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Managing dissolved methane gas in anaerobic effluents using microbial resource management-based strategies

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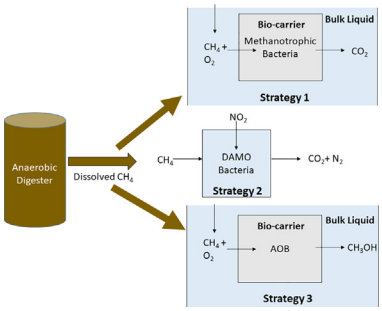
GRAPHICAL ABSTRACT



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ABSTRACT

This study reports the findings of three independent microbial resource management-based strategies to manage dissolved methane (D-CH_4) gas in anaerobic effluents. In the first approach, an aerobic methanotroph *Methylococcus capsulatus* was immobilized. A maximum of 1.75 kg COD m^{-3} d^{-1} at a hydraulic retention time of 0.5 h was recorded in the attached growth aerobic methane oxidizing reactor. In the second strategy, denitrifying methane oxidizing organisms (DAMO) were first enriched in a lab-scale batch reactor which enabled a maximum methane oxidation rate of 0.31 kg COD m^{-3} d^{-1}. In the last strategy, a mixed community of aerobic ammonia oxidizers was immobilized on sponge carriers and used to convert the D-CH_4 gas into useful biofuel methanol at a rate of 0.73 kg COD m^{-3} d^{-1} equivalent of COD with a methanol production of 31.5 g COD m^{-3} d^{-1}. On a COD basis, the amount of methanol generated could denitrify nearly 7 mg L^{-1} of NO_3-N.

1. Introduction

Under the broader paradigm of a circular economy where resource recovery is integrated with contaminant removal, anaerobic treatment of waste streams is considered an appropriate and energy-positive option for treating domestic wastewater (van Lier et al., 2015). Anaerobic processes produce energy from the waste while removing 60%–80% of chemical oxygen demand (COD), making the treatment energy-neutral or energy-positive (van Lier et al., 2015; Remi et al., 2014). Additionally, anaerobic processes are attractive to the wastewater community because they generate low excess sludge production (McCarty and Smith, 1986). Moreover, with the growing concerns over global warming, much interest has been given to wastewater treatment and energy nexus, which brings the anaerobic process into more focus for energy recovery in the form of biogas. The biogas produced in the anaerobic systems primarily consists of 50–70% methane (CH_4) and 30–50% carbon dioxide (CO_2) (Henares et al., 2016). The generated biogas is either used for heating and/or electricity generation (i.e.,

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regeneration), making wastewater treatment plants energy efficient (Cakir and Stenstrom, 2005; Henares et al., 2016). However, in engineered anaerobic environments such as in anaerobic digesters or landfills, there is always an equilibrium between the gas phase and the liquid phase CH_4 resulting in a portion of the total CH_4 being lost in the dissolved form and subsequently released to the atmosphere as a greenhouse gas (GHG) (Hartley and Lant, 2006).

Human activities have been considered accountable since the beginning of the industrial revolution for the increase in the atmospheric concentration of GHGs, such as CO_2, CH_4, and nitrous oxide (N_2O) by about 40%, 150%, and 20%, respectively (Stocker et al., 2013). Waste treatment is one of the seven sectors listed by the Intergovernmental Panel on Climate Change (IPCC) for contribution to the global anthropogenic emissions (Metz et al., 2007). As a whole, this sector is expected to be accountable for 2.8% of the global anthropogenic GHG emissions (U.S. Environmental Protection Agency, 2017). Wastewater treatment was the eighth largest anthropogenic source of CH_4 emissions in the United States in 2012 (U.S. Environmental Protection Agency, 2017) and accounts for 5% of worldwide CH_4 emissions (El-Fadel and Massoud, 2001). This is particularly poignant considering that the IPCC has increased Global Warming potential for CH_4 from 25 to 34 times that of CO_2, indicating that CH_4 emissions have a more significant environmental impact than all halocarbons combined (Stocker et al., 2013). Therefore, the loss of dissolved CH_4 (D-CH_4) in anaerobic effluents not only represents a loss of energy but also contributes to the total emission of GHG (Lobato et al., 2012). Furthermore, many investigations have focused on energy recovery from municipal wastewater in the form of biogas (McCarty et al., 2011; Smith et al., 2014). However, energy recovery from biogas might not be economical and environmentally friendly due to the loss of methane in its dissolved form, thus questioning the suitability of anaerobic processes for waste treatment (Liu et al., 2014).

Anaerobic digesters usually require post-treatment processes for polishing the effluents in order to meet water quality standards (Matsuura et al., 2010). Several post-treatment processes such as activated sludge process or aerated lagoons have been proposed, but only a few of them have paid attention to the treatment of D-CH_4 (Matsuura et al., 2010; He and Hägg, 2012; Henares et al., 2016). To diminish these GHG emissions, the D-CH_4 present in anaerobically-treated effluents should be eliminated by using alternative post-treatment processes.

Conventionally, dissolved gases are removed from liquids using vacuum-packed towers (Henares et al., 2016). Packing in these towers provides a large surface area for mass-transfer of dissolved gases from liquid to gaseous phase (Billet and Schultes, 1993). However, with direct contact of liquid and gas, this system frequently encounters problems such as flooding and foaming. Additionally, emulsions can be formed in towers, which increases the operational and maintenance costs (Billet and Schultes, 1993; Henares et al., 2016). An emerging technology for the removal of dissolved gases from the liquid is the use of degassing membranes (Henares et al., 2016). Degassing membrane is a gas-permeable membrane, which removes dissolved gases from the liquid phase by partially allowing dissolved gas particles to pass through the membrane (He and Hägg, 2012). However, it has some disadvantages, such as fouling the membrane, membrane resistance to mass transfer, and limitations concerning pressure drop (He and Hägg, 2012; Henares et al., 2016). Apart from these physical processes, innovative biotechnologies based on microbial resource management could be used for managing dissolved gases, specifically D-CH_4 using pathways or using dissolved CH_4 for beneficial uses. This allows the reduction in total GHG emissions in terms of CO_2 equivalents (Matsuura et al., 2015).

The objective of this study was to develop and test microbial resource management strategies to manage D-CH_4 from anaerobic effluents. We tested three different strategies: (1) the use of aerobic methanotrophs to directly oxidize dissolved methane in a packed bed reactor, (2) the use of anoxic methane oxidizers coupled to denitrification (DAMO), and (3) the conversion of dissolved methane to methanol using ammonia monooxygenase enzyme present in ammonia oxidizers. Because there is not much data available on dissolved CH_4 in anaerobic effluents along the treatment train, we also measured the contribution of D-CH_4 to the GHG footprint of two wastewater treatment plants (WWTP). Additionally, we also evaluated two different methods to estimate the concentrations of D-CH_4 in anaerobic effluents.

2. Materials and methods

2.1. Measurement of D-CH_4 in anaerobic effluents

Two different methods were first compared to estimate the concentration of dissolved methane gas in the liquid phase. The first method was based on gas and liquid phase equilibrium of gases based on Henry's law, and the second method was based on the effect of salt on gas solubility in liquid and was based on the salting-out method of Gal'chenko et al. (2004) with some modifications. In the first method, a 25 ml sample was collected in a 70-ml serum bottle containing 75 µL of 10% sodium azide. Care was taken to avoid any loss of D-CH_4 from the sample. Sodium azide was added to the serum bottles to inhibit any microbial activity, which could alter methane oxidation rates during the analysis of D-CH_4 (Lichstein and Soule, 1944). The serum bottles were sealed airtight. The serum bottles were then shaken at 200 rpm and 25 °C for 10 min to equilibrate between liquid and gas phases in the headspace.

