

Methane emission and methanotrophic activity in groundwater-fed drinking water treatment plants

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ABSTRACT

Groundwater for drinking water production may contain dissolved methane (CH₄) at variable concentrations. Most of this important greenhouse gas is often vented to the atmosphere during primary aeration and gas stripping processes at drinking water treatment plants (DWTPs). However, limited information exists regarding emission and fate of methane at many groundwater-fed DWTPs. This study estimates emission of methane from 1,004 DWTPs in Denmark and includes data from 3,068 groundwater wells. The fate of methane and occurrence of methane oxidizing bacteria in DWTPs was examined, including the potential role in ammonia removal. Methane emission from Danish DWTPs was estimated to be 1.38–2.95 × 10⁻⁴ Tg CH₄/y which corresponds to 0.05–0.11% of the national anthropogenic methane emission. Trace levels of methane remained in the drinking water after primary aeration and entered the sand filters as a potential microbial substrate. Methanotrophic bacteria and active methane oxidation was always detected in the sand filters at groundwater-fed DWTPs. Methanotrophic consortia isolated from DWTP sandfilters were inoculated into laboratory-scale sand filters and the activity confirmed that methanotrophic consortia can play a role in the removal of ammonia via assimilation and co-oxidation. This suggests a potential for facilitating the removal of inorganic constituents from drinking water using methane as a co-substrate.

Key words | ammonia removal, co-oxidation, groundwater, methane emission, methanotrophic consortia, waterworks

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INTRODUCTION

Groundwater is the primary source of water for drinking water production in many countries. In Europe, about 75% of all drinking water is produced from abstracted groundwater (Jørgensen & Stockmarr 2008). Some groundwater sources contain elevated concentrations of methane that occur due to the geological conditions of the aquifer. Methane in aquifers can be of biological, geological, or mixed origin although variable definitions exist regarding methane formation (Schoell 1988; Edwards & Durucan 1991). Microbial methane has a biological origin and is associated with activity of different methanogenic Archaea, which use organic and/or inorganic substrates for methane production in the subsurface (Conrad 2009). Geological methane includes thermogenic methane associated with

thermochemical processes at elevated pressures in the deep subsurface. Thermogenic methane relies on organic precursors and is often associated with coal and oil formations, and deposits can accumulate deep underground that are sometimes associated with alum shales (Schoell 1988; Edwards & Durucan 1991). Methane can also be formed in abiotic processes from inorganic precursors at low temperatures and low pressures.

Treatment of groundwater to meet drinking water standards often include different aeration and gas stripping processes at drinking water treatment plants (DWTPs). During these gas transfer processes, unwanted gases such as hydrogen sulphide (H₂S), carbon dioxide (CO₂), and methane (CH₄) are removed from the water and vented to

the atmosphere (Trussell *et al.* 2012). Methane is particularly susceptible to venting to the atmosphere because of the low aqueous solubility. Methane in the atmosphere has a long-term global warming potential (GWP) of 25–28 compared to CO₂ and is considered the second most important greenhouse gas that contributes to global warming (IPCC 2014, Dlugokencky *et al.* 2011). Methane concentrations in the atmosphere are now approaching 1.9 ppm and anthropogenic activities account for most of the global emissions of methane to the atmosphere (Conrad 2009, Dlugokencky *et al.* 2011). Anthropogenic sources associated with many processes in the urban water cycle are not well characterized with respect to their methane emission footprint. For example, scarce data exist in the literature regarding methane emission from groundwater-fed DWTPs. However, identification and evaluation of methane emissions from all anthropogenic sources is important for budget and mitigation efforts because of the increasing methane concentrations in the atmosphere.

The aims of this study were to evaluate national methane emission from groundwater during drinking water treatment in Denmark and to examine how trace amounts of methane remaining in the water can affect water treatment processes at DWTPs. The latter task involved investigation of methane oxidation kinetics and the possible role of methane oxidizing consortia for the removal of ammonia in sand filters.

