Essential metal depletion in an anaerobic reactor

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Abstract The effect of the absence of trace elements on the conversion of a mixture of volatile fatty acids by a distillery anaerobic granular sludge was investigated. Two UASB reactors were operated under identical operational conditions except for the influent trace metal concentrations, during 140 days. Experiments were carried out in three periods, where different organic loading rates (OLR) were applied to the reactors. The total trace metal concentration steadily decreased at a rate of 48 µg metal/g TS.d in the deprived reactor (down to 35% of their initial value). In contrast, trace metals accumulated in granules present in the control reactor. At the end of the experiment, the COD removal efficiencies were 99% and 77% for the control and deprived reactors, respectively, due to the lack of propionate conversion. Cobalt sorption experiments were carried out in order to study its speciation, and its effects on the speciation of other metals as well. A paper mill wastewater treating granular sludge was also included in the study as a comparison. Results obtained showed that the principal metal forms normally associated with any sludge are a function of each soluble metal concentration in the system, and the characteristics of the particular sludge.

Keywords Activity losses; granular sludge; metal deprivation; metal speciation

Introduction

The effect of the addition of trace metals on the performance of bioreactors has been an important study field in anaerobic processes, as metals are involved in the enzymatic activities of acidogenesis and methanogenesis (Scherer et al., 1983). However, as reactors are usually started up using inocula treating other wastewater types containing an ill defined stock of metals, the minimal amount of trace metals required for optimal performance of such anaerobic systems is still not well defined.

Measurement of the total concentration of trace metals does not always provide adequate information about its bioavailability and, thus, its toxicity (Oleszkiewicz and Sharma, 1990). The distribution of those metals into different fractions (organic and inorganic, free ion or chelated), that is, the speciation, determines their availability for the metabolic activity.

Behaviour of metals in methanogenic aggregates is further complicated by their biosorption to extracellular polymers as well as by their precipitation as sulphides, phosphates, and carbonates (Artola et al., 1997). The metal adsorption can be attributed to different factors the presence of extracellular polymeric substances (EPSs) excreted by the microorganisms being an important one (Lin, 1993). Granules may have a stock of trace metals, adsorbed to the granular sludge matrix, which could be used to sustain the metabolic requirements during the operation of the system, without any further trace element addition. This has an economic importance for the operation of industrial anaerobic reactors (Espinosa et al., 1995), and a more thorough understanding will allow a more economic and effective control of the process.

For this purpose, it is necessary to know the distribution of trace elements present in anaerobic methanogenic aggregates, which can be done using chemical sequential...
extraction methods. Previous test trials showed that cobalt had a tendency to accumulate in high quantities on the sludge. Its presence may interact with the behaviour of other metals.

The objective of the present study was to define the effects of specific metal deficiencies on the performance of laboratory-scale UASB reactors during the conversion of a volatile fatty acids mixture. For this purpose, the speciation of trace metals present in two different anaerobic granular sludges was determined, using a sequential extraction procedure. Also, the effect of cobalt accumulation on the global metal speciation was studied.

**Materials and methods**

**Essential metal depletion**

Experiments were performed using two UASB reactors inoculated with a methanogenic granular sludge, obtained from the full scale UASB reactor treating alcohol distillery wastewater of Nedalco (Bergen op Zoom, The Netherlands). Both reactors were operated under identical operational conditions (pH = 7, T = 30°C, HRT = 12 hours, and an upflow velocity of 1 m h⁻¹) during 140 days, except for the influent trace metal concentration. Experiments were carried out in three periods, where different organic loading rates (OLR) were applied to the reactors (Figure 1) from 2 g COD/l d (Period I) up to 10 g COD/l d (Period III). One reactor (R1) was operated as control, supplemented with a trace metals solution (Ni, Co, Fe, Se, Mo, Cu, Zn, and Mn), whereas no trace metals were supplied to the second—deprived—reactor (R2).

Along the reactor run, the evolution of the specific methanogenic activity of the sludge was determined using a VFA mixture. All trace elements were supplied at the same concentration as present in the basal medium. The effect of the presence of individual trace elements (same concentration as present in the basal medium; all other trace elements excluded) on the maximum specific activity using a specific substrate, i.e. acetate, propionate or butyrate, was also investigated. To ensure pH stability at (7.0 ± 0.2), NaHCO₃ was added in the basal medium (2.53 g/l). After closing the bottles with butyl rubber septa (Rubber B.V., Hilversum, The Netherlands) and aluminium caps, the headspace was flushed with an excess of oxygen-free N₂/CO₂ (70:30).