The salting-out method is based on the hypothesis that a supersaturated saltwater sample does not hold dissolved gases and as a result, gases partition into a gas phase. As a consequence of the oversaturation of salt in the samples, the microbial activity in the sludge/water samples is also inhibited, and the dissolved gases are salted out (Daelman et al., 2012). Previously, Gal'chenko et al., (2004) also added potassium hydroxide and/or Merthiolate to inhibit any microbial activity in the sample, but this was omitted in these samples as high salt concentrations inhibit microbial activity both in the sludge and water samples (Pernetti and Di Palma, 2005).

Before starting the analysis of headspace, phase equilibrium time was also estimated and is described below. A gas-tight syringe was used to collect headspace samples from the serum bottles, and methane concentration was measured using a gas chromatograph equipped with a thermal conductivity detector (GC-TCD, 7890A, Agilent Technologies, USA). The concentration of dissolved methane was finally calculated according to Eq. (1) (Souza et al., 2011).

$$[CH_4]_{dis} = \frac{\left[\left(\frac{[\%\,CH_4]_{gas}}{100}\right) * [d * V_{gas} + (P_T - P_V) * K_H * V_L]\right]}{V_L} \quad (1)$$

where $[CH_4]_{dis}$ is the concentration of dissolved methane (mg L^{-1}); $[CH_4]_{gas}$ is the concentration of methane in the headspace (%); d is the density of methane at 25 °C (600 mg L^{-1} at 25 °C); V_{gas} is the volume of headspace (mL); P_T is the atmospheric pressure (0.857 atm for Salt Lake City); P_V is the water vapor pressure (0.062 atm at 37 °C); K_H is the constant of Henry's Law (28.67 mg L^{-1} atm^{-1} at 37 °C); V_L is the volume of liquid phase (mL). The time required to reach the equilibrium between the gas and the liquid phase which is required for Henry's Law method was also optimized. A 25 ml sample of deionized water was taken in a 70-ml serum bottle and sealed airtight. Triplicate bottles were set up. A 5 ml mixture of CH_4 and CO_2 (95:5 vol/vol) was added into the headspace of each bottle and a 100 µL aliquot was immediately withdrawn from the headspace using a gas-tight syringe to measure the initial concentration of methane in the headspace. Thereafter, serum bottles were shaken at 200 rpm and headspace samples were analyzed at 2 min, 5 min, 10 min and 15 min. The sampling of 100 µL from a 45-ml headspace corresponds to a very small change, which is acceptable for 4 injections.

In the salting-out method, 20 ml of sample was collected in a 70-ml serum bottle containing 10 g of NaCl. While collecting the sample, the end of the tube connected to the sampling port was kept under the liquid surface to keep the liquid-gas interface as small as possible to avoid stripping. Immediately after adding the sample, a vacuum was created in the headspace by quickly sucking out the headspace air using a syringe. The pressure in the headspace was equilibrated with the atmospheric pressure by allowing the gas in the headspace to expand into a submerged graduated syringe. The increase in gas volume was used to calculate the increase in pressure in the headspace. After the gas pressure in the headspace was brought to the atmospheric pressure, the headspace was analyzed using a gas chromatograph equipped with a thermal conductivity detector (GC-TCD, 7890A, Agilent Technologies, USA). The amount of methane in the dissolved form was calculated using the ideal gas law equation.

2.2. Anaerobic effluent samples

The two methods detailed earlier were compared with real anaerobic digester effluent samples collected from two WWTPs in the State of Utah, USA. One of the treatment plants was Central Valley Water Reclamation Facility (CVWRF) with an average daily flow of about 45 million gallons per day (MGD) and with an average daily biogas production of 12,686 $m^3 d^{-1}$. The second treatment plant sampled was North Davis Sewer District (NDSD), with an average daily flow of 17.2 MGD and average daily biogas formation of 2803 $m^3 d^{-1}$. Effluents samples were collected from three locations along the anaerobic effluent line. One sample was collected at the beginning of the anaerobic digester effluent outlet, the second was collected before the blending tank where all the digested sludge blends together before dewatering, and the third sample was collected after the dewatering unit. All the samples were collected in a 500 ml PYREX® round storage bottle and were sealed using a rubber stopper just after collecting the sample onsite to avoid any loss of methane. Bottles were kept on ice and were analyzed and measured for D-CH_4 immediately after being brought to the laboratory. D-CH_4 was measured using two different methods detailed in Section 2.4.

2.3. Microbial resource management using different strategies

2.3.1. Methane-oxidizing bacteria and experimental set-up

A pure culture of methane-oxidizing bacteria (MOB), *Methylococcus capsulatus* was grown in a Nitrate Mineral Salts (NMS) medium. The composition of the NMS medium used in the experiments was obtained from Whittenbury and Wilkinson, 1970. The primary culture was grown in a 160-ml serum bottle having 80-ml NMS medium and 10% pure culture. The serum bottle was sealed airtight using 20 mm butyl rubberteflon-faced septa (Fischer, USA) and 20 mm aluminum crimp caps. The headspace of the serum bottle was purged with 12 ml (15% of headspace) of a CH_4 and CO_2 (95:5 vol/vol) gas mixture (Lee et al., 2011). By injecting only 15% methane, it was ensured that the environment inside the serum bottle remained aerobic. The serum bottle was incubated at 37 °C, 220 rpm for 24 h after which a secondary culture was grown by adding 10% primary culture to 80 ml NMS medium in another 160-mL serum bottle, which was also incubated in a similar fashion to the primary culture. Bacterial culture was grown on a daily basis to acclimatize them to methane for 1 month. After enriching the bacteria in the serum bottle, the culture was grown into a larger volume of 300 ml in a 500 ml PYREX® round media storage bottle for further use. In this larger bottle, 15% of the headspace by volume was also filled with methane gas mixture and incubated under the similar conditions.

A cylindrical packed-bed column reactor (PBR) (30 cm long × 5 cm outer diameter) with a total volume of 550 ml and a working volume of 500 ml was used in this study (as shown in the right panel in Fig. 1). The rationale behind the packed bed column was that it provides a greater surface area for microbial activities to oxidize methane, and the footprint for a packed bed column is relatively smaller in comparison to a suspended-growth system. In the PBR, the packing used were aquaporousgel (APG) bio-carriers (2 cm × 2 cm × 2 cm, obtained from Nisshinbo, as shown in Fig. 1) packed closely inside the column. The APG bio-carriers were dipped in methane-oxidizing bacteria culture for 45 days until a visible biofilm was formed on the media before they were packed in the column reactor. NMS growth medium was first bubbled with a mixture of CH_4 and CO_2 (95:5 vol/vol) gas mixture to maintain dissolved CH_4 concentration to saturation. This NMS media containing dissolved methane gas was fed to the packed bed reactor in a batch-wise manner. Another similar PBR containing fresh and autoclaved APG bio-carriers as packing were used as an abiotic control system for the experiments to account for abiotic losses of methane gas. Since the reactor was a closed system, the air was supplied to the reactor every 6 h to ensure that aerobic conditions prevail inside the reactor. Effluent samples were collected from the bottom of the reactor in a serum bottle and analyzed using methods described in Section 2.4. The hydraulic retention time (i.e., batch time) was sequentially reduced from an initial value of 24-h to 0.5-h in a stepwise manner. Reactor contents inside the reactor were maintained at a specific temperature by wrapping hot water tubing around the reactor, and the tube was fed using a hot water bath. Additionally, the experiments were conducted at two different temperatures.

2.3.2. Anoxic methane oxidation using denitrifying methane oxidizers (DAMO)

DAMO organisms were enriched in a 2 L sequencing batch reactor the details of which could be found in Bhattacharjee et al. (2016). The reactor set-up is shown in the left panel in Fig. 1. The rationale behind using DAMO was that along with oxidation of methane, this process could also reduce nitrate or nitrite to di-nitrogen gas in a single-stage process. After seeding the reactor, the SBR was operated on a 2-day cycle with each cycle including 400 ml of feed added to the reactor every second day to yield a hydraulic retention time of 5 days.

