{-1} in R1 on day 39 and in R2 to 1009 mg COD l^{-1} on day 41. At the same time, the VFA concentration in the effluent increased to 186 mg COD l^{-1} on day 39 in R1 and 224 mg COD l^{-1} on day 40 in R2 (Figure 1B). After the pH was restored to 7 (Period III), the performance of both reactors recovered within approximately 3 days and no methanol and VFA were present in the effluent (Figures 1A, B).

Although not intentional, R2 was exposed to two other short (less than one day) pH shocks i.e. at day 85 (pH 5.5) and day 97 (pH 5.4). In both cases this resulted in a sharp increase of effluent methanol and VFA concentrations (Figure 1). The accidental pH drop for less than 24 h on day 97 (pH 5.7) in R1 merely resulted in methanol accumulation in the effluent (Figure 1 A, D).

Effluent sulfur content

Before the onset of the methanogenesis, the effluent sulfide concentration reached a maximum of 12.0 (day 16) and 12.6 mg l^{-1} (day 13) in R1 and R2, respectively (Figure 2). From day 18 onwards, the sulfide concentration in the R1 effluent decreased, averaging 6.5 mg l^{-1} from day 20 to 48, but with a lot of fluctuations. During the imposed pH shock in R1 at day 37 (period II), the effluent sulfur concentration decreased, but after the pH shock the sulfur concentration returned to values similar to the values prior to the pH shock (Figure 2).

The R2 effluent sulfide concentration decreased sharply from day 13 to only 0.2 mg l^{-1} on day 20, and for a 10 day period the effluent sulfide concentrations remained around this level. Simultaneous with the sharp drop in sulfide concentration, the R2 effluent sulfur concentration increased to a maximum value of 17.3 mg l^{-1} on day 23 (Figure 2). From day 30 onwards sulfide raised again to an average of 5.7 mg l^{-1} from day 30 to 48. During the pH shock (day 40) the sulfide concentration decreased to 0.7 mg l^{-1} but a remarkable increase in the R2 effluent sulfur concentration to 34.0 mg l^{-1} manifested. Immediately after the pH was restored to 7, at day 44, the sulfide concentration had increased to the values similar to before the pH shock.

Effluent metal concentration

Immediately after the start of the experiments losses of cobalt, nickel and iron from the sludge occurred as indicated by their effluent concentrations measured (Figure 3, period I). The cobalt and nickel effluent concentration decreased steadily to values lower than 1 μg l^{-1} after 16 days of operation in both reactors. From day 16 onwards, a slight increase in both the cobalt and nickel effluent concentration occurred in both reactors, simultaneous with the onset of methanol removal and biogas formation. Iron was initially present in the effluent at relatively high concentrations (around 0.8 mg l^{-1} on day 5) in both reactors, but it decreased gradually during period I to 42 μg l^{-1} and 46 μg l^{-1} for R1 and R2 on day 34 and 38, respectively.

During the pH shock on day 37 (R1) and day 39 (R2), very discrete peaks in the effluent cobalt, nickel and iron concentrations manifested, with in R1 maximal cobalt, nickel and iron concentrations
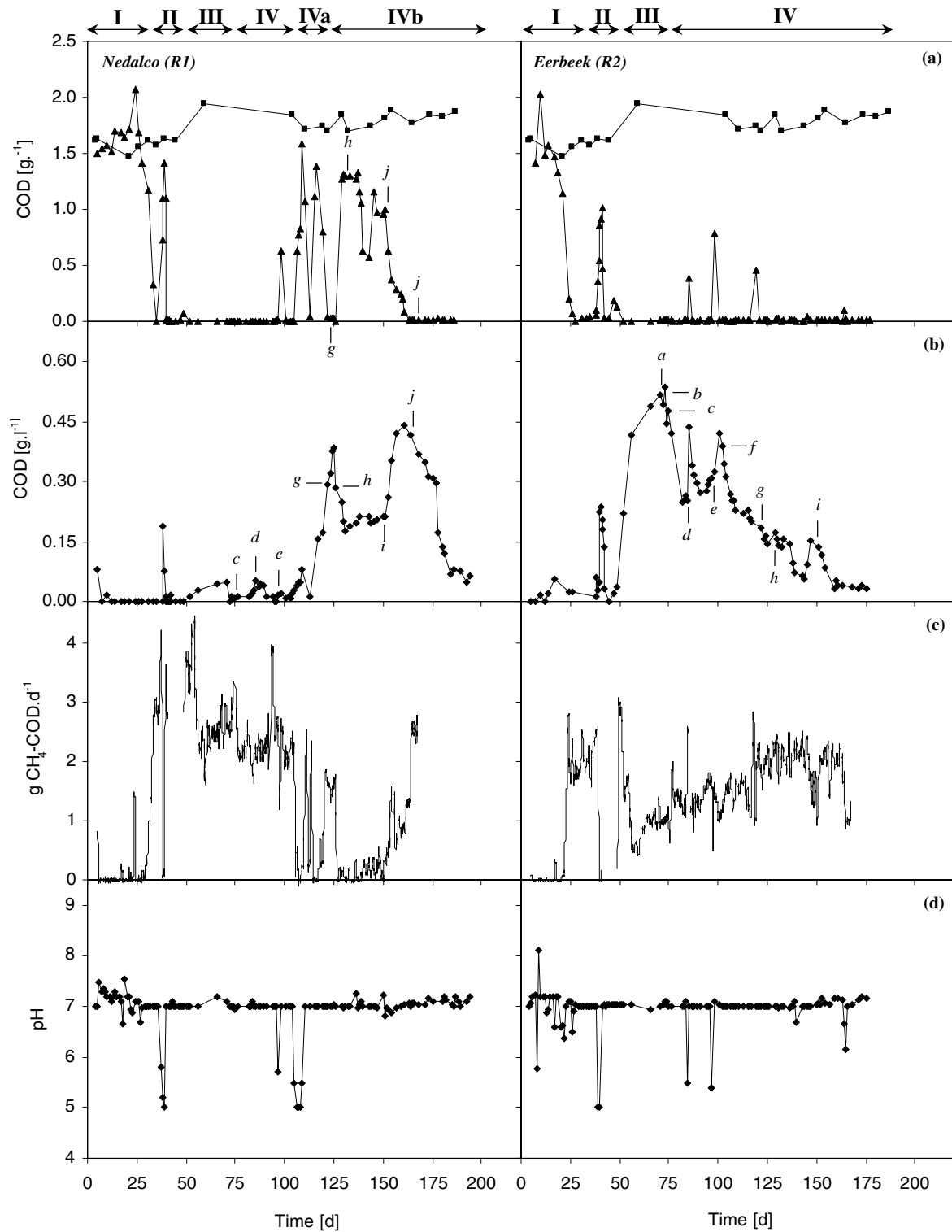
Figure 1. Evolution of the reactor performance of R1 and R2 with time. (A) Influent (■) and effluent (▲) methanol concentration. (B) Effluent VFA (♦) concentration. (C) Methane production and (D) pH. (a) Copper pulse; (b) Zinc pulse; (c) copper, zinc, manganese and nickel pulse; (d) zinc pulse; (e) nickel and manganese pulse (2 × initial dose); (f) zinc pulse (3 × initial dose); (g) zinc pulse (3 × initial dose); (h) zinc pulse (3 × initial dose); (i) start continuous zinc dosing (0.5 μM); start continuous zinc dosing (5 μM); (j) Start continuous dosing all metals except iron (5 μM).
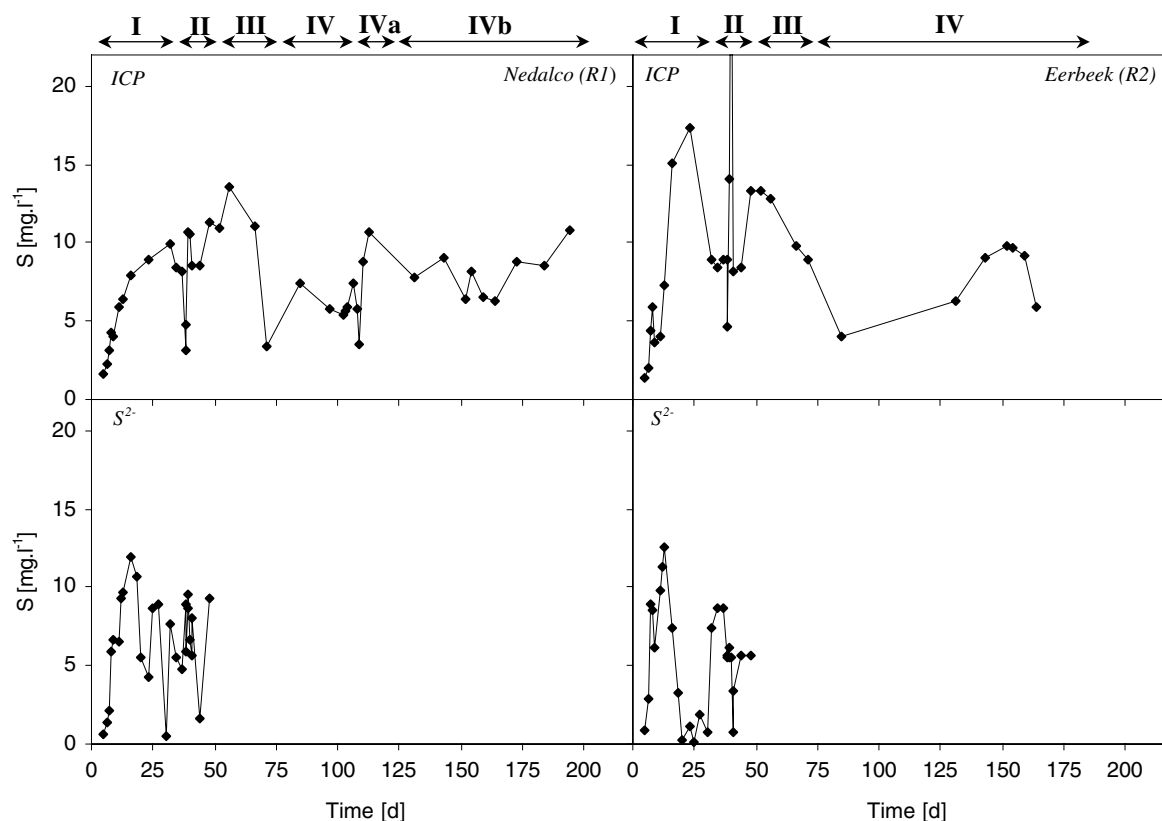
Figure 2. Evolution of the total sulfur (ICP-OES) and sulfide (S^{2-}) concentration in the effluent of R1 and R2.