**Trace metal speciation: sequential extraction**

The chemical sequential extraction method proposed by Tessier *et al*. (1979), and later on, modified by Ruiz *et al*. (1991), and Modak *et al*. (1992), was selected. The extraction scheme used with the methanogenic sludge samples differentiates the metal fraction associated to four stages, following a decreasing pattern of metal lability: exchangeable, carbonates, bound to organic matter and sulphides, and a residual stage. The procedure was applied to two different sludges: the one already used for the metal depletion experiments (distillery sludge), and a second sludge, obtained from a full scale UASB reactor treating paper mill wastewater (Eerbeek, The Netherlands). A second set of speciation tests were carried out with both sludges after sorption of cobalt. Granules were exposed for 4 days to cobalt (1 mmol l⁻¹ and 10 mmol l⁻¹) in basal medium prior to the sequential extraction. All soluble metal solutions were analysed by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Perkin-Elmer, model Elan 6000).

**Results and discussion**

**Reactor operation results**

Reactors were operated for 140 days in three periods (Figure 1). Period I, at 2.5 g COD/l d was used for the start-up. Sludge samples were taken at day 40. Both reactors showed similar behaviour, with some propionate accumulation. On Period II, from day 57 to day 74, reactors were operated at an organic loading rate of 5 g COD/l d. Propionate further
accumulated on both reactors, and the deprived reactor also showed a slight accumulation of acetate. Organic loading rate was then increased, on Period III, up to 10 g COD/l d. The control reactor had an initial accumulation of both acetate and propionate but large scale removal started after day 100 and was complete at day 133. That was not the case for the deprived reactor, and at the end of the experiment, the COD removal efficiency also decreased (99% vs. 77% for the control and deprived reactors, respectively).

**Metal depletion results**

For most metals (Co, Fe, Ni, Mn, Mo, and Se), the sludge growing in R1 was enriched with metals (up to 35% compared to inoculum), whereas metals clearly leached out from the sludge of R2 (Figure 2). Cobalt accumulated in R1 to a final concentration of 2.89 mg/g TS at the end of the experiment (140 days), compared to 0.02 and 0.01 mg/g TS, in the inoculum and the final sludge from R2, respectively. Also, at the end of the experiment the iron content was different, 3.54 and 1.32 mg/g TS for R1 and R2, respectively, as well as the nickel content in R2 sludge (0.07 mg/g TS) which was lower when compared to sludge of R1 (0.26 mg/g TS).

Manganese, molybdenum and selenium content of the deprived sludge was about 10 times lower than in the control sludge. The omission of the trace metal solution in the influent of the R2 did not have a big influence on the Ca, Mg and P concentration in the sludge (data not shown).

When specific activity tests were done using propionate as the only substrate, no conversion occurred after 400 hours of incubation of sludge from R2. In contrast, propionate was completely converted to CH₄ after 300 hours with sludge from R1 (data not shown), which had a maximum specific activity of about 100 mg COD/gVSS d in the presence of all trace elements.

Morphology of the sludge from both reactors at the end of the experiment presented some differences. As a result of metal accumulation (e.g. cobalt and iron) within the granules, the sludge in R1 showed black voluminous inclusions. In contrast, granules from the deprived reactor were pale, flat and easily broken.

**Metal speciation results**

When using distillery granular sludge, the sequential extraction procedure showed that metal content was very low (Figure 3a), according to data obtained from the total digestion (Figure 2, day = 0). Co, Mo, Ni, Se and Mn were present at concentrations below 5 µg g/TSS, and only Cu and Zn presented higher values, mainly associated to the organic and sulfide fraction. In general, the metal content in paper mill sludge (Figure 3c) was much higher than in distillery sludge and presented a different speciation. Remarkable results are the high content of Mo and Mn in paper mill sludge and also Cu and Zn distributions, mainly associated to the residual fraction (Figure 3c). The distribution between fractions of the
metal macronutrients was similar for both sludges, although, their content in the paper mill sludge was 10 times higher than in the distillery sludge (Figures 3b and 3d).