The anaerobic methane oxidation rate using the DAMO-enriched biomass was estimated in serum bottles. A quantity of 200 ml of mixed liquor from the SBR reactor was collected and centrifuged at 4500 rpm for 10 min. The supernatant was rejected, and the centrifuged pelleted biomass was washed by re-suspending it in de-ionized water. The pellet was re-suspended in 200 ml of SBR feed and was divided into 4 equal volumes, which were transferred into 160 ml serum bottles (Wheaton Science Products, USA). In all cases, the bottle contents were sealed airtight, after which they were flushed with nitrogen gas to create the anoxic environment. Two serum bottles were used to evaluate DAMO activity where a mixture of CH_4 and CO_2 (95:5 vol/vol) was maintained in the headspace. The remaining two bottles were used to evaluate the influence of any heterotrophic denitrification in the sediments. In these bottles, the anoxic environment was also maintained by purging N_2 gas in the headspace. As negative controls, a serum bottle with 95% CH_4 and 5% CO_2 gas in the headspace without biomass added to the bottles was also used. All serum bottles were incubated at 30 °C on a shaker (12,500 series, New Brunswick Scientific, Canada). The headspace was analyzed every 12–24 h for 3 days on a gas chromatograph (7890A, Agilent Technologies, USA). The concentration of NO_2-N and NO_3-N were analyzed using an ion chromatograph at the start and end of the experiments.

2.3.3. Ammonia oxidizing bacteria (AOB) and biofilm reactor

In this study, a mixed nitrifying bacterial culture was used rather than a pure culture of AOB. An enriched culture of Ammonia-Oxidizing Bacteria (AOB) was utilized from an ongoing partial nitrification (PN) lab-scale sequencing batch reactor. The details of the PN reactor are provided elsewhere (Kotay et al., 2013). The biomass sample was withdrawn from the ongoing PN reactor for further enrichment of AOBs and washed three times with de-ionized water. The washed biomass

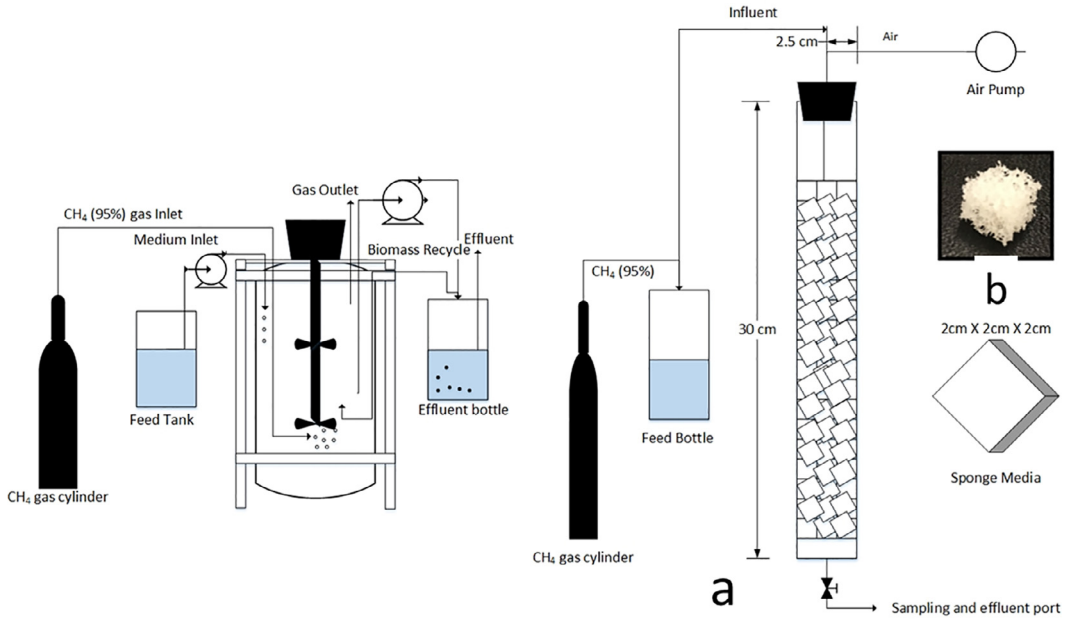

Fig. 1. Left side-schematic Diagram of SBR used in the DAMO enrichment and, right side-(a) packed bed column used for aerobic methane oxidation and (b) APG bio-carrier.

was transferred into a 500-ml PYREX® round media storage bottle and 200-ml of ATCC® medium 2265, having NH_4^+-N concentration of 700 mg L^{-1} was added for the enrichment of AOBs. Air was supplied continuously using an air pump. After a month of enrichment, the bottle was sealed using a septum, and the headspace was injected with 30 ml (10% of headspace) of CH_4 and CO_2 (95:5 vol/vol) to make the biomass acclimatized to CH_4.

After enrichment, a portion of AOB biomass was transferred to a 200 ml glass bottle. The bottle was kept airtight and injected with 30 ml (10% of headspace) of CH_4 and CO_2 (95:5 vol/vol) in the headspace periodically as needed to maintain a positive methane headspace in the bottle. An ammonium nitrogen concentration of 700 mg L^{-1} was maintained in the reactor contents. Both the methane gas in the headspace and the NH_4^+-N concentration in the liquid phase were monitored over time. When the NH_4^+-N concentration in the feed decreased to 10 mg L^{-1} over a few days, the biomass was allowed to settle down, and the supernatant was decanted gently without disturbing the biomass. This practice of replacing the supernatant continued for a month until a substantial increase in the ammonia nitrogen removal rate was recorded. After 1 month of enrichment, APG bio-carriers were added into the bottle to allow the nitrifiers to form a biofilm on APG carriers while the practice of replacing the relatively biomass-free liquid was continued. Methane concentration in the headspace was analyzed every 24 h using a gas chromatograph, and methanol concentration in the liquid phase was measured using a method reported by Zhan et al. (2010). Briefly, in this method, sodium nitroprusside (SNP) is used as a chromatographic reagent which forms a colored compound with methanol in a basic solution, and the absorbance can be measured in a UV–Visible spectrophotometer.

2.4. Microbial analysis

Genomic DNA was extracted from cell cultures or biomass/biofilm samples using the PowerSoil(R) DNA Isolation Kit (12888-50, MoBio Laboratories Inc.). The concentration of DNA samples was measured using Nanodrop 2000 (Thermo, USA). Duplicate DNA samples were extracted for each reactor. For the aerobic methane oxidizing reactor (PBR), DNA samples extracted were used for amplification of *pmoA* gene fragments with primer pairs A189f/mb661 (Costello and Lidstrom, 1999). For the DAMO reactor, a nested PCR was employed; in which case, genomic DNA was first amplified using the primer set A189_b and cmo682, and then the PCR product was used as a template with second PCR using cmo182 as a reverse and cmo568 as a forward primer (Luesken et al., 2011). In the case of AOB attached growth reactor DNA samples, amoA gene fragments were amplified with primer pairs *amoA*-1F and *amoA*-2R (Rotthauwe and Witzel, 1997).

Each PCR reaction mixture contained 12.5 µL of 2X GoTaq, 1.0 µL forward and reverse primers (each of 10 µM concentration), and 1–3 µL DNA template. Nuclease-free water was added to the mixture to bring the volume to 25 µL. Table 1 details primers and PCR programs for *pmoA* (Methanotrophs), *pmoA* (DAMO organisms), and *amoA* (AOB) target genes. Gel electrophoresis was carried out for the product obtained from PCR using 1% agarose gel. Amplified and purified PCR products obtained from PBR (aerobic methane oxidation) and SBR (DAMO) were used for Sanger sequencing. In the case of the AOB attached growth batch reactor, Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis was carried out for AOB in order to identify the species of AOB present in the reactor. The labeled primer set of amoA-1F (labeled with blue dye), and amoA-2R (labeled with

Table 1
Primers and PCR programs for Target Genes.