of respectively, 15 μg l^{-1}, 13 μg l^{-1} and 3 mg l^{-1} and in R2, 57 μg l^{-1}, 19 μg l^{-1} and 11 mg l^{-1}, respectively. However, the losses of cobalt and nickel in soluble form were minimal during the first 30 h pH shock in R1, i.e. at the maximum 37 μg of cobalt and 27 μg nickel were released from the sludge, representing 0.13 and 0.08% of the cobalt and nickel, respectively, based on the total amount of these metals present in the sludge before the shock. In contrast, losses of iron were much higher (Figure 3) with values of 4.81 and 22.48 mg lost for R1 and R2, respectively, corresponding to 2.3 and 2.9% of the total amount of iron present in the sludge before the pH shock (based on the initial sludge TSS content in the reactor).

Immediately following the pH shock the effluent concentration of these metals in both reactors quickly returned to values found prior to the shock, except for cobalt and nickel in R1, which remained elevated for more than 30 days after the pH shock (Figure 3)

Metal retention in the sludge
The pH shock did not have a significant effect on the total cobalt, nickel and iron content of the sludge (Figure 4). During the pH shock (period II), higher amounts of cobalt and nickel were extracted from the exchangeable fraction, especially in case of the R1 sludge (Figure 5). However, immediately after the pH shock (day 40), the amount extracted from this fraction was again similar to that before the 30 h pH shock (Figure 5). The iron content in the exchangeable fraction of both sludges increased during the pH shock, particularly in the R2 sludge viz. from 0.27 mg g TSS^{-1} before to 2.11 mg g TSS^{-1} during the shock. The iron concentration in this fraction remained at a higher level until day 151 (Figure 5) in the sludge of both reactors.