As previously found on trial tests, cobalt showed a very high accumulation during the R1 reactor run (Figure 2a). In order to obtain information about the maximum adsorption capacity, and the effect of such sorption on the speciation of other metals, some experiments were carried out with both sludges. The initial cobalt concentration for distillery sludge was only half of that of the paper mill sludge, with 16 µg Co/g TS, of which, 36% was bound to the organic and sulphide fractions, 28% was associated to the residual fraction, 26% to the carbonates fraction, and 10% to the exchangeable fraction (Figure 3a). The paper mill sludge (Figure 3c) presented an initial cobalt concentration of 30 µg Co/g TS, of which 33% was bound to the organic and sulphide fraction, 30% was associated to the residual fraction, around 20% to the carbonates fraction, and 17% to the exchangeable fraction (Figure 3c).

The exposure to a 1 mmol l⁻¹ concentration of cobalt for 4 days showed that both sludges had a similar sludge adsorption capacity and distribution pattern (Figure 4). The addition of cobalt leads to its adsorption into the sludge with a total concentration (after

**Figure 2** Evolution of the trace element concentration in granules from the Control reactor – (a) and (b) – and from the Deprived reactor – (c) and (d). From left to right, data for the startup, and after 40, 100 and 140 days of operation, respectively.

**Figure 3** Distribution of the metal concentration (µg/g TS) in granules from the distillery sludge – (a) and (b) – and the paper mill sludge – (c) and (d). From left to right, bars represent the following fractions: exchangeable, carbonates, organic and sulphides, and residual. Trace elements are shown on (a) and (c); macroelements, on (b) and (d), respectively, for Ca, Fe, Mg and P.
4 days of contact time) of 1,770 µg Co/g TS and 1,560 µg Co/g TS, for the paper mill and distillery sludges, respectively. The largest amount of added cobalt was located for both inocula in the exchangeable fraction, associated to the high soluble cobalt concentration. It can be seen on Figure 4 that a very small fraction gets fixed into the residual fraction.

When the cobalt concentration used for the exposure was increased to 10 mmol l–1, the total amount of cobalt found in the paper mill sludge was 12 times bigger than for the 1 mmol l–1 experiment, the increase for the distillery sludge being only 4 times (Figure 4). It should be noted that, despite the different maximum capacity, no changes in the residual fraction were observed for the distillery sludge; in contrast, this fraction increased from 189 up to 1,862 µg Co/g TS for the papermill sludge. The addition of cobalt causes a decrease on the total concentration of Mo, Ni, Se and W present in the sludge (data not shown), indicating that there was a trace metal solubilization from the sludge. Also, the total amount of iron in the sludges decreased slightly after the addition of cobalt (1 mmol l–1). However, there was a significant change in the distribution of the metal into the different fractions. The organic fraction increased, at the expense of the exchangeable fraction, which decreased significantly from 2.74 mg Fe/g TS (inoculum) to 0.60 mg Fe/g TS (1 mmol l–1 of Co) (Figure 4). There was also a mobilisation of Fe from the residual fraction and the content was distributed as organic > residual > carbonates > exchangeable fraction, by the addition of 1 mmol l–1 Co. The Ca and Mg behaviour was similar to that of Fe as they also showed an exchange of metal between the organic and residual fraction. The distribution was as follows: carbonates > exchangeable > organic > residual fraction.

Discussion
This study showed that omission of trace elements from the influent can lead to incomplete VFA degradation in UASB reactors, confirming that suboptimal metal concentrations may render the anaerobic degradation process inefficient (Figure 1). Especially, degradation of propionate was vulnerable to the continuous omission of metals. Induction of propionate degradation in the control reactor may be the result of adaptation and growth of natural populations of propionate degrading bacteria not present in the inoculum, due to its origin. Even at the end of the run (140 days), the deprived reactor was unable to degrade propionate, a probable consequence of the trace element deprivation, especially cobalt and
manganese, impeding the development of an active propionate degrading population (Öztürk, 1991). The complete removal of both butyrate and, its degradation product, acetate in both reactors during Periods I and II (Figure 3a and 3b) is not in agreement with the steady decrease in the total trace element content of the deprived reactor (Figure 2) at a washout rate of 48 µg metal/g TS.day. This suggests that previously accumulated trace elements in the inoculum sludge (an initial metal stock of 19.75 mg/g TS) were present in a high enough concentration to support the acetate and butyrate conversion during both periods (Artola et al., 1997; Leigthon and Forster, 1997; Liu et al., 1992).

