

# **Cobalt and nickel retention capacity of anaerobic granular sludge in methanol-fed UASB reactors**

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## **1 Introduction**

Mine waters and industrial effluents contain high sulphate and metal concentrations and pose significant disposal problems, which require an urgent solution to avoid serious environmental problems (Tabak et al., 2003). Anaerobic bioreactors are a promising option for treatment of mine water because the biogenically produced sulphides can react with the dissolved metals to form metal sulphide precipitates, characterized by low solubility products. Metal accumulation in these anaerobic biofilms depends on the nature of the metal and organic constituents, and the composition and pH of the influent. (van Hullebusch et al., 2003).

The first part of this work describes the retention of cobalt and nickel in anaerobic granular sludge using non-continuous batch equilibrium techniques. Anaerobic sludge granules consist of a matrix of inorganic precipitates, biomass and extra cellular polymeric substances (EPS). Metals can bind to each of these phases of the granular sludge.

The sorption data are described by langmuir isotherms, which was shown to be the most accurate model for describing metal sorption (van Hullebusch et al., 2004). This model provides information about the metal retention capacity and binding strength of the sludge. However it does not provide information about the partitioning of metals in various chemical phases. In order to make this distinction the sorption isotherms were combined with sequential extraction procedures. In this study a modified Tessier sequential extraction scheme (Osuna et al., 2004) was used to make a distinction between the preferred phases for sorption.

The second part of this work describes the metal (cobalt and nickel) retention dynamics in lab-scale up flow anaerobic sludge bed (UASB) bioreactors when fed with an influent containing methanol as the carbon source. The reactors were inoculated with anaerobic granular sludge, which was pre-loaded with cobalt. The metal sorption and retention dynamics in the granules were compared to the results of the batch sorption experiments by using the same sequential extraction scheme. Also the effect of the absence or presence of a sulphur source as well as the type of sulfur source (cysteine and sulfate) on the metal retention was studied. Cysteine is sulphur source that can be used by methanol grown *Methanosarcina barkeri* as the sole sulfur source, this uptake is accompanied by the simultaneous evolution of  $S^{2-}$  (Mazumder et al., 1986)

## 2 Materials and Methods

### 2.1 Sludge characteristics

The anaerobic granular sludge used in this research originates from a full-scale UASB reactor treating alcohol wastewater (Nedalco, Bergen op Zoom, The Netherlands). The characteristics of this sludge are described in Table 1.

**Table 1.** Characteristics and metal content of the Nedalco granular sludge (mean  $\pm$  standard deviation, n=3)

<b>Parameter</b>	
Total suspended solids	8.3 ( $\pm 0.2$ )%
Total volatile suspended solid (VSS)	91.8 ( $\pm 0.2$ )%
Carbonates (% of TSS)	0.8 ( $\pm 0.2$ )%
Total sulfur	22.7 ( $\pm 0.1$ ) mg.g TSS <sup>-1</sup>
Total phosphorous	3.3 ( $\pm 0.1$ ) mg.g TSS <sup>-1</sup>
<b>Total metal content</b>	
Cobalt	18.7 ( $\pm 0.6$ ) $\mu$ g. g TSS <sup>-1</sup>
Nickel	130.3 ( $\pm 3.6$ ) $\mu$ g. g TSS <sup>-1</sup>
Copper	690 ( $\pm 10$ ) $\mu$ g. g TSS <sup>-1</sup>
Zinc	760 ( $\pm 20$ ) $\mu$ g. g TSS <sup>-1</sup>
Manganese	54.8 ( $\pm 0.2$ ) $\mu$ g. g TSS <sup>-1</sup>
Iron	20.8 ( $\pm 0.2$ ) mg.g TSS <sup>-1</sup>

## 2.2 Batch experiments

Cobalt and nickel binding was determined in batch experiments using 20g of wet sludge submerged in 50 ml of medium in 117 ml serum bottles. NaHCO<sub>3</sub> was added in the medium to mimic bioreactor conditions. The bottles were shaken at 100 rpm. The general Langmuir sorption model is expressed by:

$$Q_e = \frac{Q_{\max} b C_e}{1 + b C_e} \quad (2.1)$$

where  $Q_e$  is the uptake of the metal ion by the biomass (mg.g TSS<sup>-1</sup>),  $Q_{\max}$  is the maximum uptake (mg.g TSS<sup>-1</sup>),  $C_e$  is the final concentration of the metal in solution (mg.l<sup>-1</sup>) and  $b$  is the langmuir adsorption equilibrium constant (l.mg<sup>-1</sup>). This model implies that saturation of the adsorptive surface is possible, so  $Q_e$  eventually can reach a maximum value ( $Q_{\max}$ ).