During the pH shock, the cobalt, nickel and iron content of the organic/sulfide fraction of the R2 sludge increased with 34, 47 and 109% respectively (Figure 5). In contrast, the pH shock did not have a significant effect on this fraction
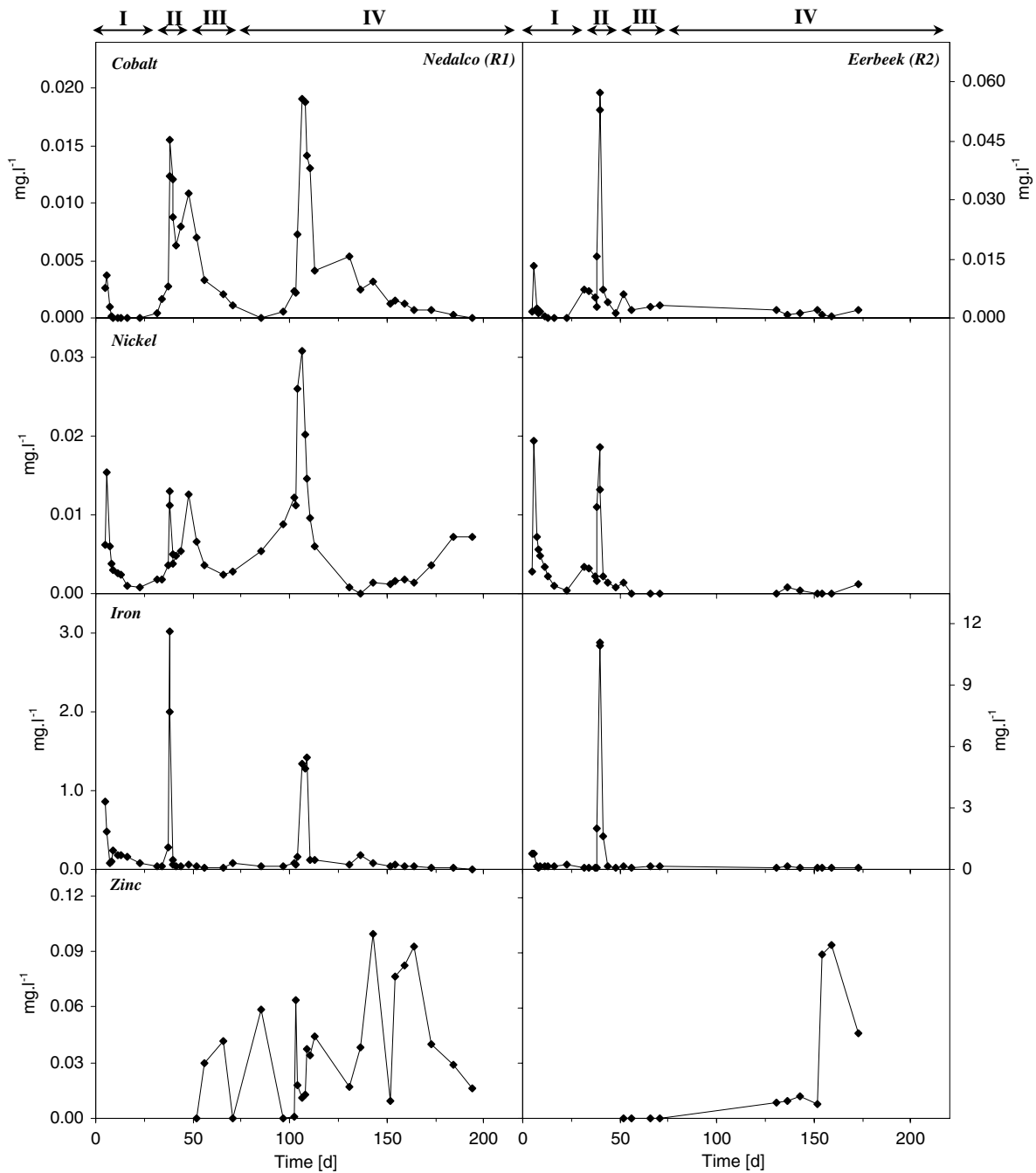
Figure 3. Evolution of the cobalt, nickel iron and zinc concentration in the effluent of R1 and R2.

of the R1 sludge (Figure 5). While the iron content of the organic/sulfide fraction of the R2 sludge increased from 4.73 to 9.89 mg g TSS^{-1} at the same time, the iron content in the residual fraction decreased from 21.22 to 18.38 mg g

TSS^{-1}. The sulfur content in the organic/sulfide fraction of the R2 sludge increased as well during the shock, i.e. from 9.79 to 16.49 mg g TSS^{-1}, while the sulfur content in the residual fraction decreased from 28.81 to 23.41 mg g
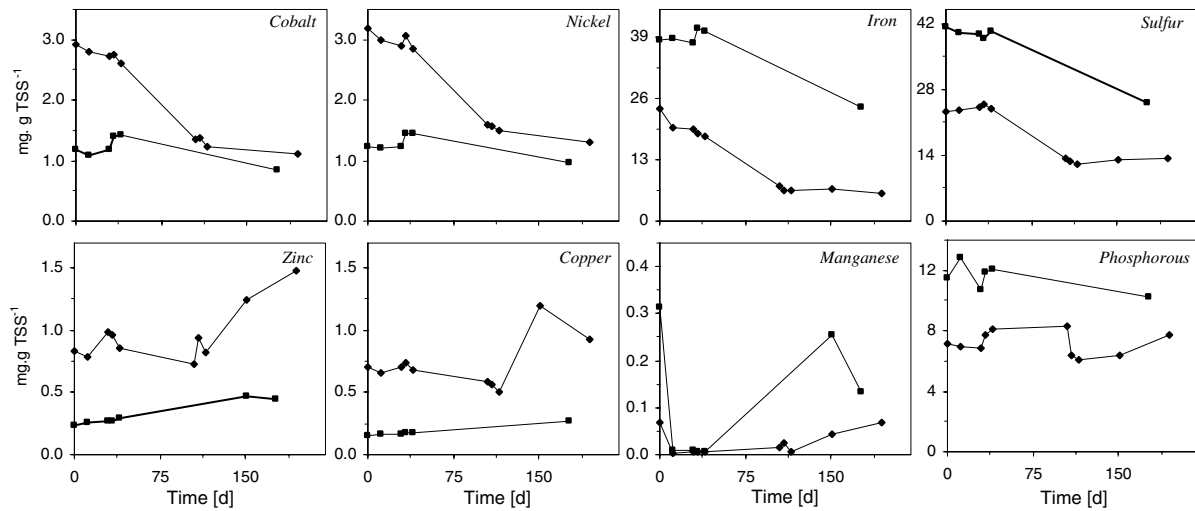
Figure 4. Evolution of the total cobalt, nickel, iron, sulfur, zinc, copper, manganese and phosphorous concentration of the R1 (♦) and R2 (■) sludge.
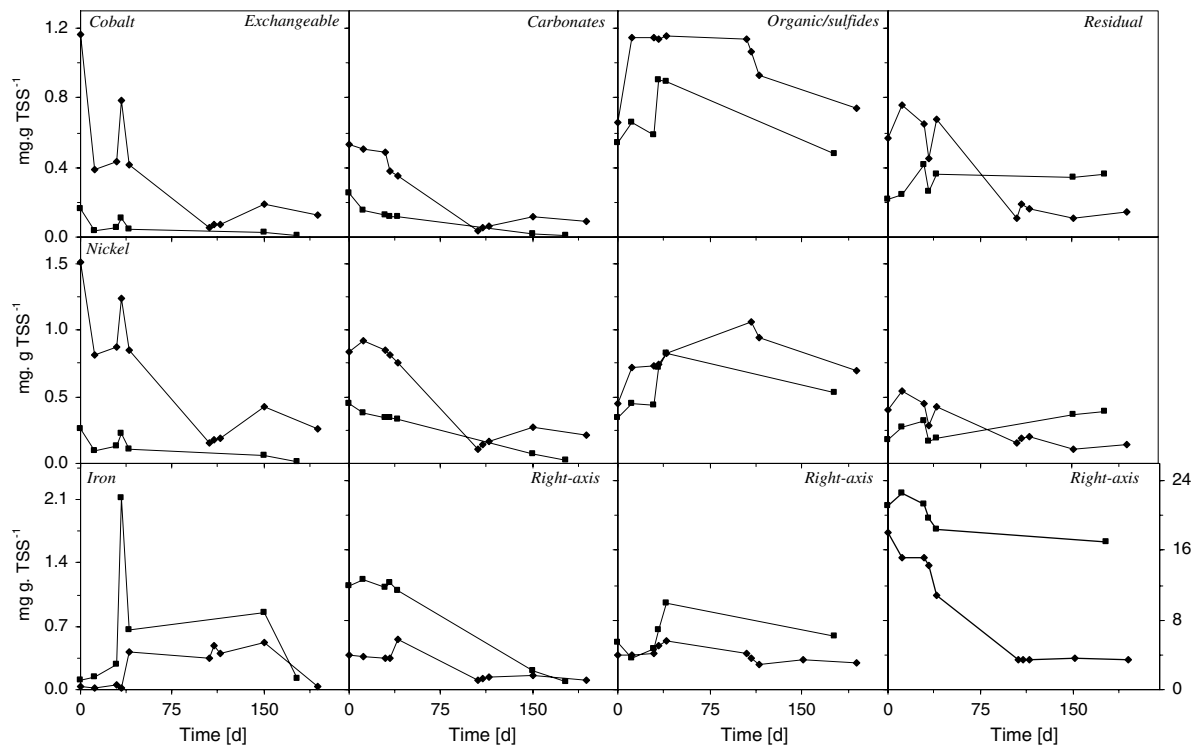

Figure 5. Evolution of the cobalt, nickel and iron content present in the extracted fractions of the R1 (♦) and R2 (■) the sludge.