METHODS

Water and filter material from DWTPs

Filter material and drinking water from 14 groundwater-fed Danish DWTPs was included in the study: five DWTPs from Aarhus municipality (Elsted, Kasted, Truelsbjerg, Østerby, Stautrup), five DWTPs from Odense municipality (Dalum, Odense, Lindved, Lunde, Holmehaven), two DWTPs from Sønderborg municipality (Huholdt, Mjang Dam), one DWTP from Fredrikshavn municipality (Skagen), and one DWTP from Aalborg municipality (Lundby Krat). Some samples were provided as part of the MUDP project ‘Smart Redesign of Drinking Water Production’. Filter material (≈500 g) was collected from active sand filters at

20-cm depth intervals using a hydrocyclone as described previously (Breda *et al.* 2019b). Water samples for measurement of methane concentrations were collected using 60 cc syringes equipped with three-way valves and flexible tubing to allow water sampling without air exposure using existing taps and hoses at wells and DWTPs. Water samples (20–60 mL) were injected directly into closed dinitrogen (N₂)-flushed and pre-evacuated 120 cc serum bottles. The serum bottles contained 5 mL 0.1 M potassium hydroxide (KOH) to halt microbial activity. The bottles were pre-sealed with butyl rubber stoppers and capped with aluminium crimps.

Methane concentration estimates

Headspace methane concentrations in the sampling bottles were determined using an Agilent 7890A gas chromatograph (GC) equipped with a HP-Plot Q column and a flame ionization detector (GC-FID). Helium was used as carrier gas and the oven, injector, and detector temperatures were 80, 200, and 300 °C, respectively. Estimation of methane concentration in the water sample was based on a headspace equilibration technique and adopted from Cédric *et al.* 2014.

$$\begin{aligned} \text{CH}_4 \text{ sample } \left(\frac{\text{mg}}{\text{L}} \right) &= \frac{\text{CH}_4 \text{ in headspace (ppmv)} * \text{Mass factor} * \text{Headspace volume}}{\text{Water volume} * 1,000} \\ &+ \frac{\text{CH}_4 \text{ in headspace (ppmv)} * \text{MW} \left(\frac{\text{g}}{\text{mole}} \right) * 55.5 \left(\frac{\text{mole}}{\text{L}} \right)}{\text{Henry's Law constant} * 1,000} \end{aligned} \quad (1)$$

Methane emissions from DWTPs

Methane emission from groundwater-fed DWTPs in Denmark was estimated from field measurements of methane concentrations in abstracted water and by analyzing the most recent data (2017) from the publically available JUPITER database (GEUS 2019; JUPITER 2019). Methane concentration in 3,068 groundwater wells for drinking water production was included, and combined with data for drinking water production at 1,004 DWTPs. Selected wells and DWTPs were visited to collect water samples and experimentally verify methane concentrations by gas chromatography.

Data from field measurements and the JUPITER database were combined on a regional scale to obtain average groundwater methane concentrations (mg/L) and average annual drinking water production (L/y) in each municipality. Annual methane emissions (g/y) were then calculated for each municipality (MUN) by assuming that methane was emitted to the atmosphere during aeration processes at the DWTPs and that methane concentration after water treatment was <0.001 mg/l (see also Figure 1):

An estimate of a national methane emission from drinking water treatment was subsequently approximated by addition of the emission from 89 Danish municipalities covering >91% of all drinking water production in Denmark. A national map with hot spots for groundwater methane was constructed using the QGIS mapping tool v.3.4. Geographical data for QGIS were downloaded from the publicly available DIVA-GIS website (www.diva-gis.org).

$$\text{CH}_4 \text{ emission (MUN)} \left[\frac{\text{g}}{\text{y}} \right] = \text{Avg. groundwater CH}_4 \text{ (MUN)} \left[\frac{\text{g}}{\text{L}} \right] * \text{Water production (MUN)} \left[\frac{\text{L}}{\text{y}} \right] \quad (2)$$