## 2.3 Reactor experiments

Two UASB reactors with a working volume of 0.75 l each were operated as described elsewhere (Zandvoort et al., 2004). Each of the UASB reactors was inoculated with anaerobic granular sludge (20 g VSS.l<sup>-1</sup>). The sludge was pre-loaded with cobalt (1 mM CoCl<sub>2</sub>.6H<sub>2</sub>O) for 24h at 30°C in the absence of substrate.

The reactors were fed with methanol as a carbon source at an organic loading rate of 5 g MeOH COD. g VSS<sup>-1</sup>.d<sup>-1</sup> during the entire experiment. During the first period of operation (Period I, day 1-37), no sulfur source was added to the influent of one of the UASB reactors (R1) while 0.41 mM of sulphate was added to the influent the other UASB reactor (R2). During period II (day 38-87), L-cysteine (0.41 mM) was added as a sulfur source to the influent of R1, while the operational condition of R2 remained unchanged. In period III (day 88-118), no sulfur source and no metals were added to the influent of either reactor.

The reactor medium contained macronutrients (Zandvoort et. al., 2002): MgCl<sub>2</sub> was used instead of MgSO<sub>4</sub> for R1 and a trace element solution without cobalt, containing Fe (50 µM) and Ni, Zn, Mn, Cu, Mo, Se at concentrations of 5 µM. To ensure pH stability 30 mM (2.52 g.l<sup>-1</sup>) of NaHCO<sub>3</sub> was added to the basal medium. To prevent oxidation, the medium was kept anaerobic under a nitrogen atmosphere.

## 2.4 Sludge metal concentrations and sequential extraction procedure

The metal concentrations in the sludge were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES; varian Vista MPX, Palo Alto, USA). The total metal content was measured as described previously (Zandvoort et al., 2002). In order to assess the metal speciation in the granular matrix, the metals were sequentially extracted using a four- step extraction scheme (Table 2), in which each next step becomes more stringent.

**Table 2.** Sequential extraction procedure

Fraction	Extracting agent	Extraction conditions	
		Shaking	Temp.
1. Exchangeable	10 ml $\text{NH}_4\text{CH}_3\text{COO}$ , (1 M, pH = 7)	1 hour	20°C
2. Carbonates	10 ml $\text{CH}_3\text{COOH}$ , (1 M, pH = 5.5)	1 hour	20°C
3.Organic matter / sulfides	5 ml $\text{H}_2\text{O}_2$ , (30%, pH = 2)	3 hour	35°C
4. Residual	10 ml demineralised water, 10 ml aqua regia ( $\text{HCl}:\text{HNO}_3$ , 3:1)	26 min	Micro-wave

## 3. Results

### 3.1 Metal sorption

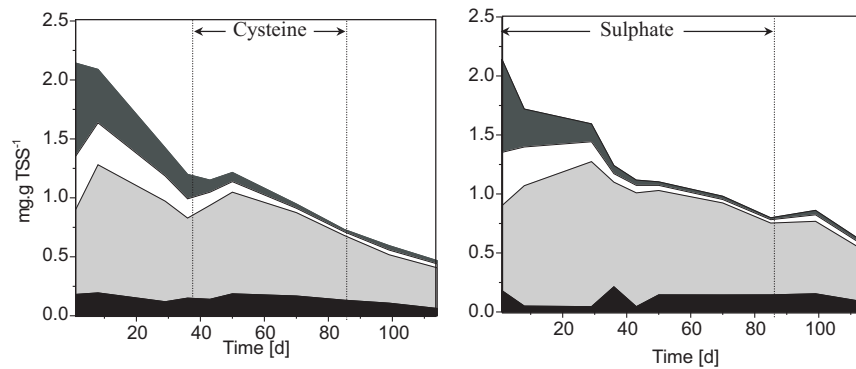
Table 3 gives the maximum adsorption capacity for each fraction extracted from the sludge, contacted with a single metal (cobalt or nickel) and competitive conditions (cobalt and nickel in equimolar amounts). The highest maximum sorption ( $Q_{max}$ ) was observed for the exchangeable fraction. However, this fraction also showed to have the lowest sorption affinity ( $b$ ) for cobalt, while the highest sorption affinity was observed for the organic matter and sulfides fraction. This was especially the case when nickel was added to the batches as a competing metal. The affinity for this fraction is then relatively high (Table 3). Nickel alone showed the lowest maximum but the highest sorption capacity in the organic matter/ sulfides fraction.