TSS^{-1} (Figures 6 and 7). In the R1 sludge, the iron content of the residual fraction decreased considerably with 4.41 mg g TSS^{-1} (29%) lower than before the pH shock.

Acidogenesis in R2

From day 42 onwards, VFA started to accumulate in the effluent of R2 and reached a maximum

concentration of 528 mg COD l^{-1} on day 73, mainly as acetate (Figure 8) while all remaining methanol was still converted to methane. In order to asses whether addition of trace metals, other than those which were initially loaded on the

sludge, could reduce the acetate formation in R2, pulses of individual metals and combinations of metals were supplied with the influent during period IV from day 70 onwards (Table 1). From day 75 onwards, these metals were also supplied to
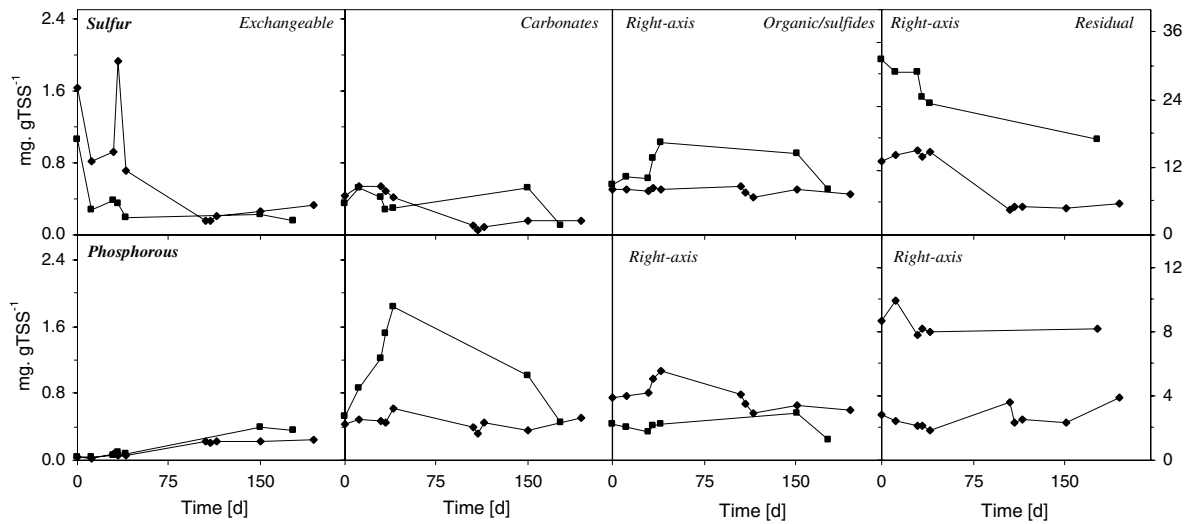

Figure 6. Evolution of the phosphorous and sulfur content present in the extracted fractions of the R1 (♦) and R2 (■) sludge.
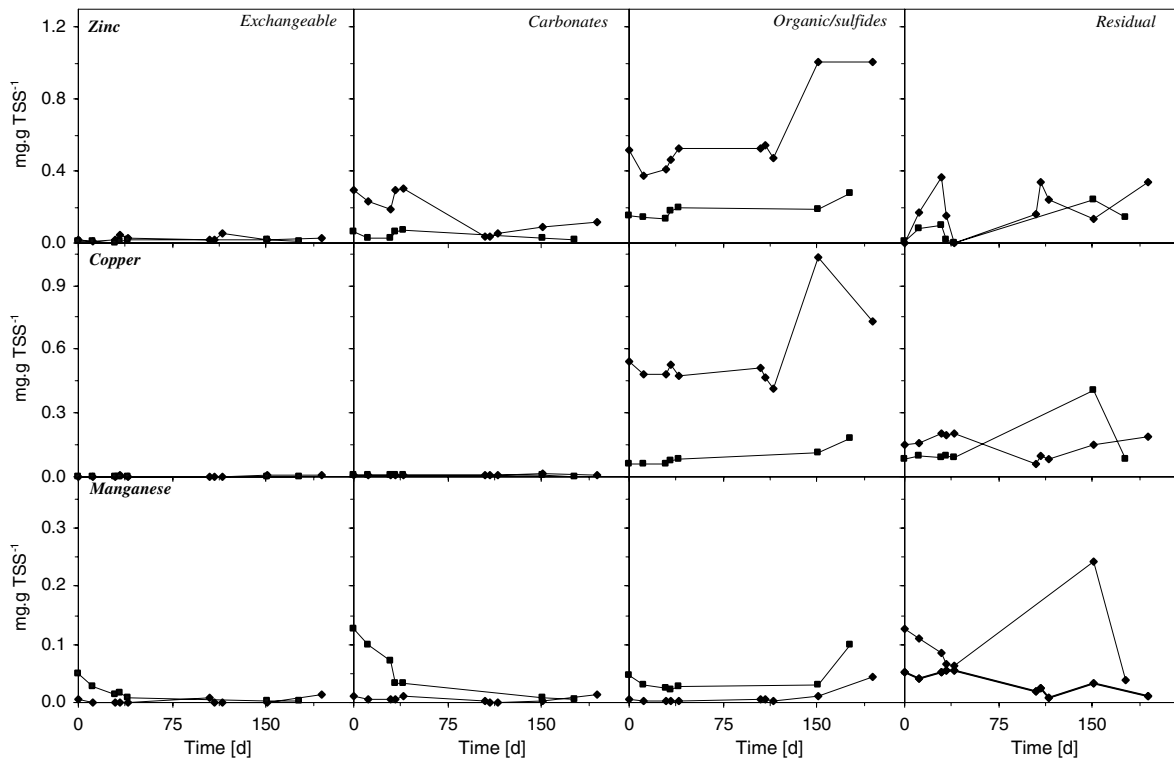

Figure 7. Evolution of the zinc, copper and manganese content present in the extracted fractions of the sludge in R1 (♦) and R2 (■).

R1 (Table 1), even though the performance of this reactor became only disturbed by a second pH shock on day 105 (Period IVa).