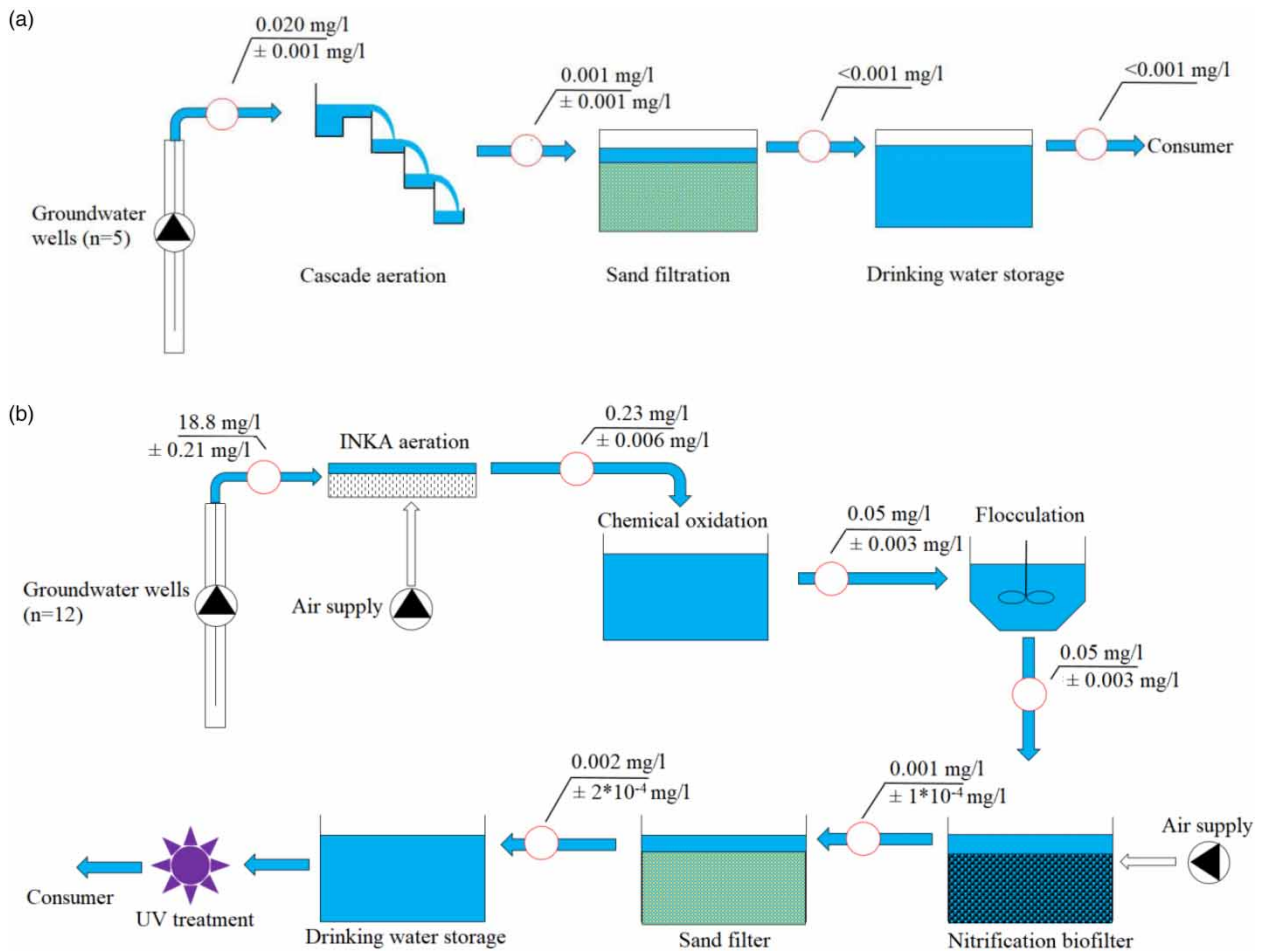


Figure 1 | Methane concentrations after different treatment steps in a traditional DWTP (a) and a DWTP with more comprehensive water treatment (b). Red circles represent sampling locations and numbers are methane concentrations ± standard error ($n = 3$). Please refer to the online version of this paper to see this figure in color: <http://dx.doi.org/10.2166/ws.2020.009>.

