Lechner P. (Hrsg.): Waste matters. Integrating views. 2nd BOKU Waste Conference 2007, 16. - 19. April 2007, Wien. Proceedings; Facultas Verlags- und Buchhandels AG, ISBN 978-3-7089-0060-5

Biological Methane Oxidation Influenced by Biochemicals

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Abstract
Lab-scale batch experiments were conducted in order to investigate the impact of selected biochemicals on microbial methane oxidation. A significant impact of these potential root exudates could be shown. Whereas organic acids tend to decrease the methane degradation rates, nitrogen containing compounds showed remarkable stimulating effects.

1 Introduction
Landfill sites are relevant sources of methane as an important greenhouse gas, even in countries with highly developed waste management strategies. In Germany for instance, landfills in 2004 still caused 10.5 m t CO_2-equivalents of methane emissions. Nevertheless, a remarkable reduction of these emissions has been achieved during the last few years (1990: 31.5 m t CO_2-Eq.) due to the pre-treatment of waste (BMU 2005). In the future a further reduction has to be expected. Thus the biological landfill gas treatment through methane oxidising bacteria (MOB) will become more and more attractive for poor gas applications.

There are numerous studies covering the factors which influence the capacity of the microbial methane oxidation in landfill cover soils. The availability of methane, oxygen and soil parameters such as moisture, pH and nutrients were found to be the most important ones (Whalen et al. 1990, Boeckx and VanCleemput 1996, Czepiel et al. 1996, Börjesson et al. 1998, Hilger et al. 2000). Only few studies exist about the interactions between MOB and plants, most of them in terms of the availability of oxygen, water and nutrients in wetland systems. However, plant roots are further known to release organic substances into the soil (Vancura 1964, Richter 1968, Kickuth 1970). Phenols, glycosides, alkaloids, amino acids, organic acids and enzymes are among some of the most important ones. The inhibiting or enhancing impacts of those biochemicals on bacterial communities has been demonstrated (Baudoin 2003). Thus, interactions with the methane oxidising organisms also have to be considered possible.

2 Materials and Methods

2.1 Soil substrate

Soil sampled from a landfill site (temporary cover of a MSW landfill) was added to 250-ml-serum bottles which were charged with methane. The soil substrate is characterised in Table 1. Gas samples withdrawn from the bottles' headspace were sampled periodically and analysed by gas chromatography. Since the soil substrate was not incubated with methane before the experiments, the degradation started typically with a lag phase followed by a linear decrease of the methane concentration in the bottles. Oxidation rates were calculated from this linear part of each degradation process. The impact of the biochemical test compounds on the methane oxidation is presented in comparison to control variants. For the particular moisture content of each experiment, see the specifications of Figures 1-3.

2.2 Testing substances

The biochemicals were added to the soil substrate under the conditions listed in Table 1. The initial concentrations of biochemicals were derived from Baudoin (2003) who states that the daily carbon input from plant roots to the rhizosphere can be estimated at around 50 – 100 μg C $d^{-1}g^{-1}$ soil. Subsequent settings were conducted by referring the supply of the testing substances to the amount of nitrogen. The substances which were selected for presentation in this paper are listed in Table 2. For the dosage of testing substances, see the figure specifications.

Tab. 1: Initial conditions of the batch experiments

Water holding capacity(WHC)	45% (g H_2O/100 g dry matter (dm))
Total organic carbon	0.3% dm
pH	5.5
Loss on Ignition	5.7% dm
Nitrite-N (Eluate)	<0.3 mg/kg dm
Nitrate-N (Eluate)	1.8 mg/kg dm
Ammonium-N (Eluate)	1.7 mg/kg dm
Total Kjeldahl-N	980 mg/kg dm
Soil Weight per Bottle	10-20 g dry matter
Volume of Serum Bottles	250 ml
Methane Concentration	8%vol in ambient air
Temperature	20°C