Following pulse doses a, b and c (Table 1) a sharp decrease in the effluent VFA concentration manifested, apparently stabilizing at a concentration of 250 mg COD l^{-1}. A second pulse dose of zinc (d) coincided with a pH drop, which resulted in a VFA increase in the effluent and confounded a possible zinc dosage effect. The same holds for the next imposed nickel/manganese pulse, which also coincided with a pH drop at day 97 (e). Thereafter two pulse doses of zinc (f, g) were supplied followed by continuous addition of zinc (h, i), which both did not affect the rate of VFA decrease in the effluent (Figure 8).

Before zinc was supplied with the influent, this element was hardly detectable in the R2 effluent (Figure 3). The effect of its continuous dosing clearly manifested in the R2 effluent, on average effluent concentrations were 8 μg l^{-1} (0.12 μM) from day 130 to 152 when it was continuously supplied at a relatively low concentration (Table 1) and an average effluent concentration of 76 μg l^{-1} (1.16 μM) was found during the second continuous zinc dosing phase at high concentrations (day 152–173). The effluent sulfur concentration of R2 was 9.2 ± 2.5 mg l^{-1} during periods III and IV (Figure 2).

Long term pH shock (4 days)

During a second 4 day pH shock (pH 5) imposed to R1 on day 105 (period IVa), the effluent methanol concentrations raised to 1579 mg COD l^{-1} on day 109, while the VFA concentration only increased slightly to a value 49 mg COD l^{-1}.

Similarly to the first 30 h pH shock again discrete peaks manifested in the effluent concentration for cobalt, nickel and iron, i.e. 19 μg l^{-1}, 31 μg l^{-1} and 1.41 mg l^{-1}, respectively (Figure 3) and concentrations of these metals remained elevated during the entire period of the shock. Losses of cobalt, nickel and iron in soluble form from the reactor during this 4 day pH shock were 0.156 and 0.197 and 12.12 mg, respectively. At termination of the pH shock, the cobalt, nickel and especially the iron concentration returned rapidly to values similar or slightly higher than those measured before the shock (Figure 3).

The cobalt content of the organic/sulfide fraction of the R1 sludge decreased with 0.20 mg g TSS^{-1} (18%). The total iron content of the sludge decreased with 0.87 mg g TSS^{-1} or 11%, losses form the organic/sulfide fraction were even higher 1.21 mg g TSS^{-1} or 29%. The iron content of the exchangeable and carbonate fraction became slightly higher, which explains the higher losses from the organic/sulfide fraction compared to the loss of the total iron content. During this 4 day pH shock, the sulfur content of the sludge decreased as well, sulfur was merely lost (1.83 mg g TSS^{-1}, 29%) from the organic/sulfide fraction.

Acidogenesis in R1

After the second 4 day pH shock, VFA accumulated in the R1 effluent, while in contrast to R2 also the methanogenesis was clearly disturbed as appeared from the higher concentration of methanol in the effluent from day 106 onwards (Figure 1A). The effluent methanol concentration of R1 started to decrease from day 151 onwards, accompanied by the onset of methane formation (Figure 1C), and methanol could not be detected anymore in the effluent on day 161. At the same time (day 151), the VFA formation started to increase from 201 mg COD l^{-1} on day 145 to 441 mg COD l^{-1} on day 161, but after day 161 the VFA concentration sharply decreased (Figure 1B).

During the poor performance of R1 (Figure1A, B), zinc was dosed to the system, either in pulses (Table 1; f and g) or continuous (Table 1; h and i). The decrease in effluent methanol concentrations with the simultaneous increase of the VFA concentration occurred when zinc was dosed continuously at higher concentrations. Once several metals were dosed (j), the VFA concentration in the effluent decreased sharply as well.

Zinc was present in the R1 effluent even before it was supplied to the system. The continuous dosing of zinc did not result in elevated effluent concentrations and the concentration averaged 37 μg l^{-1} (0.56 μM) during period III and IV.

Evolution of the specific methanogenic activity

In the SMA test conducted with the inoculum sludge (day 0), a significant methane formation

only started after ±150 h of incubation (Figure 9). This suggests methanol utilizing methanogenic populations were absent (or inactive) in both types of sludge. The assessed SMA at day 0, with a feed of only 4 g COD l^{-1}, amounted to 740 and 294 mg CH_4–COD g VSS^{-1} d^{-1} for the R1 and R2 sludge, respectively (Table 5). The imposed short term pH shock did not affect the SMA on methanol of both sludges. The SMA's at termination of the experiment of both R1 and R2 sludge were remarkably high, with values of 6619 and 2165 mg CH_4–COD g VSS^{-1} d^{-1}, respectively.

Methane formation in the SMA assay on acetate with the inoculum started after a lag phase of only ±7 h (data not shown), indicating that an active acetate utilizing methanogenic population was present in both sludges. The SMA with acetate of the R1 sludge on day 34 was 51% higher than the inoculum, but it decreased to the values found for the inoculum during and after the pH shock (Table 5). No clear effect of the pH shock was observed for the R2 sludge. The different effect of the pH shock on the acetotrophic methanogens of the R1 and R2 sludges becomes even more clear from the methane production rate curves (Figure 10): the maximum methane production rates during and after the pH shocks for the R1 sludge are lower and appear at a later stage of the assay.
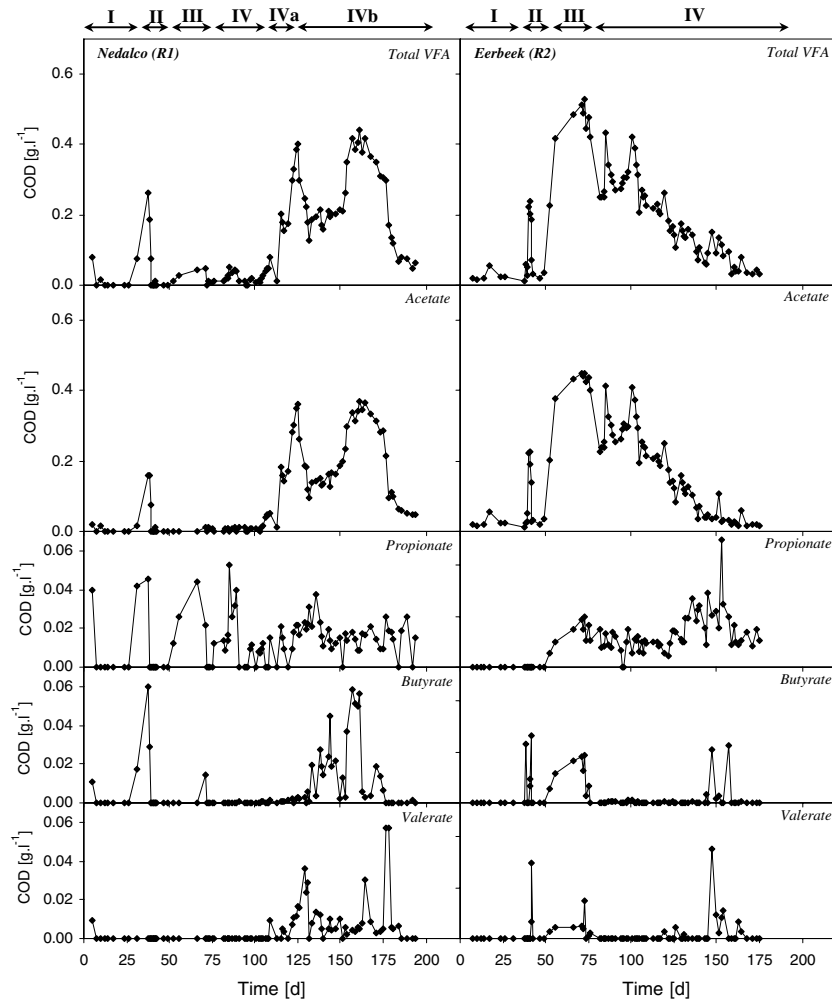

Figure 8. Evolution of the total and individual VFA concentration in the effluent.

