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Oxidation of Methane in Boggy Sediment, Industrial Biogas Plant and a Landfill Leachate Treatment Plant

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Abstract

For use in a landfill, a laboratory reactor for safe and environmentally friendly biological utilization of low-concentration methane gas will be further developed. The current principle of denitrification-coupled aerobic methane oxidation will be replaced by methane oxidation under anaerobic conditions. Anaerobic methane oxidation offers the advantage that, in addition to methane, nitrate also undergoes biodegradation. Another advantage is that the oxygen content can be significantly lower. This reduces the risk of the formation of an explosive atmosphere in the reactor. Currently, the principle of anaerobic methane oxidation is known. However, organisms capable of doing so are not yet available as a pure culture. Therefore, several biomasses were probed for the ability of anaerobic methane oxidation. It was found that moor-heavy sediment, activated sludge from the leachate treatment plant and biomass from the local biogas plant oxidize methane after the natural carbon source (C source) was been removed.

1. Introduction

When operating a landfill leachate accumulates. Leachate contains the nitrogen compounds ammonium (NH_4^+) and nitrate (NO_3^-), which are converted into elemental nitrogen (N_2) by nitrification and denitrification [1]. Denitrification requires an external C source, mostly acetic acid [2]. So cost-effective solutions to overcome carbon limitation for denitrification are needed. When operating a landfill, in addition to leachate, the greenhouse gas methane (CH_4) is also produced. In low concentrations, the energy content of methane is insufficient to utilize it energetically. At the same time, CH_4 forms an explosive atmosphere in concentrations of 4-17% (v / v) together with oxygen (O_2) [3]. CH_4 in low concentrations (<25% (v / v)) is therefore eliminated via methane oxidation [4]. As a cost-effective solution, the acetic acid is to be replaced by CH_4 by trying to couple the methane oxidation with the denitrification. A coupling of the methane oxidation with the denitrification can be achieved via the methanotrophic bacterium *Methylocystis rosea*. *M. rosea* can metabolize CH_4 to methanol with the help of atmospheric O_2 (Figure 1) [5], which can be used by the denitrifiers. Since denitrification, in contrast to classical methane oxidation, takes place under anaerobic conditions, it is difficult to run both processes unimpeded in one reactor. Recently, the phenomenon of anaerobic methane oxidation has been discovered [6], [7].

Bacteria, such as *Canidatus methylomirabilis oxyfera*, can metabolize CH_4 under anaerobic conditions by recovering O_2 for oxidation from NO_2^- (*Equation 1*).

Equation 1*:* $3\,CH_4 + 8\,NO_2^- + 8\,H^+ \rightarrow 3\,CO_2 + 4\,N_2 + 10\,H_2O\,, \Delta G = -928\,kJ\,mol^{-1}$

The AOM is explained by the example of the bacteria of the NC10 phylum and NO_2^-, as it is well described. Also, an AOM is possible with NO_3^- [8]. Bacteria of this species can be found in wetlands [6], [7]. Other sources of suitable micro biocoenoses with an anaerobic environment, a high methane and nitrogen content are landfill sites and biogas plants. Anaerobic methanotrophic bacteria could enter the leachate treatment plant (LLTP) with the leachate emanating from the landfill site. Since bacteria with denitrifying and methane oxidizing properties are not yet available, natural micro biocoenoses for the development of a laboratory reactor for the biological utilization of low-concentration methane gas should be investigated for these properties. The following should be investigated: moor-heavy sediment (BS), activated sludge from the leachate treatment plant (LLTP) and biomass from the fermentation and composting plant (BGP) at the site: metabolon.