Long term trace element deprivation led to a substantially lower methanogenic specific activity of the sludge. Goodwin et al. (1990) also found lower specific activities of Fe and/or Co deficient sludges. The direct addition of trace elements in a batch vial did not yield higher methanogenic activities (data not shown). This contrasts with the activity increase associated with the initial (first 40 days) trace element uptake by the control reactor granules, especially of Co and Fe (Figure 2). This uptake may depend on the chemical properties of the individual metal, and the characteristics of the sludge (Figures 2 and 4), which, in turn, are a function of the physical and chemical properties imposed by the particular sludge treatment process (Alibhai et al., 1985). Carbohydrate and protein components of Extracellular Polymeric Substances (EPS) are reported to sorb dissolved trace metals from aqueous media in biotechnological applications and suggest a high capacity of these groups for metal binding (Flemming, 1995; Gould and Genetelli, 1984).

After the reactors run, differences in physical appearance and degree of granulation of the distillery sludge for both reactors agreed with the larger solid washout from the deprived reactor. The metal concentration in the granules from the control reactor increased steadily as a result of the formation of mineral precipitates. Black ferrous sulphide might contribute to stabilisation of bacterial aggregates within granules (Artola et al., 1997). These accumulated either inside or on the surface of the granules. This happened because the bacterial growth rate is controlled by the COD loading rate and the availability of micronutrients, while the formation rate of inorganic precipitates is mainly related to the concentration of metals in the feed (Shen et al., 1993).

Sorption of cobalt experiments may be considered as a way of testing the binding capacities of the sludge (Figure 4). The cobalt addition to the sludge produced a cation exchange process, as there was Mo, Se, Ni, W (data not shown) solubilization from the sludge as well as a solubilization of the total Ca, Mg and Fe concentration (to an extent of 50%). According to that, studies on biological systems such as humic materials have shown that the metal uptake occurs by ion exchange of aqueous metal ions with anions sites associated with metals such as Ca and Mg, and by proton displacement.

Also, during the cobalt sorption experiments, the cobalt bound to organics and sulphides and to the residual fraction for the paper mill sludge had a high increase, associated to inorganic precipitates, as that sludge had a significant amount of sulphide, not found in the distillery sludge. When 10 mmol l⁻¹ Co was added (Figure 4), a great increase of the exchangeable fraction was found, associated to the high soluble cobalt concentration.

Several interactive effects between biomass and metals have been described, for instance, complexation and adsorption. However, the biologically active form of metals is reported to be the free metal ions. This warrants further research towards the bioavailability of metals in anaerobic sludges, e.g. using adsorption stripping voltammetry or ¹H Nuclear Magnetic Resonance imaging.

**Conclusions**

The lack of trace metals affected the overall process due to the sensitivity of the
metabolic pathway of propionate conversion in a UASB reactor operated at an OLR of 10 g COD/l.day, a pH \((7.0 \pm 0.2)\) and an HRT of 12 hours.

The addition of metals to the reactor influent resulted in a steady accumulation of these metals in the granules, especially cobalt. Their omission resulted in a continuous leaching of the metals. The continuous addition of metals to the influent stimulated the conversion of acetate, and achieved the full conversion of propionate, the sludge of the control reactor having higher maximum specific activity values compared to that of sludge from the deprived reactor. However, both sludges had a similar maximum specific activity using butyrate as substrate.

As cobalt showed a very important sorption of the sludge (up to 140 times the initial concentration), sorption experiments, done with two different sludges, showed that the total sorption of the metal depends on both the concentration of the metal during the exposure, and on the nature of the granule. Some of that metal remains on the exchangeable fraction, displacing other metals from the adsorption sites and/or producing a cation-exchange effect.

Also, an important fraction of the metal gets fixed onto the sludge as inorganic precipitates, depending on the chemical composition of the sludge and the medium. Speciation studies offer an insight into the distribution of metals in the granules and may help to understand the metal bioavailability under deprivation conditions.

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