Gene	Primers Used	PCR program	Reference
pmoA (Methanotrophs)	A189f/mb661	95 °C, 5-min (95 °C, 30 s; 56 °C, 30 s; 72 °C, 60 s) × 25; 72 °C, 10 min	Costello and Lidstrom (1999) and Hatamoto et al. (2010)
pmoA (DAMO organisms)	A189_b/cmo682 for the first PCR, and cmo182/cmo568 for the second PCR	For both PCR: 95 °C, 5-min (95 °C, 60 s; gradient from 50 °C–60 °C, 60 s; 72 °C, 90 s) × 35; 72 °C, 10 min	Luesken et al. (2011)
amoA (AOB)	*amoA*-1F/ *amoA*-2R	95 °C, 5-min (95 °C, 60 s; 56 °C, 90 s; 72 °C, 90 s) × 34; 72 °C, 10 min	Rotthauwe and Witzel (1997)

green dye) were used for TRFLP. Sanger sequencing samples and TRFLP samples were sent to the DNA Sequencing CORE facility at the University of Utah. The resulting Sanger sequencing results were blasted against the nucleotide collection database and compared with the literature to determine the species. Also, TRFLP electropherogram demonstrated the diversity of species present in the AOB attached growth batch reactor.

3. Results and discussion

3.1. Comparison of methods to measure dissolved methane

We modified the salting-out method proposed by Gal'chenko et al. (2004). Due to oversaturation and vigorous shaking, dissolved gases such as CH_4 and CO_2 escape from the liquid phase to the headspace of the serum bottles. This causes a building-up of pressure in the headspace, which was not accounted for by Gal'chenko et al. (2004). In the present study, the pressure build-up was taken into account as the sludge samples contained substantially higher amounts of dissolved gases.

Two methods to estimate the concentration of dissolved CH_4 in the liquid phase were compared, and a one-way ANOVA analysis was conducted. The analysis showed a good correlation between the data obtained through the two methods with an R^2 value of 0.97 (Fig. 2a). The one-way ANOVA analysis also indicated that there is not much evidence to report any statistical difference between the results obtained using the two methods ($p > 0.05$). Furthermore, while measuring the dissolved methane concentration using both methods, it was important to inhibit any microbial activity that might affect the results. In Henry's method, sodium azide was used to inhibit the microbial activity (Lichstein and Soule, 1944), whereas, in the salting-out method, salt itself acts as an inhibitor (Pernetti and Di Palma, 2005). To confirm the complete inhibition of microbial activities, Live and Dead analysis was conducted. The analysis showed nearly a complete inhibition of microbial activities during analysis using both methods, which was concluded based on the presence of nearly all dead cells after the live and dead staining (results not included). These results are in accordance with previously-reported results which showed inhibition of microbial activities with sodium azide at 0.03% concentration, which acted as a biocide agent for both gram-negative and gram-positive bacteria (Lichstein and Soule, 1944). The high salt concentration is known to inhibit microbial activities both in activated sludge and anaerobic digester process (Pernetti and Di Palma, 2005). Based on these results, it can be concluded that both methods could be used for quantification of D-CH_4. Since both methods enabled compatible results; we decided to use Henry's method for further analysis of D-CH_4 in the rest of the analysis.

Both methods rely on incubating the samples in serum bottles for a specified period of time such that an equilibrium is established between liquid- and gas-phase concentrations. Because the accuracy of the results is highly dependent on the equilibrium time, tests were also conducted to optimize the equilibrium time. The equilibrium phase time was determined to evaluate the time required for equilibration between the liquid and gaseous phase. Fig. 2(b) shows methane concentration in the headspace at different time intervals in three different bottles that were started simultaneously. Based on time-dependent gas sampling in the headspace of batch tests, a 5-min duration was deemed optimum for equilibrium to be reached between dissolved and gas-phase concentrations of CH_4.

3.2. Dissolved methane in anaerobic effluents

The concentration of dissolved methane (D-CH_4) in anaerobic effluents was measured in liquid waste streams at two treatment plants at different locations along the anaerobic effluent line. Firstly, the absolute concentrations of CH_4 were measured in effluents from digesters of CVWRF and NDSD treatment plants. Secondly, samples from the effluents between the anaerobic digester (AD) and blending tank (BT) and from the BT to the dewatering unit (DW) were also collected to estimate the degree at which CH_4 could possibly be stripping off from liquid waste streams when the digester effluent travels between these locations. Table 2 shows the concentration of D-CH_4 in anaerobic effluents of CVWRF and NDSD. Methane in the dissolved form was compared with the saturated concentration calculated using Henry's Law, which allowed for the determination of the degree of methane supersaturation in the effluent. The saturation concentrations were calculated from the percentage of methane in the biogas, considering atmospheric pressure to be 0.857 atm for Salt Lake City, water vapor pressure to be 0.062 atm at 37 °C, and Henry's Law constant to be 28.67 mg L^{-1} atm^{-1} at 37 °C (Sander, 2015). Since Henry's law constant is not available for raw or treated wastewater, the value derived for pure water was used (Souza et al., 2011).

It can be noticed from Table 2 that the concentration of methane in the liquid phase was about 4.7–5.4 times higher than the saturation concentrations calculated using Henry's law, confirming supersaturation of methane in anaerobic effluents (Hartley and Lant, 2006; Pauss et al., 1990). These supersaturation degrees are in the range of estimation reported by Hartley and Lant (2006). These values are also in the range of reported values published by investigators in which case a COD mass balance was used and the supersaturation degree varied between 1.9 and 6.9 times (Hartley and Lant, 2006; Souza et al., 2011). Pauss et al. (1990) also reported that the supersaturation of dissolved

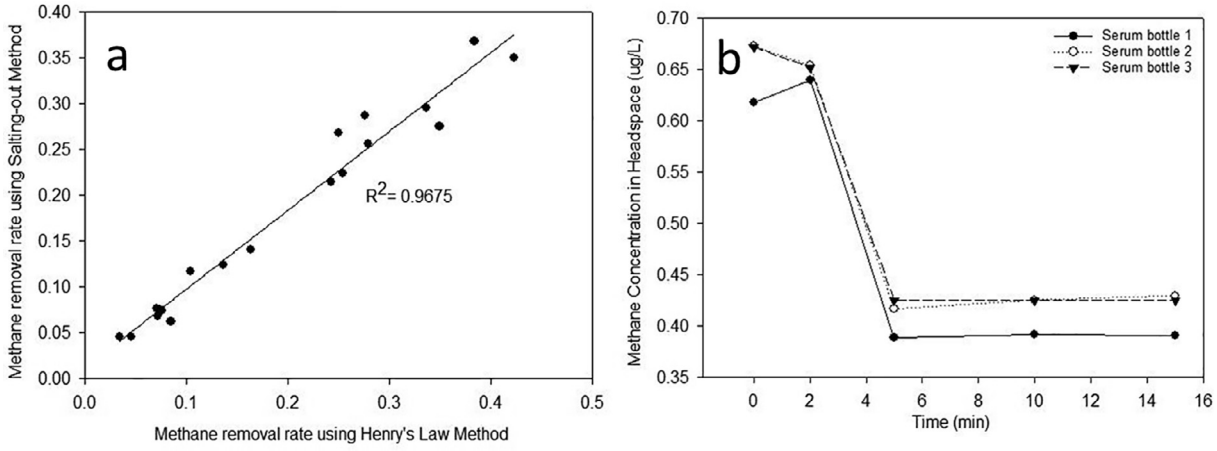

Fig. 2. (a) Correlation between Henry's Law Method and Salting-out Method and (b) dependence of headspace concentration on the time of phase equilibrium between headspace and water.

Table 2
Degree of methane saturation in the liquid phase of CVWRF and NDSD anaerobic digesters.

Parameter	CVWRF	NDSD
% methane in the biogas	61	63
Saturation concentration of methane (mg L^{-1})	13.90	14.36
Dissolved methane concentration (mg L^{-1})	75.34 ± 4.12	67.36 ± 1.78
Degree of methane saturation in the liquid phase	5.42 ± 0.3	4.69 ± 0.12

methane in the anaerobic effluents could reach as high as 12 times.