Presence and activity of methane oxidizing bacteria

Groundwater, drinking water and filter material were examined for culturable methanotrophic bacteria using a most probable number technique (MPN). Samples were serially diluted in 96-well microplates using half strength nitrate minimal medium (NMM) (Hesselsoe *et al.* 2005). Microplates were incubated for ≥ 4 weeks at 15 °C in an atmosphere with 10% CH₄ and 1% CO₂. Growth was measured as changes in absorbance using a Multiskan FC plate reader and by subculturing onto NMM agar. MPN values were calculated as described by Jarvis *et al.* (2010). Selected cultures were subcultured in NMM with and without copper in Erlenmeyer flasks with 20% methane and 1% CO₂ on a shaker at 60 revolutions per minute. These methanotrophic consortia were subsequently used as inoculum in column experiments (see below). The methanotrophic consortia were characterized by 16S rDNA amplicon sequencing to identify dominant bacteria as described previously (Breda *et al.* 2019a).

Intact filter material from DWTP sand filter depth profiles were assayed for methane oxidation activity and methane oxidation kinetics. Subsamples (5 g) were incubated in 120 cc serum bottles at 15 °C with different initial concentrations of methane. Methane removal was measured as described above. Kinetic values for methane oxidation were estimated based on Michäelis–Menten type kinetics (apparent K_m and V_{max}).

Sand column experiment

Laboratory experiments with a methanotrophic consortium spiked into virgin sand columns were conducted to examine potential effects on removal of ammonium. Columns (40 × 250 mm) were packed with 260 g virgin quartz sand (0.71–1.25 mm; Dansand A/S) and operated in downflow mode at 0.4 m/h. Methane (1%) was introduced to the bottom of the feeding water tank using a peristaltic pump to obtain dissolved methane in the feeding water of 0.15–0.2 mg CH₄/L (see Figure 4). Untreated groundwater spiked with 1 mg/L NH₄⁺ was used as feeding water to the sand columns. The experiment included columns with methane and ammonium feeding with and without inoculation with methanotrophic consortia enriched from DWTPs

(approximately 10⁶ cells/mL). The consortium included the following dominant microbial taxa as determined by 16S rDNA amplicon sequencing: an unknown methanotrophic bacterium, *Pseudomonas* sp., *Variovorax* sp., and *Rhodoferrax* sp. The experiment was conducted in triplicates in a climate chamber at 15 °C. The empty bed contact time was 53 min and the filter bed contact time was 34 min. Ammonium in the influent and effluent samples was determined colorimetrically according to standard methods using the Berthelot reaction (Indophenol blue method) and an automatic nitrogen autoanalyzer (Technicon TRAACS 800).

Statistics

Comparisons of results were carried out using the non-parametric Kruskal–Wallis H test for evaluating differences among multiple treatments, and the Mann–Whitney U test (Wilcoxon rank-sum test) for evaluating differences between two defined treatments. Statistical analyses were carried out using KaleidaGraph 4.5.4 (Synergy Software) and GraphPad Prism 8.0.1 (GraphPad Software) with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Fate of methane at DWTPs

Groundwater is the major source of raw water for drinking water production in Denmark (>99%). The main drinking water treatment comprises of variable forms of aeration (e.g., cascade, trickling, plate, spray) and different forms of filtration (e.g., one or two filters with one or several filter media types). The fate of methane was investigated at two DWTPs that produce municipal drinking water from groundwater (Figure 1). DWTP 1 (Figure 1(a)) represents the most common type of water treatment in Denmark (aeration and sand filtration), whereas DWTP 2 (Figure 1(b)) represents a more advanced water treatment (aeration, chemical oxidation, flocculation, nitrification, sand filtration, UV disinfection). Water samples were collected and analyzed for methane after each treatment step, and confirmed that most methane in the source water is vented

to the atmosphere during primary aeration at the DWTPs (95–99%). The measurements also showed that trace amounts of methane persist in the water entering nitrification biofilters (0.05 mg/l) and sand filters (0.001 mg/L). The DWTP shown in Figure 1(b) used INKA aeration with a ratio of 200 L atmospheric air to 1 L groundwater, and about 1% of the incoming methane remained in the water after this gas stripping step.