Table 5. Evolution of the SMA with methanol, acetate and H_2/CO_2 as the substrate of both sludges

Time	R1 (mg CH_4–COD g VSS^{-1} d^{-1})			R2 (mg CH_4–COD g VSS^{-1} d^{-1})		
	Methanol	Acetate	H_2/CO_2	Methanol	Acetate	H_2/CO_2
0	740/268[a]	671/639[a]	577	294/243	242/237[a]	330
34	1411	998	513	700	359	185
37/39	1594	663	290	832	366	170
45	1496	690	382	792	336	189
84	n.d	n.d	n.d	n.d	0	n.d
194/178	6619	0	n.d	2165	0	n.d

[a] SMA before pre-loading with cobalt, iron and nickel; n.d not determined

In contrast, the methane production rate curves assessed for the sludge in R2 remain almost the same (Figure 10). Surprisingly, no SMA on acetate could be determined for the R2 sludge anymore on day 84. At termination of the experiment, no SMA on acetate was present in the sludge of both reactors anymore.

The SMA on H_2/CO_2 of the R1 sludge decreased from 513 mg CH_4–CODg $VSS^{-1}d^{-1}$, before the pH shock to 290 mg CH_4–COD.g $VSS^{-1}d^{-1}$ during the pH shock (Figure 11, Table 5), but then the SMA increased again to 382 mg CH_4–COD g VSS^{-1} d^{-1} 7 days after the pH shock. However, the pressure in the headspace of the bottles decreased at a similar rate during and after the pH shock (Figure 11). The assessed higher SMA can be attributed to the higher methane percentage (81%) in the headspace found at termination of the experiment after the pH shock, compared to the 62% methane found during the pH shock. Prior to the pH shock the methane percentage in the headspace was 82 and

86% during, respectively, the start up and at day 37. For the R2 sludge, a less pronounced effect of the pH shock on the SMA with H_2/CO_2 as the substrate was found (Table 5). A similar reduction of the methane percentage in the headspace of the assay bottles was observed: 62% during, 90% before and 83% after the pH shock, respectively. The sludge sample during the pH shock had a longer lag phase before a faster pressure decrease occurred (Figure 11).

Evolution of the metal retention in the sludge during the reactor run

The total amount of cobalt (2.930 and 1.182 mg g TSS^{-1} for the R1 and R2 sludge, respectively) and nickel (3.187 and 1.235 mg g TSS^{-1} for the R1 and R2 sludge, respectively) of both inoculum sludges differed considerably (Table 6). A significant amount of cobalt (1.166 mg g TSS^{-1}; 40%) and nickel (1.511 mg g TSS^{-1}; 47%), was
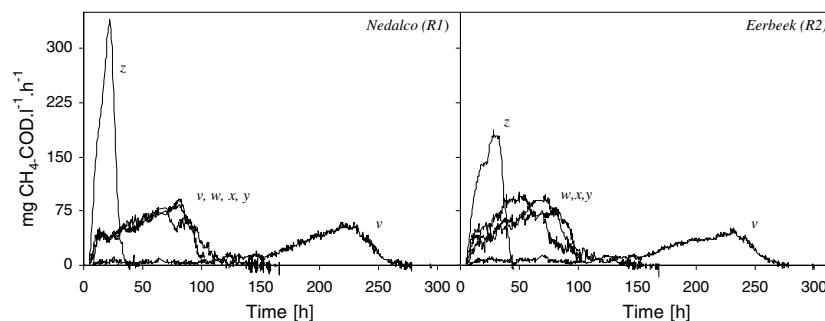

Figure 9. Methane production rate curves of methanogenic activity tests with methanol as the substrate. (v) initial rate (day 0), (w, x, y) rate curves before, during and after the first pH shock (day 34, day 37/39 and day 45). (e) the methane formation rate at termination of the experiment (day 194/178, respectively).
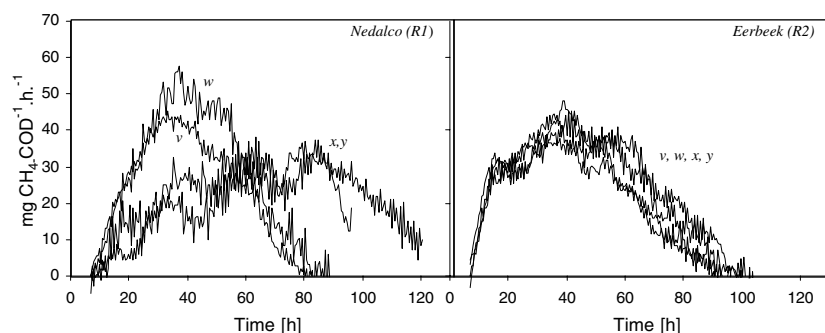
Figure 10. Methane production rate curves of the methanogenic activity tests with acetate as the substrate. (v) initial rate (day 0), (w, x, y) rate curves before, during and after the first pH shock (day 34, day 37/39 and day 45, respectively).

present in the exchangeable fraction of the R1 sludge, while the relative contribution of this fraction was small in the R2 sludge, 0.161 mg g TSS^{-1} (14%) and 0.265 mg g TSS^{-1} (21%) for cobalt and nickel, respectively (Table 6).

Absolute cobalt and nickel losses were considerably higher from the R1 sludge, mainly because of depletions of the exchangeable and carbonate fraction, which initially contained high amounts of these metals (Table 6, Figure 5). The pattern of the cobalt and nickel losses from the different fractions during the reactor run, however, was similar for the R1 and R2 sludge. Depletion of the exchangeable and carbonate fraction occurred in both the R1 and R2 sludge and the cobalt and nickel content of the organic/sulfide fraction was maintained in both sludges or even increased during operation (Table 6, Figure 5).

The total iron content of the R1 and R2 sludge at the termination of the experiment were, respectively, 76 and 37% lower than the initial content. The relatively large iron losses of the R1 sludge can mainly be attributed to depletion of the

residual fraction (81%), which initially contained 76% of the total iron content (Table 6, Figure 5). For the R2 sludge, the main loss of iron was from the carbonate fraction, which contained 31% of the iron and was for 92% depleted. Also significant losses from the residual fraction occurred (Table 6).

The total zinc content of the R1 sludge remained approximately the same until day 115. In the inoculum of R1, some (0.29 mg g TSS^{-1}) zinc was present in the carbonate fraction, which was depleted during operation (Figure 7). On day 151 however, due to the continuous supply of zinc with the effluent (1,12 μmol day^{-1}), the zinc content of the sludge had increased from 0.82 to 1.24 mg g TSS^{-1} and increased further to 1.48 mg g TSS^{-1} on day 194 (after continuous dosing at 11.25 μmol d^{-1} from day 151 onwards). The zinc supplied with the effluent mainly accumulated in the organic/sulfide fraction (Figure 7). In R2 upon dosing, zinc also accumulated in the sludge from 0.29 to 0.45 mg g TSS^{-1} (also mainly in the organic/sulfide fraction).