Fig. 3(a, b) depicts the results of the percentage of dissolved methane stripped off (in terms of concentration) from the effluents between the anaerobic digester (AD) and blending tank (BT) and from the BT to the dewatering unit (DW), respectively, for both treatment plants used in this study estimated using Henry's method. It can be observed that the amount of CH_4 stripped from the anaerobic effluent between the AD and the BT varies between 35 and 45% and 55–65% between BT and DW, for both the treatment plants. Also, as per the calculations made by Hartley and Lant (2006), methane losses could be up to 80% of the total methane production in the digester. However, as Fig. 3(b) depicts, methane losses (in terms of concentration) in the effluent of the digesters from the two treatment plants reached 45 to 48% of the total methane produced.

3.3. Performance of the reactors

3.3.1. Dissolved methane oxidation in packed bed column reactor

In this study, we used a Packed Bed Column reactor (PBR), containing APG bio-carriers for immobilizing *M. capsulatus*. Severe washout of the immobilized biomass and clogging of the reactor were not observed during the experimental period, which is sometimes reported as potential operational problems in methane-oxidizing biofilters (Wilshusen et al., 2004; Machdar et al., 1997). For a post-treatment process of anaerobic wastewater, it is important that there be easy maintenance requirements, such as no/minimal need for backwash, low energy consumption, and low maintenance costs, so as not to weaken the advantages of anaerobic wastewater treatment.

The PBR was first initiated at a residence time of 24-h and a temperature of 25 °C. Methane was supplied in dissolved form in the feed. Fig. 4 shows the CH_4 removal rate in terms of COD equivalent as kg COD m^{-3} d^{-1} on the left axis and the overall CH_4 removal efficiency as a percentage on the right axis. Fig. 4 shows that the methane Removal Rates (MRR) in phase 1 was 0.09 kg COD m^{-3} d^{-1}. In phase 2, the temperature of the reactor was increased to 37 °C as the growth and activity of methanotrophs increases at 37 °C (Soni et al., 1998). MRR in

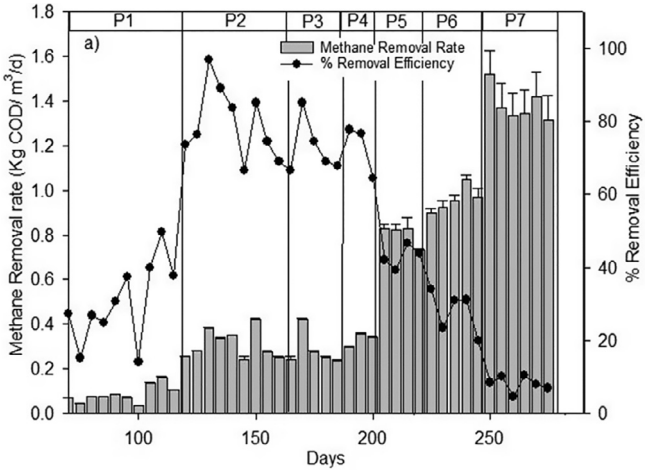

Fig. 4. Methane removal rate (vertical bar) and the corresponding removal efficiencies (black circles) in aerobic methane oxidizing biofilm reactor estimated using Henry's law method.

phase 2 increased to 0.31 kg COD m^{-3} d^{-1}, calculated Henry's method. In the next phases from 3 to 7, the residence time was successively decreased to 22 h, 16 h, 4 h, 2 h, and 0.5 h, respectively, in order to optimize the operating conditions for the reactor. During these periods, the average MRR increased from 0.29 to 1.28 kg COD m^{-3} d^{-1}. The maximum MRR of 1.75 kg COD m^{-3} d^{-1} (18.3 g CH_4 m^{-3} h^{-1}) was achieved during phase 7.

These values are low compared to the MRR obtained in previous studies for biofiltration of methane from landfills with a removal rate of ca. 30–280 g-CH_4 m^{-3} h^{-1}. These studies used soil, a mixture of compost and fibers, and peat as biofilters media (Park et al., 2009). The key reason for the lower MRR in this study might be the supply of methane as the dissolved methane. The gas flow rate has the main impact on methane removal efficiency, especially in biofilters that treat gaseous methane (Josiane and Michèle, 2009). However, for dissolved methane treatment, the methane supply rate was controlled to avoid the formation of a potentially flammable mixture of CH_4 and oxygen. Therefore, the appropriate ratio of dissolved methane supply rate to air-supply rate remains to be determined. Also, in this study, APG bio-carriers were used as supporting carrier materials. According to a previous down-flow hanging sponge study, the dissolved oxygen concentration drops significantly from the sponge surface toward the center, and anaerobic conditions prevail inside the sponge-cubes (Araki et al., 1999). Potentially, the aerobic MOB was not active deep inside the biofilm due to oxygen diffusion limitations. Therefore, an

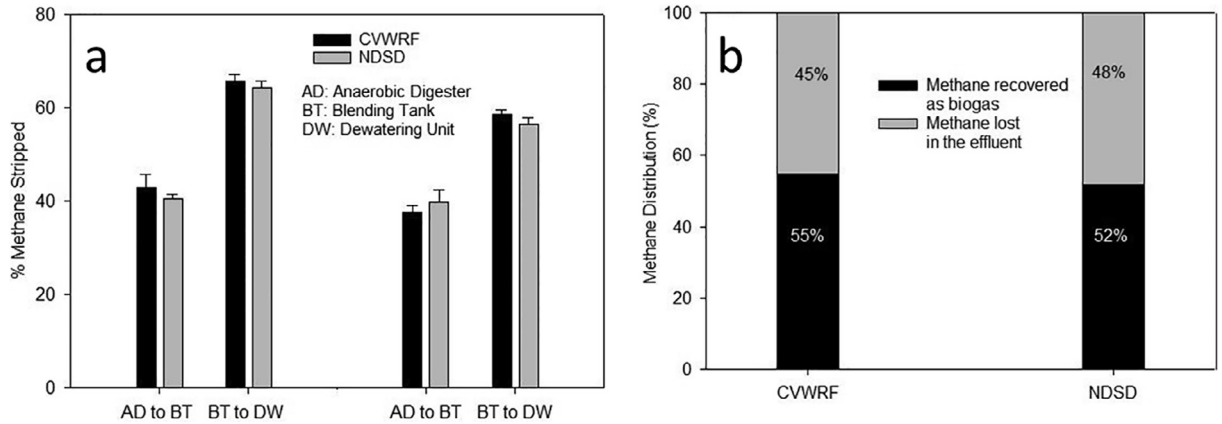

Fig. 3. a) Percentage of dissolved methane stripped off from the effluents at various locations and b) Percent distribution of methane recovered as biogas and methane lost in the effluent for CVWRF and NDSD anaerobic digesters.

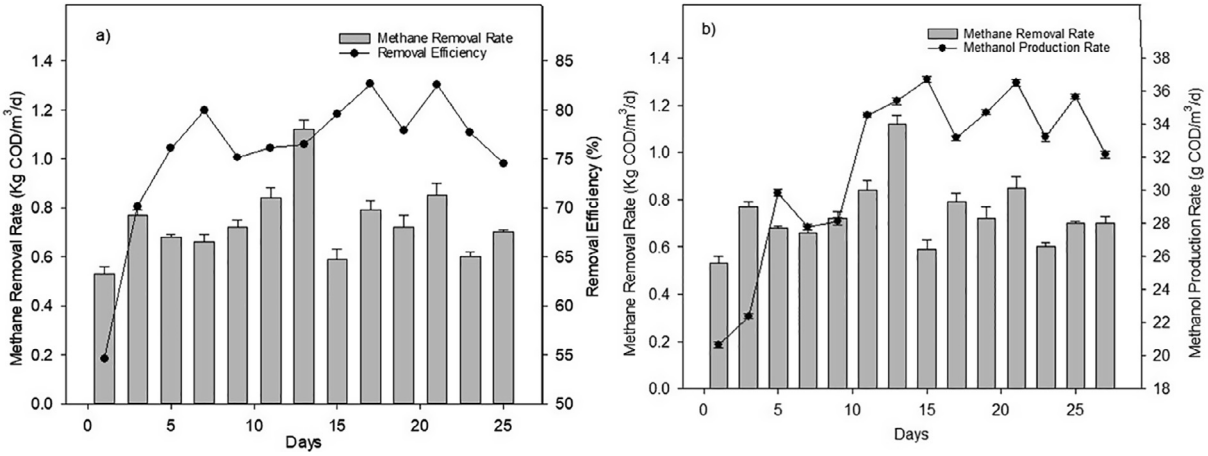

Fig. 5. a) Methane removal rate (vertical bars) with removal efficiency (black circles) by mixed community nitrifying biomass, b) Methane removal rate with methanol production rate using mixed community nitrifying biomass.

improvement of carrier structures or better oxygen supply strategy to increase effective oxygen supply is an important design consideration for future studies.