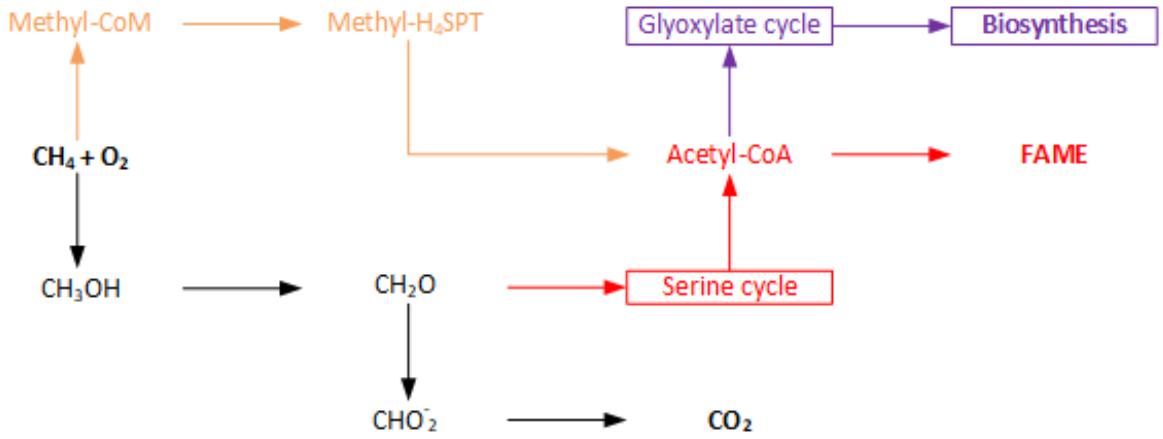

Figure 1: Classic methane oxidation using the example of Methylocystis rosea: reverse methanogenesis using methyl coenzyme M (orange), serine cycle and formation of fatty acid methyl esters (FAME) (red) and glyoxylate cycle and biosynthesis (violet) [5].

2. Material and Methods

For investigation, sediment samples and samples of LLTP and BGP with a high solids content were taken. Samples BS 1 to BS 3 were all taken at different locations (of different nature) at the same location. Sample BS IV was taken for comparison in another boggy area. Subsequently, the samples were diluted with a 0.9% NaCl solution. For trial A (with natural C source), 1 mL of diluted biomass was transferred to 9 mL of medium (0.9% NaCl solution with a NO_3^- content of 500 mg L^{-1}) in a methane atmosphere. For trial B (without natural C source), the bacteria were isolated from the diluted biomass with a sterile filter (pore size 0.45 µm) and dissolved in 10 mL of medium (0.9% NaCl solution with a NO_3^- content of 500 mg L^{-1}) resuspended. The culture tubes also contained a CH4 atmosphere. At the

beginning, the gas composition in the samples was measured and it was ensured that the methane content in the batches was at least 90% (v/v). After 2 months, the atmospheres in the culture tubes were again measured by gas chromatography. To measure the gases, a modified version of the type 1310 gas chromatograph from ThermoFisher Scientific was used. The modified version is designed in terms of detection limits of relevant gaseous molecules specifically for the analysis of biogas and landfill gas. The gas chromatograph uses the columns HayeSep Q and Rtx®-1 from RESTEK (size exclusion), so that in a series connection of both columns and the carrier gas helium, the compounds CH_4, CO_2, O_2, N_2, N_2O, H_2S, and H_2O can be separated and analysed.

3. Results

The results of the gas phase measurements of incubation trials with natural C source are shown in Table 1, the results of the gas phase measurements of the incubation trials without natural C source inTable 2.

Table 1: Changes in the atmospheres in the incubation approaches with native C source. It is shown an average of 5 replicas. BS = bottom sediment, LLTP = biomass from a landfill leachate treatment plant, BGP = biomass from a biogas plant. "Samples SP 1 - 3 belong to the same location, SP IV was taken elsewhere.

sample	CO_2 mmol	O_2 mmol	N_2 mmol	CH_4 mmol	N_2O mmol
BS 1	0,95	-1,82	3,17	-12,43	1,79
BS 2	0,05	-2,59	-5,99	-4,55	0,69
BS 3	1,58	-6,20	-7,33	-2,35	2,50
BS IV	1,27	-5,89	-7,41	-1,08	2,14
LLTP	1,04	-4,02	-4,85	1,08	1,84
BGP	-0,37	-4,47	-7,20	-0,59	0,22

Table 2: Changes in the atmospheres in the incubation approaches without natural C source. It is shown an average of 5 replicas. BS = bottom sediment, LLTP = biomass from a landfill leachate treatment plant, BGP = biomass from a biogas plant. "Samples SP 1 - 3 belong to the same location, SP IV was taken elsewhere.