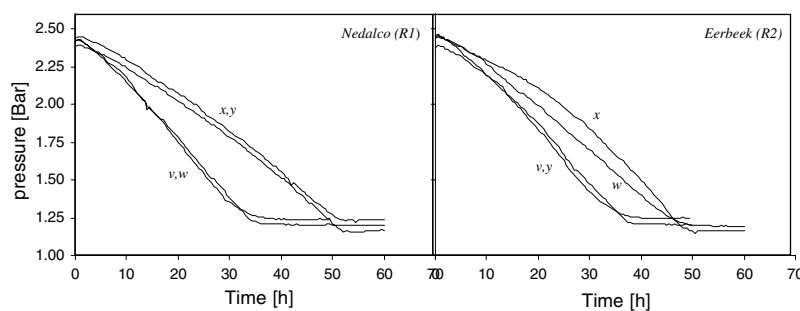*Figure 11.* Pressure decrease during methanogenic activity tests with H_2/CO_2 as the substrate. (v) day 0, (w, x, y) rate curves before, during and after the first pH shock (day 34, day 37/39 and day 45, respectively.

Table 6. Cobalt, nickel and iron content at the start (day 0) and end (day 194 and 177 of operation, for R1 and R2, respectively) of the experiment and percentage of the metals lost (or accumulated) in total and in individual extracted fractions

	R1			R2		
	Day 0 (mg g TSS^{-1})	Day 194 (mg g TSS^{-1})	Loss/acc. (%)	Day 0 (mg g TSS^{-1})	Day 177 (mg g TSS^{-1})	Loss/acc. (%)
Cobalt						
Total	2.930	1.101	−63	1.182	0.847	−28
Exchangeable	1.166	0.122	−90	0.161	0.008	−95
Carbonates	0.536	0.093	−83	0.256	0.007	−97
Organic/sulfide	0.657	0.738	+13	0.545	0.475	−13
Residual	0.571	0.148	−74	0.220	0.357	+62
Nickel						
Total	3.187	1.315	−59	1.235	0.970	−21
Exchangeable	1.511	0.259	−83	0.265	0.017	−94
Carbonates	0.834	0.209	−75	0.453	0.027	−94
Organic/sulfide	0.445	0.702	+58	0.346	0.533	+54
Residual	0.397	0.145	−63	0.172	0.393	+128
Iron						
Total	24.006	5.783	−76	38.350	24.094	−37
Exchangeable	0.035	0.037	+5	0.096	0.125	+31
Carbonates	4.037	1.127	−72	11.875	0.962	−92
Organic/sulfide	3.931	3.113	−21	5.359	6.093	+14
Residual	18.141	3.461	−81	21.058	16.914	−20

Discussion

Metal retention

This research showed that both the pH of the reactor mixed liquor and the sludge source strongly affect the metal retention dynamics in UASB reactors. The losses resulting from the pH shocks of metal pre-loaded sludge (cobalt and nickel) were limited, except for iron, which was significantly solubilised from the sludge. A significant effect of the short term pH shock was the translocation of metals between the different operationally defined fractions (Figures 5 and 6). Particularly in the R2 sludge, the iron and sulfur content increased in the organic/sulfide fraction with 5.15 and 6.53 mg g TSS^{-1} (molar ratio Fe:S, 1:2.2), respectively (Figures 5 and 6). At the same time, the iron and sulfur content in the residual fraction decreased with 2.83 and 5.4 mg g TSS^{-1}, respectively. Apparently, a transformation of the stability of the iron sulfides present in the solid

phase occurred during the pH shock. Anderko and Shuler (1997) showed indeed that the pH conditions may cause such a transformation of crystalline into more amorphous iron sulfide solids. This means that iron and sulfide are already extracted from the sludge by less stringent reagents, i.e. they are extracted in an earlier extraction step of the sequential extraction procedure (Figure 5).

Cobalt and nickel were concomitantly translocated from the residual fraction to the organic/sulfide fraction during the pH shock. Morse & Luther (1999) showed that cobalt and nickel tend to adsorb on or co-precipitate with iron sulfide, which may explain why the nickel and cobalt content of the organic/sulfide fraction of the R2 sludge also increased during the pH shock. Watson et al. (1995) showed that iron sulfide is a very efficient sorbant (high binding energy) for heavy metals such as cobalt and nickel.

In the sludge of R1, the cobalt and iron concentration during the first pH shock did not

increase in the organic/sulfide fraction, while also no effect on the sulfur concentration in both the organic/sulfide and the residual fractions was observed. In contrast, during the 4 day pH shock a decrease of the cobalt, iron and sulfur content occurred from the organic/sulfide fraction. This indicates that cobalt and iron sulfides present in this organic/sulfide fraction dissolve and are then lost with the effluent during a longer pH shock. Various readily soluble minerals can be expected to be present (Maes et al. 2003), e.g., amorphous FeS and/or crystalline FeS (such as mackinawite and greigite), which are the precursors of pyrite (Billon et al. 2001). According to Maes et al. (2003), dissolution of iron starts around a pH of 6 in a sulfidic canal sediment, an anoxic environment with similar physico-chemical characteristics to those in anaerobic granular sludge. This pH lies between the theoretical solubility of iron carbonate (siderite) and iron sulfide (mackinawite), Cobalt dissolution can also occur directly as the dissolution of cobalt sulfides is complete at pH 5 (van der Lee 2000). In order to explain the differences in metal retention, more insight in the sulfur speciation within the sludge is required with respect, to for instance, the amorphous and crystalline state of the sulfides present in the sludge. These can be determined by coupling the determination of acid volatile sulfide (AVS) with sequential extraction (Jong & Parry 2004) or X-ray adsorption near edge structures spectroscopy (XANES) (van Hullebusch et al. 2003).

The loss of cobalt, nickel and iron from the carbonate fraction (Table 6) during reactor operation is remarkable, but this has been observed previously for cobalt after pre-loading Nedalco sludge (Zandvoort et al. submitted). When nickel (5 μM) and iron (50 μM) were dosed with the influent, a constant nickel content in the carbonate fraction prevailed during reactor operation, while the iron content of this fraction even increased (Zandvoort et al. submitted). Sorption studies using the same sludges (van Hullebusch et al. submitted) showed that the exchangeable and carbonate fraction have a lower affinity for cobalt and nickel compared to the organic/sulfide fraction, which may explain why these fractions were depleted first.

Previous experiments conducted with cobalt pre-loaded R1 sludge showed a similar biphasic release of cobalt from the sludge during reactor operation (Figure 4), i.e. a relatively fast depletion of the exchangeable and carbonate fraction then followed by a slower depletion of the organic/sulfide fraction (Zandvoort et al. submitted). The R2 inoculum sludge did not accumulate cobalt well in the exchangeable and carbonate fraction (Figure 5), which explains its relatively better cobalt retention (Table 6) and the absence of such biphasic cobalt loss (Figure 4).