Methane emissions from DWTPs in Denmark

Methane emission from groundwater-fed DWTPs in Denmark was estimated from field measurements of methane in groundwater and drinking water, and by analyzing water quality data and abstraction amounts listed in the public JUPITER database (GEUS 2019; JUPITER 2019). Estimated average groundwater concentrations of methane in 89 Danish municipalities are shown in Figure 2. Groundwater with high methane concentrations were mainly located in the northern part of mainland Jutland and on

the island of Zealand (Figure 2). The hotspots for methane occurrence in Danish groundwater corresponds with several hot spots for occurrence of shale gas (Nielsen *et al.* 2016).

An initial estimate of a national methane emission from municipal drinking water treatment was approximated by considering emissions from the 89 municipalities shown in Figure 2. These municipalities cover >91% of annual municipal drinking water production in Denmark with groundwater methane concentrations >0.01 mg/L. Methane emission was calculated by multiplying the annual groundwater production for each municipality by the average (arithmetic) groundwater methane concentration in each municipality. Methane emission from these Danish DWTPs was estimated to be 1.38×10^{-4} Tg CH_4/y (138 t CH_4/y). The greatest contribution to methane emission (71%) were from the 11 municipalities marked in red in Figure 2 (0.98×10^{-4} Tg CH_4/y). The 78 municipalities marked in yellow and green contributed only 29% to the estimated annual methane emission. Considering the amount of produced water (m^3) in the different regions,

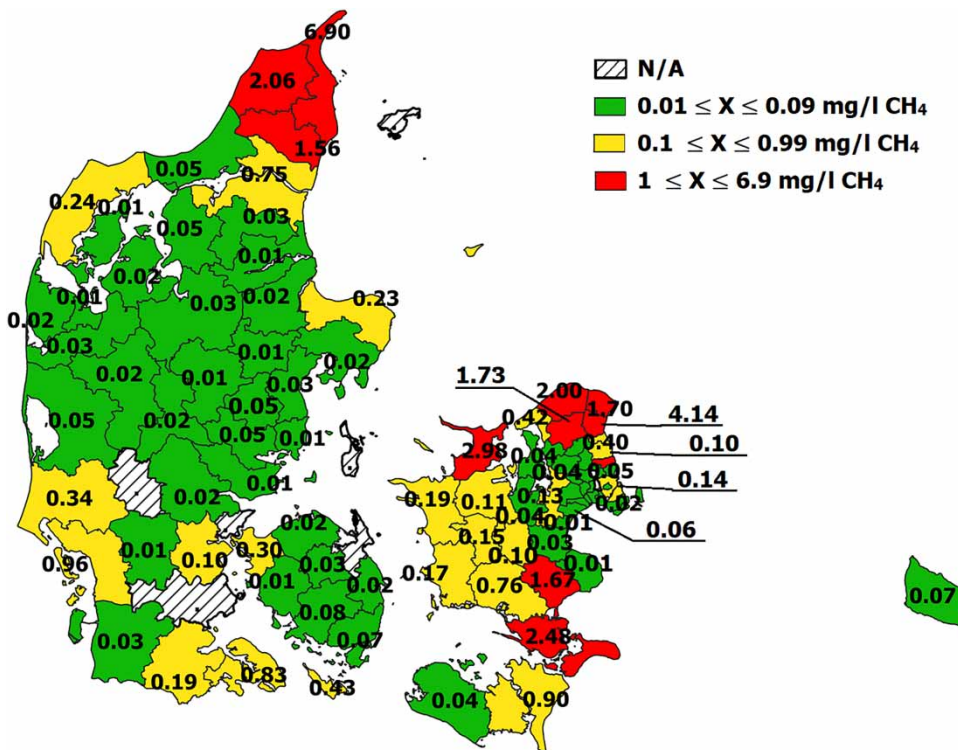


Figure 2 | Methane concentrations in groundwater wells in 89 municipalities in Denmark that supply water for drinking water production. Average values for each municipality include field measurements and methane concentrations from 3,068 groundwater wells listed in the public JUPITER database. Green color: 0.01 mg/l < CH₄ ≤ 0.09 mg/l; yellow 0.1 < CH₄ ≤ 0.99 mg/l; red 1 < CH₄ ≤ 6.9 mg/l CH₄. Hatched areas indicate municipalities with no available data in 2017–2018.

the red hot-spot areas with methane concentrations ≥ 1 mg/L emitted ≈ 2.6 g CH₄/y per m³ whereas municipalities marked in yellow and green emitted ≈ 0.35 and 0.03 g CH₄/y per m³, respectively. Hence, targeting the relatively few DWTPs with the highest methane concentrations to mitigate emissions could significantly reduce overall methane emissions from drinking water production.