Sample	CO_2 mmol	O_2 mmol	N_2 mmol	CH_4 mmol	N_2O mmol
BS 1	-0,15	5,02	21,16	-25,81	0,46
BS 2	1,85	0,82	21,48	-26,62	2,74
BS 3	0,73	0,83	11,83	-15,78	1,47
BS IV	0,52	-0,12	8,12	-13,99	1,24
LLTP	4,59	-7,99	6,33	-3,68	5,93
BGP	5,31	-5,37	18,94	-26,36	6,74

4. Discussion

Figure 2 shows the changes in the atmospheres in the natural C source incubation approaches. BS 1 shows a CO2 formation of 0.5 mmol, which can occur independently of aerobic (Figure 1) [5] and anaerobic (Equation 1) [9] metabolic pathways. An explanation for the CO_2 formation can be the metabolism of the natural C source in the incubation approach. In addition to the formation of CO_2, the consumption of -1.82 mmol O_2 is also observed. An O_2 consumption was not expected because the air in the approach should be completely replaced by methane. Because the control of the atmosphere in the 20 mL batches was difficult due to the small size of the batches, it is possible that some residual O_2 was present in the batches. BS 1 also shows formation of N_2, which may have been caused by denitrification [1] as well as by AOM [6], [7], [9]. N_2O, as an intermediate of denitrification, in the samples also indicates denitrification [1], [10]. Consumption of -12.43 mmol CH_4, was not expected. The approach included natural C source, so it should be metabolized first. It is possible that CH_4 after the natural C source was also metabolized. Assuming an AOM, the formation of N_2O is not only an indicator for denitrification [1], but also of methanotrophic organisms [11]. The incubation approach BS 3 behaves in a similar way to BS 2. However, the large error on the measured values of N_2 and CH_4 is noticeable. This error can be explained by a possible inhomogeneity of the number of bacteria and carbon amount in the 5 trials. Also noteworthy is the consumption of 5.99 mmol N_2. This can only be explained by a measurement error or by nitrogen-fixing organisms or plants. BS 3 and BS IV show the same tendency as BS 2. The trial LLTP leads to similar results as the trials BS 2 to BS IV with the difference that a smaller amount of CH_4 was formed. The formation can be explained by a possible measurement error. Another explanation would be the

formation by methanogenic bacteria from the landfill body. Because especially in the non-ventilated dead spaces of the plant, in which organic material is deposited, fermentation due to O_2-deficiency can take place. Figure 2 shows that BGP is also similar to trials BS 2 to BS IV. The BGP is the only sample of the trials with natural C source and a CO_2-consumption. This was not to be expected, since CO_2 should be formed as a metabolite product. One explanation would be algae in the landfill leachate [12], which metabolize CO_2 to O_2. Even if the results of the incubation approaches with natural C source are not very clear and the changes in atmosphere are not pronounced, they can be explained quite well. In addition, Figure 2 shows a small CH4 degradation, as was due to the natural C source.