To analyze the differences due to the operating temperature, MRR values were compared using one-way ANOVA between phase 1 and phase 2. ANOVA results indicate that MRR in phase 1 and phase 2 are statistically different ($p < 0.05$) and is more in phase 2 as compared to phase 1, which indicates that at 37 °C methanotrophs shows more activity of methane oxidation than at 25 °C. This result is similar to a previous result by Soni et al. (1998), which showed that methanotroph, especially *M. capsulatus*, grew best at 37 °C with maximum methane consumption.

The polymerase chain reaction (PCR) was performed for *pmoA* genes, to confirm the purity and the presence of methane-oxidizing bacteria in the reactor. The PCR for *pmoA* (Methanotrophs) were conducted using A189f/mb661 primer pair, which resulted in a PCR product of 470 base-pairs (bp), similar to a previous report (Costello and Lidstrom, 1999). Sanger sequencing was performed for methanotrophs; the results from Sanger sequencing showed that *Methylococcus capsulatus* was the prominent species present in PBR.

3.3.2. Sequencing batch reactor for DAMO

The reactor was started with the aim to enrich nitrite- and nitrate-dependent anaerobic methane oxidizers. For the methane oxidation experiment performed in this work, serum bottle tests were conducted for calculating MRR. The serum bottles contained mixed liquor from the reactor with nitrite and nitrate in the feed and methane in the headspace. The MRR calculated was 0.31 ± 0.11 kg COD m^{-3} d^{-1}. Also, the nitrite and nitrate removal in the serum bottle which did not hold methane gas was insignificant, confirming that the activity was dependent on methane as an electron donor, and not on other internal electron donor/s. The value of MRR is higher than results reported by Ettwig et al. (2010), having MRR of 61 × 10^{-3} kg COD m^{-3} d^{-1} for a batch-scale experiment performed with 380 ml of an anoxic enriched culture of DAMO bacteria. The reason for higher MRR in this study might be the amount of NO_2-N spiked for the batch experiments. Ettwig and colleagues spiked the batch experiment with 0.7 mg L^{-1} of NO_2-N, and the experiment was conducted for 30 min (Ettwig et al., 2010). In our study, the batch experiment was spiked with about 16 mg L^{-1} of NO_2-N and was conducted for 3 days, allowing the bacteria to grow and consume more methane and nitrite. *Methylomirabilis oxyfera related organisms* in NC 10 phylum have been shown to be dominant DAMO organisms in denitrifying methane oxidation reactors with nitrite as the electron acceptor (Ettwig et al., 2010; Raghoebarsing et al., 2006). A PCR based hunt using *Methylomirabilis oxyfera* related biomarkers followed by cloning and Sanger sequencing of the purified product confirmed the presence of *Methylomirabilis oxyfera* related organisms in the reactor.

3.3.3. AOB attached growth batch reactor for methanol production

The thought of using AOB over MOB is rather upfront and straightforward. MOB oxidizes methane entirely to CO_2, which cannot be readily utilized as a fuel. Hence, if MOB are to be used for methanol (CH_3OH) production, then the pathways that further process CH_3OH will be required to be selectively inhibited, which may not be easy to do. However, AOB only oxidizes CH_4 partially to CH_3OH as reported by different studies (Jones and Morita, 1983) and possibly to trace amounts of formaldehyde (HCHO) (Voyseyt and Wood, 1987).

Mixed microbial AOB biomass from the PN reactor was withdrawn and enriched for methane oxidation in this study. Initially, AOB biomass was enriched with a high amount of NH_3 (700 mg NH_4^+-N L^{-1}) and 10% headspace of CH_4 and CO_2 (95:5% mixture). About 258.67 ± 20.03 mg L^{-1} d^{-1} of NH_3-N was oxidized to nitrite in case of batch enrichment. On subsequent decreases in NH_3-N concentration to 10 mg L^{-1} and keeping the methane concentration constant, the rate of NH_3-N oxidation was found to be 3.94 ± 1.62 mg NH_4^+-N L^{-1} d^{-1}.

An attached growth batch reactor was used in this strategy to evaluate methane oxidation by AOB. APG bio-carriers were enriched with the mixed microbial culture of AOB and used for the attached growth reactor, and 10 mg L^{-1} of NH_4^+-N was maintained in the reactor with methane in the headspace of the reactor bottle. Fig. 5(a) shows the methane removal rates (MRR) in terms of COD equivalent in the left axis and the overall methane oxidation efficiency on the right axis, both as a function of time on the x-axis. Average MRR over 25 days of the experiment was 0.73 ± 0.03 kg COD m^{-3} d^{-1} with an average removal efficiency of 76%. Also, Fig. 5(b) shows the methanol production rate along with MRR, with an average methanol production rate of 31.5 ± 0.19 g COD m^{-3} d^{-1} equivalent over the same period. Methanol being a cheap external carbon source having a low COD:N requirement of 4.5 is extensively used for denitrification (Exponent and Theis, 2012). Hence, on calculating for the amount of NO_3-N reduction using methanol, the amount of methanol produced in our study can theoretically reduce approximately 7 g NO_3-N m^{-3} d^{-1}. Moreover, the onsite production of CH_3OH can reduce the cost for denitrification, qualifying the treatment plant for the circular economy and making the process energy-efficient (Exponent and Theis, 2012).

The result of methanol production rate from this study was lower than the results reported by Taher and Chandran (2013). In their study, the methanol production rate was 80.45 ± 1.7 g COD m^{-3} d^{-1} for mixed nitrifying culture with 10 mg L^{-1} of NH_3-N in the feed. The primary reason for the lower methanol production rate could be the way methane was supplied in current experiments. The methane and

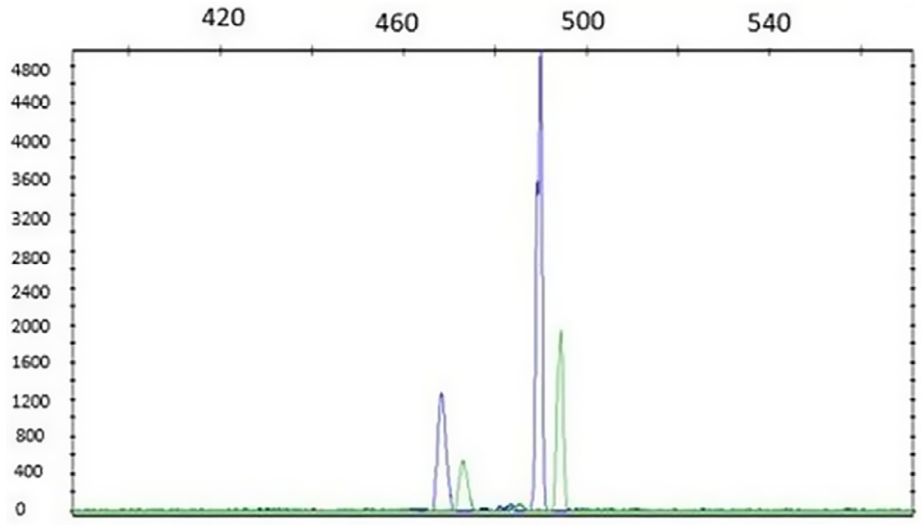

Fig. 6. Electropherograms of amoA Gene from attached growth batch reactor for oxidation of methane to methanol.