Total Danish groundwater abstraction is around 6.6×10^8 m³ per year of which about 1/3 is used in agriculture, aquaculture and industry, and about 2/3 are used for drinking water production. The average (arithmetic) methane concentration in groundwater wells for drinking water production was 0.67 mg/L (wells with $0.002 \text{ mg/L} \leq \text{CH}_4 \leq 61 \text{ mg/L}$). If one considers all national groundwater abstraction for drinking water production and also includes sites with low methane concentrations (< 0.01 mg/L), the total national methane emission associated with drinking water production then amounts to $\approx 2.95 \times 10^{-4}$ Tg CH₄/y (295 t CH₄/y). This is equivalent to 82.6×10^{-4} Tg CO₂/y considering a GWP potential of 28.

The estimated methane emission from Danish drinking water production (138–295 t CH₄/y) is minor compared to emission from national wastewater treatment (4,360 t CH₄/y) but comparable or greater than emission from food processing industries (150 t CH₄/y) and passenger cars (259 t CH₄/y) (Nielsen *et al.* 2017). The estimated methane emission from the investigated Danish municipalities and DWTPs correspond to 0.05–0.11% of the total annual anthropogenic methane emission of 275,000 t CH₄/y (Nielsen *et al.* 2017). Interestingly, the estimated emissions of methane from groundwater use in the UK has been estimated to 0.05% of the national methane budget (Goody & Darling 2004). In the USA, methane emission from groundwater pumping has recently been estimated to 0.2% of the total annual methane emissions (Kulongoski & McMahon 2019). In the present study, only groundwater pumping for drinking water production was considered and direct abstraction of groundwater by agriculture and industry was not included. Hence, the total methane emission associated with groundwater pumping may be slightly greater than estimated above. Collectively, the results from the USA, UK, and Denmark suggest a minor but non-trivial and relatively comparable contribution to national methane emissions from

anthropogenic use of groundwater. In addition, natural (passive) emission of methane from groundwater probably also occurs due to degassing from shallow aquifers and groundwater-fed lakes and streams (Kulongoski & McMahon 2019).

Methane oxidation activity in sand filter material

Measurements at DWTPs suggest that trace amounts of methane (0.001 mg/l) remain in the drinking water after primary aeration and enter sand filters as a potential microbial substrate (Figure 1). The presence of methane oxidation activity and methane oxidizing bacteria was investigated in raw water, sand filter material and treated water from 12 Danish DWTPs. Active methane oxidation activity and/or enrichment of methane oxidizing bacteria was achieved from all 12 DWTPs. The number of culturable methane oxidizing microorganisms determined by a most probable number technique suggested at least 3×10^3 to 8×10^4 MPN/g in sand filter material from these sites. Pink-pigmented methane oxidizing bacteria were frequent among enrichments and were tentatively identified as Type I methanotrophs. Bacterial community analysis by 16S rDNA amplicon sequencing of enrichments from the DWTPs confirmed that Type I methanotrophs (*Methylomonas*) were present in some samples. 16S rDNA amplicon sequencing of parallel samples has also now shown that bacteria belonging to the novel methylotrophic genus *Methyloglobulus* are widespread in sand filter material from these groundwater-fed DWTPs (Søborg *et al.* in preparation). In other groundwater-fed DWTPs, it has been observed that some of the most abundant bacterial taxa are probably methanotrophic bacteria (*Methylococcales*) (Gülçay *et al.* 2016). These bacteria were found in groundwater, pre-filtration, and after filtration indicating their presence throughout the water treatment process (Gülçay *et al.* 2016). Collectively, these data suggest that methanotrophy and methanotrophic bacteria are probably widespread in many groundwater-fed DWTPs.