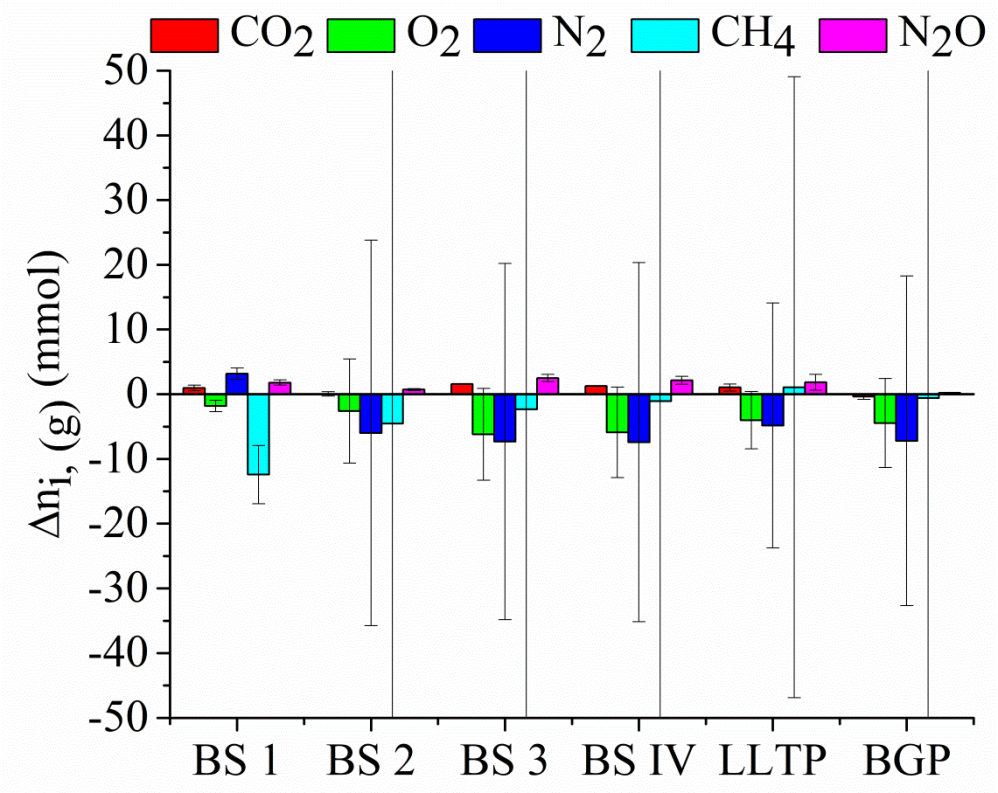

Figure 2: Changes in the atmospheres in the incubation approaches with native C source. The average and standard deviation of 5 replicates are shown. BS = bottom sediment, LLTP = biomass from a landfill leachate treatment plant, BGP = biomass from a biogas plant. "Samples SP 1 - 3 belong to the same location, SP IV was taken elsewhere.

This becomes particularly clear in comparison with Figure 3. Figure 3 shows changes in the atmospheres in the incubation trials without natural C source. Compared to Figure 2, significantly more N_2 is produced and CH_4 is significantly more eliminated. Furthermore, in comparison to Figure 2, a significantly lower error is observed on the mean values in the approaches. This can be explained, on the one hand, by the larger changes in the trials. On the other hand, a sterile filter was used to isolate the bacteria. The filtration ensured that the bacteria and the C source were much more homogeneous in the samples. As a result, the CH_4 consumption began at the same time and much earlier, whereby the error was

significantly reduced. In Sample BS 1 a low CO_2 consumption was detected. The consumed CO_2 is probably the small amount, which is in the ambient air. The consumption can be caused by algae. The formation of about 5 mmol O_2 is also an indication for algae. Algae in the samples make the balancing difficult because they change the O_2 and CO_2 content unchecked. Another explanation for the low amount of CO_2 may be the solubility, since CO_2 can be dissolved in aqueous solution as carbonic acid.

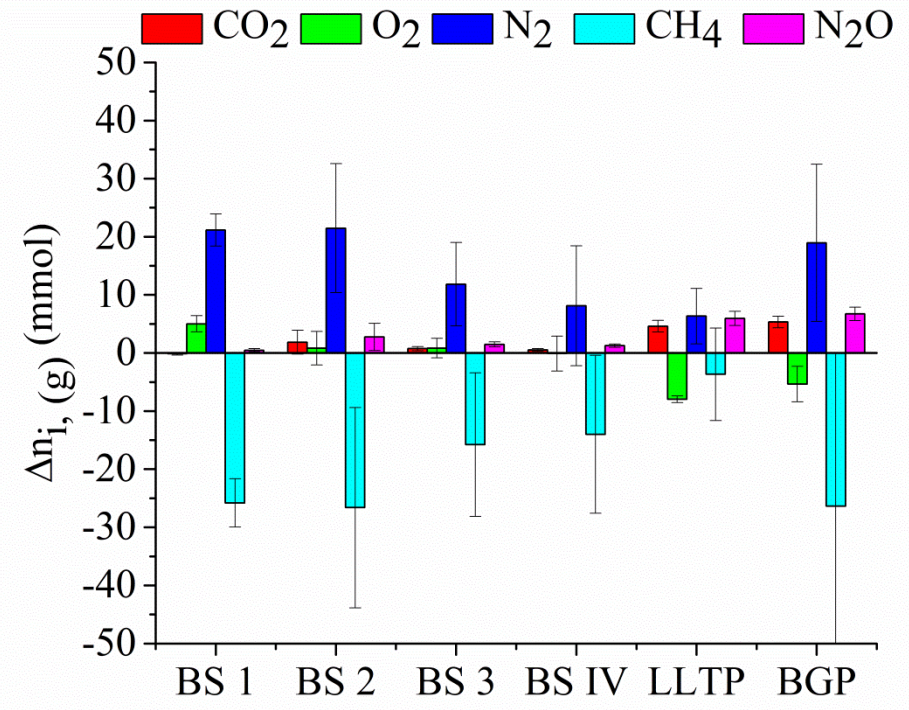

Figure 3: Changes in the atmospheres in the incubation batches without native C source). The average and standard deviation of 5 replicates are shown. BS = bottom sediment, LLTP = biomass from a landfill leachate treatment plant, BGP = biomass from a biogas plant. "Samples SP 1 - 3 belong to the same location, SP IV was taken elsewhere.

