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# Effect of vitamin $B_{12}$ pulse addition on the performance of cobalt deprived anaerobic granular sludge bioreactors

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ABSTRACT

The effect of a pulse addition of vitamin $B_{12}$ as cobalt source to restore the performance of cobalt depleted methanol-fed bioreactors was investigated. One upflow anaerobic sludge bed (UASB) reactor was supplied with a pulse of vitamin $B_{12}$, and its operation was compared to that of another cobalt depleted UASB reactor to which a pulse of $CoCl_2$ was given. The addition of cobalt in the form of $CoCl_2$ supplies enough cobalt to restore methanogenesis and maintain full methanol degradation coupled to methane production during more than 35 days after the $CoCl_2$ pulse. Similar to $CoCl_2$, pulse addition of vitamin $B_{12}$ supplies enough cobalt to maintain full methanol degradation during more than 35 days after the pulse. However, the specific methanogenic activities (SMAs) of the sludge in the vitamin $B_{12}$ supplied reactor were around 3 times higher than the SMA of the sludge from the $CoCl_2$ supplied reactor at the same sampling times. An appropriate dosing strategy (repeated pulse dosing) combined with the choice of vitamin $B_{12}$ as the cobalt species is suggested as a promising dosing strategy for methanol-fed anaerobic bioreactors limited by the micronutrient cobalt.

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## 1. Introduction

Cobalt plays a key role in anaerobic methanol degradation (Fermoso et al., 2008b; Florencio et al., 1993; Zandvoort et al., 2002). Lack of cobalt in the influent of an anaerobic reactor leads to a decrease in the specific methanogenic activity (SMA) of the sludge, resulting in methanol accumulation, and later acidification and ultimately complete process failure of methanol-fed reactors (Fermoso et al., 2008b).

Dosing strategies have been studied for methanol-fed upflow anaerobic sludge bed (UASB) reactors using cobalt chloride ($CoCl_2$) as the cobalt species dosed, including continuous addition (Zandvoort et al., 2002), pre-loading of the granular sludge (Zandvoort et al., 2004) or pulse addition of high amounts (77.5 μmol per litre) of $CoCl_2$ (Zandvoort et al., 2004). All these dosing protocols have as drawback that high amounts of cobalt are lost with the effluent. Fermoso et al. (2008a) studied the addition of $CoCl_2$ in repeated pulse additions of low amounts (5 μmol per litre) of cobalt, which minimizes the cobalt losses. Although cobalt in the form of $CoCl_2$ was well retained in the UASB reactor (93–95% retention) and the reactor function was sustained by this addition, the restoration of SMA by $CoCl_2$ was little efficient and also the high retention of cobalt in non-bioavailable forms (precipitates, sorption) decreased the overall efficiency of the cobalt addition (Fermoso et al., 2008a). To study the role of metal bioavailability of the dosed cobalt, Fermoso et al. (2008a) studied the addition of $CoEDTA^{2-}$ in repeated pulses as well. $CoEDTA^{2-}$ is retained much less, only about 5% of the dosed cobalt, compared to a retention of about 90% of the dosed cobalt in the granular sludge when dosing $CoCl_2$. Moreover, repeating $CoEDTA^{2-}$ pulses was not sustainable and the quality of the granular sludge as well as its activity deteriorated after four $CoEDTA^{2-}$ pulses (Fermoso et al., 2008a).

To overcome the drawbacks of $CoCl_2$ and $CoEDTA^{2-}$ pulses, another suitable cobalt source to dose cobalt for restoration of methanogenesis of methanol might be the cofactor cobalamin (vitamin $B_{12}$). Using cobalamin (vitamin $B_{12}$) brings two important advantages, first the molecule of vitamin $B_{12}$ can be actively transported across cell membranes (Zhang and Gladyshev, 2009) and second vitamin $B_{12}$ can be directly utilized as an enzyme in the process of methanogenesis (Matthews, 2001). Vitamin $B_{12}$ plays a key role in the first methyl transfer reaction that takes place in the anaerobic degradation of methanol to methane, as the cobalt(I) form of the cofactor serves as the methyl acceptor (Matthews, 2001). Therefore, the aim of this study was to evaluate the pulse addition of vitamin $B_{12}$ as cobalt source for methanol-fed UASB reactors and compare it to $CoCl_2$ pulsing. Moreover, the possibility of a vitamin $B_{12}$ pulse addition to restore methanogenesis in an acidified methanol-fed bioreactor was investigated. The evaluation was carried

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**Table 1**
Requrired amount of cobalt needed per day to support adequate methanogenesis.

| Cobalt source | Dosing strategy | Cobalt needed (μmol Cobalt $L_{Reactor}^{-1}$ $day^{-1}$) | Reference |
|---|---|---|---|
| $CoCl_2$ | Continuous | 0.15 | Zandvoort et al. (2002) |
| $CoCl_2$ | Pulse | 0.36 | Fermoso et al. (2008a) |
| $CoEDTA^{2-}$ | Pulse | 0.77 | Fermoso et al. (2008a) |
| $CoCl_2$ | Pulse | 0.12 | This study |
| Vitamin $B_{12}$ | Pulse | 0.12 | This study |

out by monitoring the performance of the UASB reactors, quantification of the cobalt retention into the granular sludge and determination of the evolution of the specific activity profile of the biomass present in the bioreactor over time.

## 2. Methods

### 2.1. Source of biomass

The UASB reactors were inoculated with 1.5 L anaerobic granular sludge obtained from a full-scale UASB reactor treating alcohol distillery wastewater of Nedalco (Bergen op Zoom, The Netherlands). The total suspended solids (TSS) and volatile suspended solids (VSS) content of the wet sludge amounted to 7.2 (±0.1)% and 6.7 (±0.1)%, respectively. The initial TSS and VSS content in the UASB reactors amounted to 7.5 (±0.3) and 6.5 (±0.1) g $L_{Reactor}^{-1}$.

