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Enrichment of denitrifying methanotrophic bacteria for application after direct low-temperature anaerobic sewage treatment

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HIGHLIGHTS

- A new concept for low-temperature anaerobic sewage treatment is proposed.
- In this concept, denitrification and methane oxidation are performed by *Methylomirabilis oxyfera*.
- The bacteria were enriched from fresh water sediment using sequencing fed-batch reactors.
- The volumetric consumption rate has to be increased by an order of magnitude for practical application.
- Further research should focus on systems with improved biomass retention.

GRAPHICAL ABSTRACT

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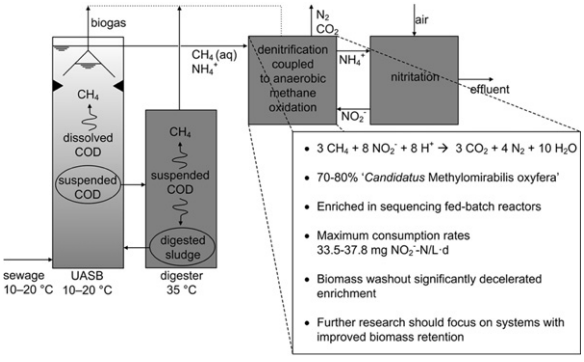

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ABSTRACT

Despite many advantages of anaerobic sewage treatment over conventional activated sludge treatment, it has not yet been applied in temperate zones. This is especially because effluent from low-temperature anaerobic treatment contains nitrogen and dissolved methane. The presence of nitrogen and methane offers the opportunity to develop a reactor in which methane is used as electron donor for denitrification. Such a reactor could be used in a new concept for low-temperature anaerobic sewage treatment, consisting of a UASB-digester system, a reactor for denitrification coupled to anaerobic methane oxidation, and a nitritation reactor. In the present study denitrifying methanotrophic bacteria similar to '*Candidatus* Methylomirabilis oxyfera' were enriched. Maximum volumetric nitrite consumption rates were 33.5 mg NO_2^--N/L d (using synthetic medium) and 37.8 mg NO_2^--N/L d (using medium containing effluent from a sewage treatment plant), which are similar to the maximum rate reported so far. Though the goal was to increase the rates, in both reactors, after reaching these maximum rates, volumetric nitrite consumption rates decreased in time. Results indicate biomass washout may have significantly decelerated enrichment. Therefore, to obtain higher volumetric consumption rates, further research should focus on systems with complete biomass retention.

1. Introduction

Anaerobic sewage treatment has many advantages over conventional activated sludge treatment. These include energy recovery as biogas instead of energy consumption, reduced sludge production and a smaller footprint (e.g. [1,2]). Despite these advantages

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and successful application of anaerobic sewage treatment in tropical regions it has not yet been applied in temperate zones [3]. For lower temperatures reactor systems with solid retention times (SRT) long enough for hydrolysis and growth of methanogens, but still relatively short hydraulic retention times (HRT) are required. Also, to comply with discharge standards, effluent from anaerobic treatment requires further treatment. This is required for remaining chemical oxygen demand (COD), but especially for nitrogen and phosphorus, which are largely conserved during anaerobic treatment. In addition, the effluent from a low-temperature anaerobic sewage treatment system contains a considerable amount of dissolved methane [4,5]. The concentration of dissolved methane can be 20 mg/L assuming Henry's law (calculated for atmospheric pressure, 10 °C and 70% methane in the biogas), and frequently methane supersaturation occurs [4,5]. If effluent containing dissolved methane would be discharged, methane would be released to the atmosphere. As it is a gas with a high global warming potential, dissolved methane has to be removed to reduce the greenhouse gas emissions of low-temperature anaerobic sewage treatment compared with conventional treatment.

Pilot-scale application of the combination of an upflow anaerobic sludge bed (UASB) reactor and a sludge digester, referred to as UASB-digester system, was successful for anaerobic sewage treatment at low temperatures [6–8]. In the UASB reactor (at 10–20 °C) dissolved COD is converted; solids are entrapped in the flocculent sludge bed and transported to the digester. In the digester (at 35 °C) suspended COD is hydrolyzed and the sludge is enriched in methanogens. The sludge is recirculated to the UASB reactor to provide methanogenic activity. With this system a total COD removal efficiency of 66% was achieved at a temperature of 15 °C and an HRT of 6 h while a long SRT of 21 d was maintained in the digester [7]. Although conventional technologies can be applied to remove remaining COD and phosphorus from the effluent, conventional nitrogen removal is not a preferred option. Effluent from an anaerobic system contains ammonium, which is usually removed by a sequence of nitrification to nitrate and heterotrophic denitrification. However, during anaerobic treatment the readily available carbon sources are removed and addition of an external electron donor, e.g. methanol, would be required to sustain heterotrophic denitrification. Anaerobic ammonium oxidation, an autotrophic process, would be an alternative [9]. However, this process will not remove dissolved methane. Instead, a new treatment concept is proposed, in which dissolved methane is used as electron donor for denitrification via nitrite. Such a system would solve two problems, viz. removal of nitrogen and dissolved methane. To provide nitrite a nitritation reactor is required. To conserve methane for denitrification and to save on aeration energy this reactor is positioned after the reactor for denitrification coupled to anaerobic methane oxidation. Combined, the UASB-digester, a reactor for denitrification coupled to anaerobic methane oxidation, and a nitritation reactor, to supply nitrite required for the denitrifying methanotrophic bacteria, offer a new opportunity for energy-efficient wastewater treatment with a reduced carbon footprint (Fig. 1).