Another explanation for missing carbon in the gas phase can also be the metabolism to fatty acid methyl esters (FAME) or the formation of biomass, as shown in Figure 1. The formation of about 21 mmol N_2 can be attributed to denitrification or to the AOM. Since CH_4 was also used, in addition to the denitrification an AOM is assumed. A reason for the increased consumption of CH_4 is the removal of the natural C source. As already observed in the test series with natural C source, N_2O was also formed in BS 1 after the removal of the natural C source, presumably as an intermediate product of denitrification [11]. BS 2 and BS 3 behave like BS 1. The changes in the gas phase of BS 3 are less pronounced than in BS 1. Of the BS samples, the changes in the gas phase are lowest in BS IV. This can be explained by the age of the sample, since BS IV was already taken 2 weeks before the other samples. In contrast to BS 1 to BS IV LLTP and BGP show a significantly higher CO_2 formation and a higher O_2 consumption. The formation of N_2O is also increased in the LLTP and BGP. The increased O_2 consumption is due to an increased amount of O_2 at the beginning of the incubation. This shows that the method and the practical implementation can still be optimized. In addition,

an uneven gas composition initially makes balancing difficult. However, it makes the distinction between aerobic and anaerobic methane oxidation particularly difficult. As long as O_2 is present, aerobic metabolic pathways including aerobic methane oxidation can occur. After consumption of O_2, anaerobic methane oxidation and denitrification can additionally take place. An increased O_2 consumption is also an explanation for the increased amount of CO_2. Particularly noteworthy is the small amount of N_2 in LLTP. The mixed culture in LLTP specialized in denitrification should form the most N_2. At this point one might assume that the specialized mixed culture is particularly sensitive to the presence of O_2 or the lack of usable C source. Although the biomass in BGP is anaerobically adjusted, unlike LLTP it does not react sensitively. This suggests that biomass of BGP is much more robust to changes in the environment.

Summary and Outlook

In this work boggy sediment, biomass from the Landfill Leachate Treatment Plant and a biogas plant have been investigated. It was found that significantly more methane was metabolized after removal of the natural C source. Samples with naturally C source, except for BS 1, metabolised no CH_4. The changes in the gases CO_2, O_2, N_2 and N_2O were also minimal (Figure 2). An explanation was the untreated biomass, which allowed an original metabolism. In contrast, in all samples without a natural C source a CH_4 degradation and N_2 formation were observed Figure 3. No O_2 was measured in BS 1 to BS IV, so that an anaerobic methane oxidation is possible. Since a N_2 formation and a CH_4 degradation are approximately reciprocal, a stoichiometric correlation is supposed. A stoichiometric relationship between CH_4 degradation and N_2 formation is required for anaerobic methane oxidation. However, it is not excluded that the formation of N_2 was due (inter alia) to denitrification. LLTP and BGP also showed CH_4 degradation and N_2 formation. However, as O_2 was metabolized in these two samples, it is uncertain whether aerobic or anaerobic methane oxidation occurred. It is quite possible that as long as O_2 was present, CH_4 was oxidized aerobically and subsequently anaerobically. Since CH_4 degradation was found in all of the investigated biomass, the assumptions made at the beginning are considered to be true. According to the literature [6], [7] anaerobic methane oxidizers were found in sediments of a wet area. Anaerobic conditions and natural methane formation are the reasons for the methane-oxidizing property of the sediment. The activated sludge of the LLTP also showed a methane-oxidizing property. As previously suggested, landfilling is also a good environment for the development of methanotrophic organisms due to its anaerobic character and increased methane content. Such a finding can be confirmed by the literature [13]. Bacteria from the landfill can then be rinsed out and carried in leachate treatment plant. But the methanotrophic bacteria do not have to come exclusively from the landfill. These could also settle in the leachate treatment plant, as is the case in sewage treatment plants. As previously assumed, the methanotrophic property was also found in the biomass of the biogas plant [14]. It is not clear if this is an aerobic or anaerobic methane oxidation. But independent studies have also detected methanotrophic bacteria in biogas plants [15].

For identification, a quantitative polymerase chain reaction (qPCR) was made. A qPCR would be an evidence. Since a qPCR is not yet possible at this time, it should be scheduled for later. For the following series of experiments, it is advisable to isolate the methanotrophic bacteria directly from the biogas plant. Furthermore, a repetition in the 1 L scale with an improved experimental procedure is recommended. Care should be taken to ensure complete replacement of the air with methane. This step can also be skipped, as a 10 L scale-up is planned. Performing in a 10 L scale-up reactor also provides better process monitoring with ion and gas chromatography. At the same time, the pH value and the redox potential can be monitored. While running in a 10 L scale-up reactor, not only an absolute difference in methane change, but also a gas kinetics of degradation can be measured.

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