The total amount of cobalt and nickel lost from R1, based on its average effluent concentration from day 37 until day 103, were 0.89 and 1.21 mg cobalt and nickel, respectively. Based on the initial sludge content of the reactor, 13.3 and 13.2 mg cobalt and nickel was lost from the R1 sludge during this period, comprising a loss of approximately, 6.7 and 9.0% of the cobalt and nickel in a soluble form. This indicates that the main part of the cobalt and nickel is lost from the sludge in particulate form, e.g., as precipitates or associated to biomass.

Following the 4 day pH shock the cobalt concentration in the effluent returned slowly to the original values measured before the shock (Figure 3). This could be related to losses of cobalt containing enzymes like vitamin B_{12}, which can be overproduced by metabolising cells and/or released from decaying cells (Mazumder et al. 1987), likely due to the pH shock.
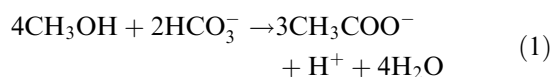
Reactor start up

The start up of both reactors proceeded considerably slower than observed in previous experiments with the same inoculum. The inoculum sludge from both R1 and R2, not pre-loaded with cobalt, nickel and iron completely removed methanol after 17 and 12 days, respectively (unpublished results), while it only needed 10 days for the Nedalco sludge (R1) when pre-loaded with a 1 mM cobalt solution for 24 h (Zandvoort et al. 2004). Apparently, the pre-loading of the sludge with a 1 mM cobalt, iron and nickel solution for 4 days initially inhibited the growth or activation of the methanol degrading methanogens. Also the relatively long lag-phase of $\pm$150 h needed for methane formation on methanol in the SMA assays (day 0) is an indication that these organisms are inhibited (Figure 9). Remarkable is also the close similarity found for the lag-phases for both innoculum

sludges and for the shape of the rate curves with methanol (Figure 9). Apparently, the growth/activation of the methanol degrading methanogens in both sludges, which had previously not been exposed to methanol, proceeds at a similar rate. The acetotrophic methanogens already present in the sludge remained unaffected by the metal loading procedure: because the SMA before and after loading was similar (Table 5) while also a lag phase was almost absent in the SMA assays after loading.

Reactor acidification

For the formation of acetate from methanol, CO_2 is required as a cosubstrate (Equation 1). Because no bicarbonate was supplied with the influent merely the CO_2 generated by the direct formation of methane from methanol (Equation 2) will be available for acetogenesis.

$$4CH_3OH + 2HCO_3^- \rightarrow 3CH_3COO^- \\ + H^+ + 4H_2O \qquad (1)$$

$$4CH_3OH \rightarrow 3CH_4 + HCO_3^- + H^+ + H_2O \qquad (2)$$

Based on the stoichiometry of these conversion reactions at the maximum one third of the methanol supplied can be converted to acetate. This implies that, when methanogenesis is not the limiting factor, at the maximum 600 mg COD l^{-1} of acetate can be formed from the 1800 mg COD l^{-1} of methanol supplied. This explains the limited extent of the acidification proceeding in both reactors, as well as the observation that in R1 higher acetate concentrations in the effluent only prevail when methanogensis from methanol occurs (Figure 1; day 121–126 and day 151–161). The amount of acetate formed in R1 ($\pm$165 mg l^{-1}) in the absence of methane formation (Figure 1; day 117–119 and day 132–151) therefore can only be attributed to the presence of some CO_2 in the system.

The question why the acetate formed in both reactors is not converted to methane remains to be elucidated, the more because just 4 days before the onset of acetate accumulation in R2 (day 49), the SMA on acetate of the R2 sludge was still considerable (336 mg g VSS^{-1} d^{-1}) and a negative effect of the pH shock on the SMA was not observed (Table 5, Figure 10). Consequently, in principle the acetate formed could have been converted to methane. On the other hand, a seri-

ous loss of SMA on acetate was observed for the R2 sudge at day 84 and for the R1 sludge at the termination of the experiment (but this most probably already occurred in an earlier stage). Loss of the SMA on acetate has been observed previously after prolonged (260 days) UASB reactor operation with methanol as the substrate (Zandvoort et al. 2002; Zandvoort et al. 2003). Apparently, the loss of methanogenic activity on acetate can occur rather abruptly. *Methanosarcina sp.*, the methanogen responsible for the direct conversion of methanol, in principle is also capable to use acetate, but looses this ability after prolonged cultivation on methanol (Boone et al. 1987; Smith & Mah 1978).

Effect of metal dosage on reactor performance

Upon continuous zinc dosage at 11.25 μmol d^{-1} (day 152), the elevated methanol concentration in the R1 effluent, which occurred after the second pH shock, dropped rapidly (Figure 1A). This may indicate that the recovery of the methanol degradation can be attributed to the zinc addition. Because zinc is involved in methanogenesis as it is part of coenzyme M, which catalyses the first step in the methanogenic pathway of methanol conversion, it is indeed an essential trace element (Sauer & Thauer, 2000). The high amount of zinc in the sludge (0.821 mg g TSS^{-1}) present at the start of the zinc supply (Figure 4), is either not bioavailable or incidentally the onset of methanogenesis coincided with the continuous supply of zinc.

From day 163 onwards, when a mixture of metals was supplied continuously (Table 1), the VFA concentration in the effluent of R1 decreased. Except for the addition of these metals, another reason for this decrease might be the outcompetition of the acetogens by the methanogens, because of the big differences in apparent substrate affinity coefficients (K_s) for acetogens (777 mg COD l^{-1}) and methanogens (12 mg COD^1). This favors the growth of methanogens at low methanol concentrations (Florencio et al. 1994) and such low methanol concentrations prevailed in R1 on day 161, when the VFA decrease started.

The decrease of the effluent acetate concentration in R2 proceeded slower compared to R1, and a clear impact of the zinc supply at different concentrations is not apparent. The acetate concentration of the R2 effluent decreased slowly from

day 105 until day 140 at a more or less constant rate of 6 mg l^{-1} per day. Likely this drop in R2 effluent VFA concentrations is also related to the outcompetition of the acetogens by methanogens and not to the methanogenesis of the formed acetate as a result of the zinc supply, because the SMA on acetate after 84 days and at the end of the experiment was nihil.

Conclusions

- The iron losses in soluble form with the effluent induced by the 30 h pH shock (pH 5) were considerable: 2.3 and 2.9% for R1 and R2, respectively, based on the initial iron content in the reactors, while cobalt and nickel losses in soluble form were limited.
- The 30 h pH shock (pH 5) in R2 induced a translocation of cobalt, nickel, iron and sulfur from the residual to the organic/sulfide fraction in the R2 (Eerbeek) sludge.
- Translocation of metals was not observed in the R1 (Nedalco) sludge during the first 30 h pH shock, but a second 4 day pH shock (pH 5) induced significant losses of cobalt (18%), iron (29%) and sulfur (29%) from the organic/sulfide fraction of the sludge.
- The SMA of the sludge on acetate in both R1 and R2 was lost during prolonged reactor operation.

Acknowledgements

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