Depth profiles of methane oxidation activity and kinetics of methane oxidation for sand filter material from a DWTP is shown in Figure 3. The material originated from the DWTP shown in Figure 1(a). The influent water to the sand filter at this DWTP contained 0.001 mg/L. There was an apparent stratification with the greatest oxidation activity

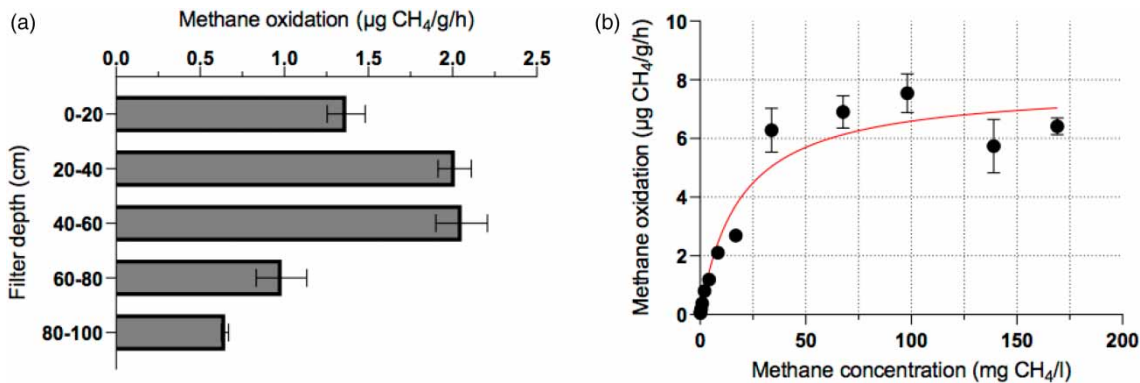


Figure 3 | Methane oxidation activity at different depths in a sand filter from DWTP (a) and methane oxidation kinetics (b). The red curve represents non-linear Michaelis–Menten style kinetics. Error bars indicate standard errors ($n = 3$). Please refer to the online version of this paper to see this figure in color: <http://dx.doi.org/10.2166/ws.2020.009>.

detected at 20–60 cm depth in the filter. However, oxidation was detected at all filter depths, which probably reflects the mixing that occurs during regular backflushing events. Filter material was subsequently examined for oxidation kinetics to determine maximum oxidation rates and methane affinity (Figure 3(b)). The saturation curve for methane consumption at different concentrations (V–S) followed Michaelis–Menten type kinetics indicative of microbial methane oxidation (Figure 3(b)). The maximum methane oxidation activity per gram of filter material was estimated to be $8 \mu\text{g CH}_4/\text{g/h}$ and the apparent half-saturation constant ($K_{m(\text{app.})}$) was 19 mg/L . This suggests a methanotrophic community adapted to relatively high methane concentrations and with a significant capacity for methane oxidation.

Sand column experiment

Biological nitrification in DWTP sand filters is an inherent slow process during start-up, and mature filters can be susceptible to disturbances. The aim of the sand column experiment was to investigate if methanotrophic consortia isolated from DWTPs were able to facilitate the removal of ammonium in the presence of methane. It was hypothesized that ammonium assimilation and co-metabolic oxidation of ammonia (NH_3) by the methanotrophic consortia could decrease ammonium concentrations because some methane oxidizing bacteria have been shown to co-oxidize ammonia (NH_3), which is the non-protonated form of ammonium (O'Neill & Wilkinson 1977; Bedard & Knowles 1989). The

ammonia/ammonium equilibrium is pH sensitive and more co-oxidation of ammonia has been observed at slightly alkaline pH (O'Neill & Wilkinson 1977). The pH in our sand column experiment was 7.5 and this pH will result in relatively greater concentrations of non-protonated ammonia (NH_3) compared to the equilibrium at lower pH, even though the overall equilibrium at this pH favors ammonium (NH_4^+).