Table 3
Terminal Fragment sizes and corresponding AOB groups based on TRFLP.

Species	Growth at 0 °C	Salt Requirement	TRFLP Electropherogram peak
N. europaea	No	No	491/491
N. oligotropha	No	No	48/135, 354/135
N. cryotolerans	Yes	Yes	48/441, 354/48
N. marina	No	Yes	48/441, 48/135
N. communis	No	No	491/491
Nitrosospira sp. NpAV	–	–	470/470

oxygen supplies in this study were not continuous, unlike in the study by Taher and Chandran (2013), where continuous CH_4 was fed at 30 ml/min with the pulsing of pure oxygen at 500 ml/min for 30 min every hour. Our result for CH_3OH production obtained in this study is approximately 1.5 times higher than the previous study, which employed a pure culture of immobilized *Nitrosomonas europaea* for the oxidation of methane (Thorn, 2007). However, our methanol production rate is similar to what Su et al., (2019) recently reported in a continuous flow process at a hydraulic retention time of 2 h, It could be concluded based on these results that the primary objective of this strategy to oxidize D-CH_4 and convert it into methanol was accomplished. Additionally, CH_3OH as a stimulant for denitrification could be directed to a downstream anoxic reactor to boost denitrification.

The polymerase chain reaction (PCR)-based TRFLP was conducted to reveal the identity of AOBs. Fig. 6 shows the electropherograms for AOBs present in the reactor. According to the literature as shown in Table 3, the T-RF peaks for AOBs were observed *at* 469/470 and 488/491, which corresponds to *Nitrosospira* sp. NpAV and *Nitrosomonas europaea/N. communis*, respectively (Junier et al., 2008; Whang et al., 2009). Both *Nitrosomonas europaea/N. communis* and *Nitrosospira* sp. NpAV dominated the reactor biomass for the oxidation of methane to CH_3OH.

3.3.4. Comparison of three microbial resource management based strategies

Table 4 summarizes methane oxidation in terms of COD equivalent and absolute number in mM for three management strategies. As seen from this table, the packed bed column reactor for aerobic methane oxidation by methanotrophic bacteria provided the maximum removal rate. In our lab scale reactor, about 1.14 mM CH_4 h^{-1} can be oxidized to CO_2 with a residence time of 30 min. Also, with the use of APG biocarriers there is a negligible washout of the biomass. However, there is a limitation on oxygen transfer to the interior of the biocarrier, which decreases the efficiency of the system. DAMO was the slowest process in term of methane oxidation. This is perhaps because of the slow growth of DAMO organisms. Nevertheless, this method is useful because dissolved methane gas is used to denitrify nitrate. Hence, methane management using DAMO is enables managing dissolved nitrogen in liquid wastes. The third method is the aerobic methane oxidation by AOBs to methanol. The in-situ methanol could be used to enhance the denitrification process. In our study, about 0.48 mM of methane h^{-1} was oxidized, and about 31.5 g COD m^{-3} d^{-1} was produced. Implementation of this strategy could potentially allow WWTPs to offset some of their CH_3OH costs.

4. Conclusions

In this study, we evaluated three strategies to manage D-CH_4 in anaerobic effluents. In all cases, the D-CH_4 was successfully removed from its dissolved form with more than 80% removal efficiency. The use of DAMO organisms to simultaneous oxidize D-CH_4 and denitrify, and the conversion of D-CH_4 to methanol by aerobic ammonia oxidizers provide sustainable approaches to manage D-CH_4 in anaerobic effluents. However, both DAMO and aerobic methane conversion to methanol by aerobic ammonia oxidizers are relatively slow processes; hence, efficient process engineering can perhaps help integrate these useful bioprocesses into mainstream treatment for efficient carbon and nitrogen management. Overall, our results indicate that each of the tested strategies can be employed individually as a post-treatment process and could make an anaerobic treatment process eco-friendlier

Table 4
Methane removal rates using three microbial resource management strategies.

Method	Methane Removal Rate (kg COD m^{-3} d^{-1})	Methane Removal Rate (mM h^{-1})
Aerobic Methane Oxidation by Methanotrophic bacteria	1.28 ± 0.16	1.14 ± 0.10
Anaerobic methane oxidation by DAMO	0.31 ± 0.11	0.2 ± 0.07
Aerobic methane oxidation by Ammonia-oxidizing bacteria	0.73 ± 0.03	0.48 ± 0.02

by oxidizing D-CH_4.