Though denitrification coupled to aerobic methane oxidation was studied extensively (reviewed by [10]), the progress on denitrification coupled to anaerobic methane oxidation is slow due to a limited number of enrichment cultures [11]. However, denitrification coupled to anaerobic methane oxidation (Eq. (1)), would have several advantages over aerobic processes. These include that no oxygen is required for partial methane oxidation and methane is used more efficiently. This implies that more nitrogen can be removed using the methane dissolved in the effluent from UASB-digester systems.

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \quad (1)$$

A few years ago a denitrifying methanotrophic culture consisting of a bacterium and an archaeon was obtained under anaerobic conditions [12]. Further research has shown that the process also proceeds without the archaea, indicating that the dominant bacterium, '*Candidatus* Methylomirabilis oxyfera' (*M. oxyfera* hereafter) can catalyze the methane oxidation on its own [13,14], expressing a unique intra-aerobic pathway [15].

Typically effluent from anaerobic sewage treatment plants contains 50 mg N/L. Using the 20 mg/L of dissolved methane, 47 mg N/L could be removed according to the stoichiometry presented in Eq. (1). The maximum volumetric nitrite consumption rate of enrichment cultures coupling denitrification to anaerobic methane oxidation reported is 36 mg NO_2^--N/L d [14]. This rate would translate to an HRT of 1.4 d. Conventional denitrification typically has an HRT of 3–4 h. Thus, for a practical application of denitrification coupled to anaerobic methane oxidation for sewage treatment, volumetric nitrite consumption rate needs to be increased by an order of magnitude. However, a stagnating rate was observed in two enrichment cultures [13,14]. It was hypothesized this could be due to production of an inhibiting compound, or absence of an unknown growth factor. Since a completely stirred tank reactor with external settler and a sequencing batch reactor were applied, inefficient biomass retention may also have been a cause for the stagnating conversion rates.

The objectives of this study were (1) to enrich denitrifying methanotrophic cultures and (2) to increase the volumetric conversion rates of the enrichment cultures, so eventually the process can be integrated in the proposed concept for anaerobic sewage treatment at low temperatures.

Denitrifying methanotrophic bacteria were enriched for a period of 651 d in two sequencing fed-batch reactors. To increase maximum volumetric conversion rates, a long settling time was applied to improve biomass retention and effluent from a sewage treatment plant was fed to one of the reactors as a source of potential growth factors. The reactors were mixed by gas recirculation, providing sufficient transfer of methane. In both reactors, nitrite consumption rates were followed in time and whole culture batch tests were performed to measure denitrifying methanotrophic activity. Washout of biomass with the effluent was quantified to evaluate biomass retention of the systems. The practical applicability of a process with denitrifying methanotrophic bacteria for nitrogen and methane removal after direct low-temperature anaerobic sewage treatment is discussed.

2. Materials and methods

2.1. Inoculum

Two sequencing fed-batch reactors (SFBRs) were inoculated with sediment (3.7 ± 0.6 g protein each) from ditches in Ooijpolder, The Netherlands, similar to [14]. Prior to inoculation the sediment was sieved (1.0 mm) and diluted with ditch water to obtain a homogeneous slurry.

2.2. Medium

Medium contained (g/L) 0.1–1.0 $KHCO_3$, 0.05 KH_2PO_4, 0.30 $CaCl_2{\cdot}2H_2O$, 0.22 $MgSO_4{\cdot}7H_2O$, 0.069–4.83 $NaNO_2$ (0.014–0.980 NO_2^{-1}–N), 0.085–0.765 $NaNO_3$ (0.014–0.126 NO_3^{-1}–N), 0.6 mM HCl, 0.5 mL acidic trace element solution and 0.2 mL alkaline trace element solution (adapted from Ettwig et al. [14]). The acidic trace element solution contained (g/L) 2.085 $FeSO_4{\cdot}7H_2O$, 0.068 $ZnCl_2$, 0.12 $CoCl_2{\cdot}6H_2O$, 0.5 $MnCl_2{\cdot}4H_2O$, 0.32 $CuSO_4$, 0.048 $NiCl_2{\cdot}6H_2O$ and 100 mM HCl. The alkaline trace element solution contained (g/L) 0.067 SeO_2, 0.05 $Na_2WO_4{\cdot}2H_2O$, 0.284 $Na_2MoO_4{\cdot}2H_2O$ and 10 mM NaOH.