The results showed that the inoculated methanotrophic consortium affected ammonium concentrations (Figure 4). The consortium was enriched with methane as the sole carbon and energy source and included the following dominant taxa as determined by 16S rDNA amplicon sequencing: an unknown methanotroph, *Pseudomonas* sp., *Variovorax* sp., and *Rhodoferrax* sp. Ammonium concentrations in the effluent of the inoculated sand columns decreased significantly over time (Kruskal–Wallis; $p < 0.024$), whereas ammonium concentrations in the effluent from the control columns did not change significantly (Kruskal–Wallis; $p > 0.860$). Effluent ammonium concentrations at day 1 and at day 2 in the inoculated sand columns were both significantly different from the influent ammonium concentrations (Mann–Whitney; $p < 0.026$).

The results from the column experiment suggested that methanotrophs in drinking water could facilitate oxidation of common water constituents, such as ammonium. Although it is likely that it is the unionized form of ammonia (NH_3) that is being co-oxidized by methanotrophs, this process will subsequently affect the ammonium concentration due to the ammonia/ammonium equilibrium. Other studies

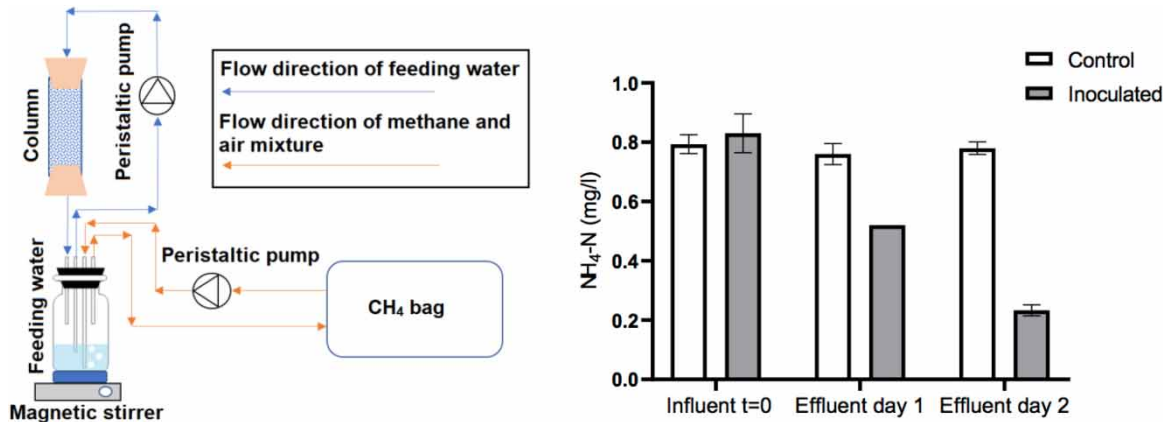


Figure 4 | Ammonium concentrations in influents and effluents of sand filter columns with and without inoculation with a methanotrophic consortium isolated from drinking water. Error bars indicate standard errors ($n = 3$).

have shown that methanotrophic bacteria can also play a role in the removal of other drinking water constituents, such as nitrate, manganese, and different pesticides (Zhu *et al.* 2016; Hedegaard *et al.* 2018; Matsushita *et al.* 2018; Papadopoulou *et al.* 2019). The removal involved direct interactions (e.g., co-metabolism) and indirect mechanisms (e.g., cross-feeding), and the processes were stimulated by continuous or intermittent supply of methane. These results corroborate previous studies reporting that variants of the key enzyme methane monooxygenase in methanotrophic bacteria can co-oxidize different pollutants while obtaining energy from oxidation of a primary substrate such as methane (O'Neill & Wilkinson 1977; Bedard & Knowles 1989; Hesselsoe *et al.* 2005). Collectively, these results suggest that methanotrophic bacteria may have a somewhat underexploited potential for application in drinking water treatment.

CONCLUSIONS

- Groundwater-fed DWTPs can emit non-trivial amounts of methane to the atmosphere during primary aeration and gas stripping.
- Methane emission from Danish DWTPs was estimated to 0.05–0.11% of the national anthropogenic methane emission.
- Trace amounts of methane often enter sand filters and serve as a microbial substrate. Methanotrophic bacteria

and active methane oxidation was detected at all sand filter depths at groundwater-fed DWTPs.

- Methanotrophic consortia in sand filters can play a role in the removal of ammonia through assimilation and co-oxidation.

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