**Table 3.** Langmuir adsorption maxima ( $Q_{max}$ ) and affinity parameters ( $b$ ) of individual phases for the sorption of cobalt, cobalt in the presence of equimolar amounts of nickel (Co+Ni), and Nickel alone

	Co			Co+Ni			Ni		
	$Q_{\max}$ mg/g	b l/mg	$r^2$	$Q_{\max}$ mg/g	B l/mg	$r^2$	$Q_{\max}$ mg/g	b l/mg	$r^2$
Exch.	3.97	1.04	0.996	2.47	0.40	0.988	3.37	0.86	0.998
Carb.	3.45	1.21	0.991	1.59	3.35	0.999	2.16	0.59	0.995
OM/ S.	3.09	2.05	0.997	1.76	13.40	0.999	1.50	1.82	0.999
Res.	1.12	2.86	0.999	1.86	0.28	0.976	3.07	0.38	0.997

### 3.2 Metal retention in UASB reactors

The pre-loading of cobalt resulted in an initial concentration of 1.99 mg.g TSS<sup>-1</sup>. During the reactor operation no cobalt was added, the cobalt content of the sludge decreased considerably in both reactors at overall rates of 14 and 12 µg.g TSS<sup>-1</sup> in R1 and R2, respectively (Fig. 1). At termination of the experiment 22 % and 28% of the initial amount was present in R1 and R2, respectively. In the inoculum, 62% of the cobalt was present in the exchangeable and carbonate fractions. The initial depletion of cobalt from the sludge was fast and mainly from these fractions (Fig. 1). During the first 36 days, the average depletion rates were 22 and 21µg.g TSS<sup>-1</sup> for R1 and R2, respectively.

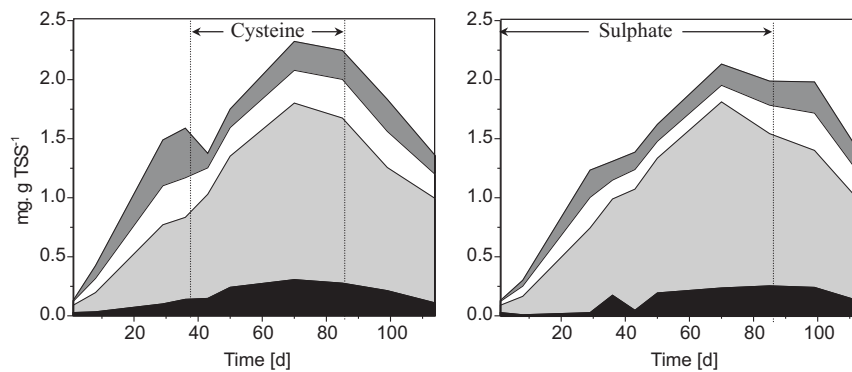


**Fig. 1.** Evolution of the cobalt concentration in the extracted fractions of R1 (left) and R2 (right). Residual fraction (black), organic/sulfides fraction (light grey), carbonate (white) and exchangeable (dark grey)

The depletion of cobalt occurred in two phases, an initial fast depletion rate, governed by the exchangeable and carbonate fraction, was followed by a slower phase governed by the organic/sulfides fraction (Fig. 1), apparently independent of the sulfur source. This confirms the results of the

sorption experiments (Table 3), as the affinity for these fractions is relatively low. The only effect of the sulfur source on cobalt retention was that some transfer of cobalt from the exchangeable and carbonate fraction to the organic/sulfides phase occurs in the presence of a sulfur source from day 38 onwards and from start-up in R1 and R2, respectively (Fig. 1).

The nickel content of the sludge in both reactors increased constantly with time (Fig. 2), independent of the sulfur source. Nickel mainly accumulated in the organic/sulfides fraction, reaching a maximum on day 70 of 1.50 and 1.57  $\text{mg.g TSS}^{-1}$  for this fraction in the R1 and R2 sludge, respectively. This is similar to the maximum sorption capacity of this sludge for this fraction (table 3). This result is also in agreement with the relatively high affinity for sorption of this fraction (Table 3). When nickel was omitted from the feed (day 88), the content of the sludge decreased at rates of 22 and 18  $\mu\text{g.g TSS}^{-1}$



**Fig. 2.** Evolution of the nickel concentration in the extracted fractions of R1 (left) and R2 (right). For explanation of the fractions: see Figure 1

#### 4. Summary and conclusions

The maximum nickel and cobalt sorption and the affinity for the sorption on operationally defined sorption phases in anaerobic granular sludge can be described by the langmuir sorption isotherm. The fraction with the highest affinity, organic matter/sulphides governed the cobalt retention and nickel accumulation in lab-scale UASB reactors. Cobalt losses from the

sludge were hardly influenced by the presence of a sulfur source and occurred mainly from the low affinity fractions (exchangeable and carbonates). Not only the sorption but also the long-term retention will determine the applicability of granular sludge as a metal sorbent, therefore future work will focus on the characteristics of the granular sludge required for maximum as well as the long-term retention of metals by anaerobic granular sludge.

## 5. References

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