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References

Araki, N., Ohashi, A., Machdar, I., Harada, H., 1999. Behaviors of nitrifiers in a novel biofilm reactor employing hanging sponge-cubes as attachment site. Water Sci. Technol. 39 (7), 23–31.
Bhattacharjee, A.S., Motlagh, A.M., Jetten, M.S.M., Goel, R., 2016. Methane dependent denitrification- from ecosystem to laboratory-scale enrichment for engineering applications. Water Res. Elsevier Ltd 99 (2), 244–252.
Billet, R., Schultes, M., 1993. Predicting mass transfer in packed columns. Chem. Eng. Technol. 16 (1), 1–9.
Cakir, F.Y., Stenstrom, M.K., 2005. Greenhouse gas production: a comparison between aerobic and anaerobic wastewater treatment technology. Water Res. 39 (17), 4197–4203.
Costello, A., Lidstrom, M., 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Appl. Environ. 65 (11), 5066–5074.
Daelman, M.R.J., van Voorthuizen, E.M., van Dongen, U.G.J.M., Volcke, E.I.P., van Loosdrecht, M.C.M., 2012. Methane emission during municipal wastewater treatment. Water Res. Elsevier Ltd 46 (11), 3657–3670.
El-Fadel, M., Massoud, M., 2001. Methane emissions from wastewater management. Environ. Pollut. 114 (2), 177–185.
Ettwig, K.F., Butler, M.K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M.M.M., Schreiber, F., Dutilh, B.E., Zedelius, J., De Beer, D., Gloerich, J., Wessels, H.J.C.T., Van Alen, T., Luesken, F., Wu, M.L., Van De Pas-Schoonen, K.T., Op Den Camp, H.J.M., Janssen-Megens, E.M., Francoijs, K.J., Stunnenberg, H., Weissenbach, J., Jetten, M.S.M., Strous, M., 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature Nature Publishing Group 464 (7288), 543–548.
Exponent, Theis, T.L., 2012. Methanol Use in Wastewater Denitrification.
Gal'chenko, V.F., Lein, A.Y., Ivanov, M.V., 2004. Methane content in the bottom sediments and water column of the Black Sea. Microbiology 73 (2), 211–223.
Hartley, K., Lant, P., 2006. Eliminating non-renewable CO_2 emissions from sewage treatment: an anaerobic migrating bed reactor pilot plant study. Biotechnol. Bioeng. 95 (3), 384–398.
Hatamoto, M., Yamamoto, H., Kindaichi, T., Ozaki, N., Ohashi, A., 2010. Biological oxidation of dissolved methane in effluents from anaerobic reactors using a down-flow hanging sponge reactor. Water Res. 44 (5), 1409–1418.
He, X., Hägg, M.B., 2012. Membranes for environmentally friendly energy processes. Membranes 2 (4), 706–726.
Henares, M., Izquierdo, M., Penya-Roja, J.M., Martínez-Soria, V., 2016. Comparative study of degassing membrane modules for the removal of methane from expanded granular sludge bed anaerobic reactor effluent. Sep. Purif. Technol. Elsevier B.V. 170, 22–29.
Jones, R.D., Morita, R.Y., 1983. Methane oxidation by Nitrosococcus oceanus and Nitrosomonas europaea. Appl. Environ. Microbiol. 45 (2), 401–410.
Josiane, N., Michèle, H., 2009. The influence of the gas flow rate during methane biofiltration on an inorganic packing material. Can. J. Chem. Eng. 87 (1), 136–142.
Junier, P., Junier, T., Witzel, K., 2008. In: TRiFLe, a Program for In Silico Terminal Restriction Fragment Length Polymorphism Analysis with User-Defined Sequence Sets, pp. 6452–6456.
Kotay, S.M., Mansell, B.L., Hogsett, M., Pei, H., Goel, R., 2013. Anaerobic ammonia oxidation (ANAMMOX) for side-stream treatment of anaerobic digester filtrate process performance and microbiology. Biotechnol. Bioeng. 110 (4), 1180–1192.
Lee, E.H., Yi, T., Moon, K.E., Park, H., Ryu, H.W., Cho, K.S., 2011. Characterization of methane oxidation by a methanotroph isolated from a landfill cover soil, South Korea. J. Microbiol. Biotechnol. 21 (7), 753–756.
Lichstein, H.C., Soule, M.H., 1944. Studies of the effect of sodium azide on microbic growth and respiration: I. the action of sodium azide on microbic growth. J. Bacteriol. 47 (3), 221–230.
Liu, Z.H., Yin, H., Dang, Z., Liu, Y., 2014. Dissolved methane: a hurdle for anaerobic treatment of municipal wastewater. Environ. Sci. Technol. 48 (2), 889–890.
Lobato, L.C.S., Chernicharo, C.A.L., Souza, C.L., 2012. Estimates of methane loss and energy recovery potential in anaerobic reactors treating domestic wastewater. Water Sci. Technol. 66 (12), 2745–2753.
Luesken, F.A., Zhu, B., van Alen, T.A., Butler, M.K., Diaz, M.R., Song, B., Op den Camp, H.J.M., Jetten, M.S.M., Ettwig, K.F., 2011. pmoA primers for detection of anaerobic methanotrophs. Appl. Environ. Microbiol. 77 (11), 3877–3880.
Machdar, Izarul, Harada, Hideki, Ohashi, Akiyoshi, Yuji, Sekiguchi, Okui, Hiroyuki, Ueki, K., 1997. A novel and cost-effective sewage treatment system consisting of UASB pre-treatment and aerobic post-treatment units for developing countries. Water Sci. Technol. 36 (12), 189–197.
Matsuura, N., Hatamoto, M., Sumino, H., Syutsubo, K., Yamaguchi, T., Ohashi, A., 2010. Closed DHS system to prevent dissolved methane emissions as greenhouse gas in anaerobic wastewater treatment by its recovery and biological oxidation. Water Sci. Technol. 61 (9), 2407–2415.
Matsuura, N., Hatamoto, M., Sumino, H., Syutsubo, K., Yamaguchi, T., Ohashi, A., 2015. Recovery and biological oxidation of dissolved methane in effluent from UASB treatment of municipal sewage using a two-stage closed downflow hanging sponge system. J. Environ. Manage. Elsevier Ltd 151, 200–209.
McCarty, P.L., Bae, J., Kim, J., 2011. Domestic wastewater treatment as a net energy producer-can this be achieved? Environ. Sci. Technol. 45 (17), 7100–7106.
McCarty, P.L., Smith, D.P., 1986. Anaerobic wastewater treatment. Environ. Sci. Technol. 20 (12), 1200–1206.
Metz, B., Davidson, O., Bosch, P.R., Dave, R., Meyer, L.A. (Eds.), 2007. Mitigation of Climate Change: Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Park, S., Lee, C.-H., Ryu, C.-R., Sung, K., 2009. Biofiltration for reducing methane emissions from modern sanitary landfills at the low methane generation stage. Water Air Soil Pollut. 196 (1), 19–27.
Pauss, A., Andre, G., Perrier, M., Guiot, S.R., 1990. Liquid-to-gas mass transfer in anaerobic processes: inevitable transfer limitations of methane and hydrogen in the biomethanation process. Appl. Environ. Microbiol. 56 (6), 1636–1644.
Pernetti, M., Di Palma, L., 2005. Experimental evaluation of inhibition effects of saline wastewater on activated sludge. Environ. Technol. 26 (6), 695–703.
Raghoebarsing, A.A., Pol, A., Van De Pas-Schoonen, K.T., Smolders, A.J.P., Ettwig, K.F., Rijpstra, W.I.C., Schouten, S., Sinninghe Damsté, J.S., Op Den Camp, H.J.M., Jetten, M.S.M., Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440 (7086), 918–921.
Remi, C., Boulestreau, M., Lesjean, B., 2014. Proof of concept for a new energy-positive wastewater treatment scheme. Water Sci. Technol. 70 (10), 1709–1716.
Rotthauwe, J., Witzel, K., 1997. The ammonia monooxygenase structural gene amoA as a functional marker molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63 (12), 4704–4712.
Sander, R., 2015. Compilation of Henry's law constants (version 4.0) for water as solvent. Atmos. Chem. Phys. 15 (8), 4399–4981.
Smith, A.L., Stadler, L.B., Cao, L., Love, N.G., Raskin, L., Skerlos, S.J., 2014. Navigating wastewater energy recovery strategies: a life cycle comparison of anaerobic membrane bioreactor and conventional treatment systems with anaerobic digestion. Environ. Sci. Technol. 48 (10), 5972–5981.
Soni, B.K., Conrad, J., Kelley, R.L., Srivastava, V.J., 1998. Effect of temperature and pressure on growth and methane utilization by several methanotrophic cultures. Appl. Biochem. Biotechnol. 70–72, 729–738.
Souza, C.L., Chernicharo, C.A.L., Aquino, S.F., 2011. Quantification of dissolved methane in UASB reactors treating domestic wastewater under different operating conditions. Water Sci. Technol. 64 (11), 2259–2264.
Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. The Physical Sciences Basis. University Press, Cambridge, New York.
Su, Yu.-C., Sathyamoorthy, S., Chandran, L., 2019. Bioaugmented methanol production using ammonia oxidizing bacteria in a continuous flow process. Bioresour. Technol. 79, 101–107.
Taher, E., Chandran, K., 2013. High-rate, high-yield production of methanol by ammonia-oxidizing bacteria. Environ. Sci. Technol. 47 (7), 3167–3173.
Thorn, G.J.S., 2007. Development of An Immobilized Nitrosomonas europaea Bioreactor for the Production of Methanol from Methane. University of Canterbury, Christchurch, New Zealand.
U.S., and Environmental Protection Agency, Washington DC, 2017. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2015 38(4).
van Lier, J.B., van der Zee, F.P., Frijters, C.T.M.J., Ersahin, M.E., 2015. Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. Rev. Environ. Sci. Bio/Technol. Springer, Netherlands 14 (4), 681–702.
Voyseyt, P.A., Wood, L.M., 1987. Methanol and formaldehyde oxidation by an autotrophic nitrifying bacterium. J. Gen. Microbiol. 33, 283–290.
Whang, L.M., Yang, K.H., Yang, Y.F., Han, Y.L., Chen, Y.J., Cheng, S.S., 2009. Microbial ecology and performance of ammonia oxidizing bacteria (AOB) in biological processes treating petrochemical wastewater with high strength of ammonia: effect of Na_2CO_3 addition. Water Sci. Technol. 59 (2), 223–231.
Whittenbury, R., Wilkinson, J.F., 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. J. Gen. Microbiol. 61 (1970), 205–218.
Wilshusen, J.H., Hettiaratchi, J.P.A., De Visscher, A., Saint-Fort, R., 2004. Methane oxidation and formation of EPS in compost: effect of oxygen concentration. Environ. Pollut. 129 (2), 305–314.
Zhan, Y.Y., Zhang, Y., Li, Q.M., Du, X.Z., 2010. A novel visible spectrophotometric method for the determination of methanol using sodium nitroprusside as spectroscopic probe. J. Chin. Chem. Soc. 57 (2), 230–235.