### 2.2. Influent composition

The reactors were fed a basal medium consisting of methanol, macronutrients and a trace metal solution (Table 1). The inorganic macronutrients contained (in milligrams per litre of basal medium): $NH_4Cl$ (280), $K_2HPO_4$ (250), $MgSO_4{\cdot}7H_2O$ (100) and $CaCl_2{\cdot}2H_2O$ (10). To ensure pH stability around 7.0, 2.52 g (30 mM) of $NaHCO_3$ was added per litre of basal medium. To avoid precipitation in the storage vessels, the influent was composed of three streams: (1) macronutrients and trace metal solutions without $K_2HPO_4$ and $NaHCO_3$, (2) methanol with $NaHCO_3$ and $K_2HPO_4$ and (3) dilution water. Demineralised water was used to prepare the influent and as dilution water.

### 2.3. Experimental set-up

The experiments were performed using 3.25 L glass cylindrical UASB reactors as described by Fermoso et al. (2008c). The reactors were operated at 30 (±2) °C, at a hydraulic retention time (HRT) of 8 h and a superficial liquid upflow velocity of 0.5 m $h^{-1}$.

### 2.4. Experimental design

Three UASB reactors were run to which cobalt was added in pulses. The day at which the cobalt pulse was done in each reactor was defined as day 0. Every reactor had a start-up period of about 10 days prior to the cobalt pulse. One reactor (R1) was supplied with one pulse addition of $CoCl_2$. Its operation was compared to that of another UASB reactor (R2) to which one pulse of vitamin $B_{12}$ was given. The effect of vitamin $B_{12}$ pulsing on the performance of an already acidified reactor was investigated in R3.

Every pulse addition consisted of 17.5 μmol of cobalt, corresponding to 5 μmol cobalt per litre of reactor volume. This value supports stable methylotrophic methanogenic UASB bioreactors inoculated with the same inoculum sludge as used in this study (Zandvoort et al., 2003).

Methanol was fed to the reactors at an organic loading rate (OLR) of 8.5 g COD $L_{Reactor}^{-1}$ $day^{-1}$ for R1 and R2 and 12 g COD $L_{Reactor}^{-1}$ $day^{-1}$ for R3.

### 2.5. Specific maximum methanogenic activity tests

The SMA of the sludge was determined in duplicate at 30 (±2) °C using on-line gas production measurements as described by Fermoso et al. (2008b). SMA values reported refer to measurements done in the absence of cobalt in the test medium, unless specified otherwise. Gas production measurements were plotted in a rate versus time curve, using moving average trend lines with an interval of 15 data points. The samples were always taken from the same place (the mid-height) in the sludge bed.

### 2.6. Metal composition of the sludge

The metal composition of the sludge was determined after destruction with Aqua Regia (mixture of 2.5 ml 65% $HNO_3$ and 7.5 ml 37% HCl) as described by Fermoso et al. (2008c).

### 2.7. Analytical procedures

Methanol and volatile fatty acids (VFA) were determined using gas liquid chromatography as described by Fermoso et al. (2008b). Total dissolved metal concentrations in the influent and effluent were determined by ICP-OES (Varian, Australia) in samples acidified with 0.1 M $HNO_3$. The samples were centrifuged at 10,000 rpm to remove particles from the liquid phase. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations were determined according to Standard Methods (APHA/AWWA, 2005). All chemicals were of analytical or biological grade and purchased from E. Merck AG (Darmstadt, Germany).

## 3. Results

### 3.1. Pulse addition of cobalt to methanogenic UASB reactors

#### 3.1.1. Pulse addition of cobalt chloride to a methanogenic UASB reactor

No methanol or VFA accumulation was observed in the R1 run (Fig. 1A) besides a slight methanol accumulation during the reactor start-up (Fig. 1A), which was more than 5 days before the pulse was applied. The SMA with methanol as the substrate with cobalt present was 1.75 times higher compared to SMA medium without cobalt: 1380 and 800 mg COD-$CH_4$ g $VSS^{-1}$ $day^{-1}$, respectively (Fig. 1D) just before the $CoCl_2$ pulse was applied in the reactor.

The cobalt content of the sludge increased from 15 to 30 μg Co g $TSS^{-1}$ after the cobalt pulse (Fig. 1C). The cobalt content of the sludge was decreasing through the whole run from 30 μg Co g $TSS^{-1}$ on day 1 till 15 μg Co g $TSS^{-1}$ on day 42 (Fig. 1C). Interestingly, the SMA with methanol as the substrate without cobalt in the medium did not increase after the cobalt pulse. The SMA with methanol as the substrate without cobalt in the medium (the same conditions as in the reactor medium) was slightly decreasing from day 1 after the pulse (796 mg COD-$CH_4$ g $VSS^{-1}$ $day^{-1}$) till the end of the reactor run (42 days after the pulse; 650 mg COD-$CH_4$ g $VSS^{-1}$ $day^{-1}$). The SMA with methanol as the substrate, but in the presence of cobalt in the medium, increased strongly during the 42 days of reactor operation after the $CoCl_2$ pulse (from 1280 to 2359 mg COD-$CH_4$ g $VSS^{-1}$ $day^{-1}$, Fig. 1D).

#### 3.1.2. Pulse addition of vitamin $B_{12}$ to a methanogenic UASB reactor

No methanol or VFA accumulation was observed in R2 during the whole run (Fig. 2A). The SMA with methanol as the substrate

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