Tab. 2: Testing substances

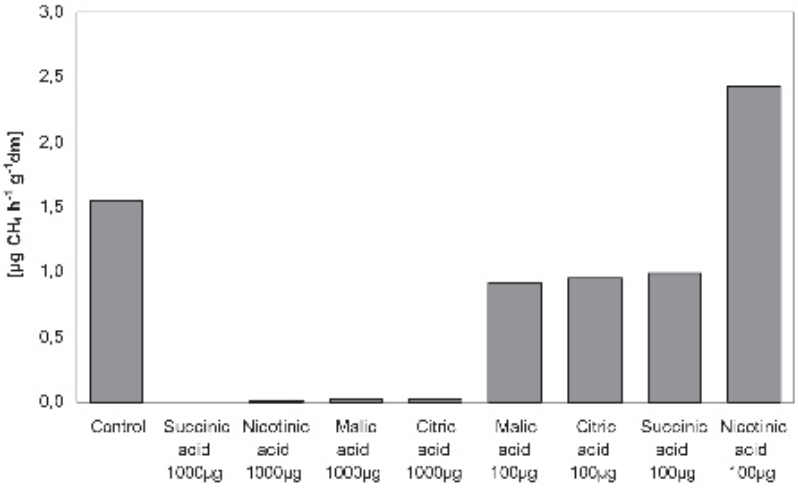


Fig: 1: Methane oxidation rates under the impact of selected organic acids (Soil moisture content: 57% of the WHC; Testing substance concentration is indicated in [µg C g^{-1}dm])

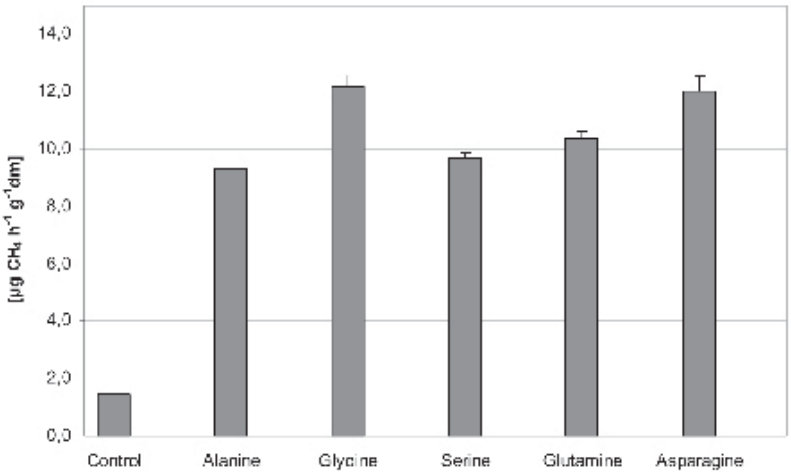


Fig. 2: Methane oxidation rates under the impact of selected amino acids (Soil moisture content: 57% of the WHC; Testing substance concentration: 100µg C g^{-1}dm)

Organic Acids	Amino Acids
Malic Acid	Alanine
Citric Acid	Glycine
Succinic Acid	Serine
Nicotinic acid	Glutamine, Asparagine

3 Results

Figure 1 shows the calculated oxidation rates of methane oxidation under the impact of the selected organic acids. The control variant without supply of biochemicals showed an oxidation rate of 1.5 µg CH_4 $h^{-1}g^{-1}$dm. Methane oxidation was clearly inhibited in all variants with high test compound concentrations (1000 µg C g^{-1}dm). The inhibition rate ranged from ca. 35% (Succinic acid at 100 µg C g^{-1}dm) to a complete inhibition (Succinic acid at 1000 µg C g^{-1}dm) in comparison to the control variant. The variants, representing the daily carbon input that is to be expected in rhizospheres, showed much lower impacts but nevertheless a significant reduction in methane oxidation. Nicotinic acid was the only substance that showed a positive effect on the methane oxidation, (increased rate by 57%). This effect could be verified by testing different nicotinic acid concentrations (not presented here).

Figure 2 shows the calculated oxidation rates under the impact of the selected

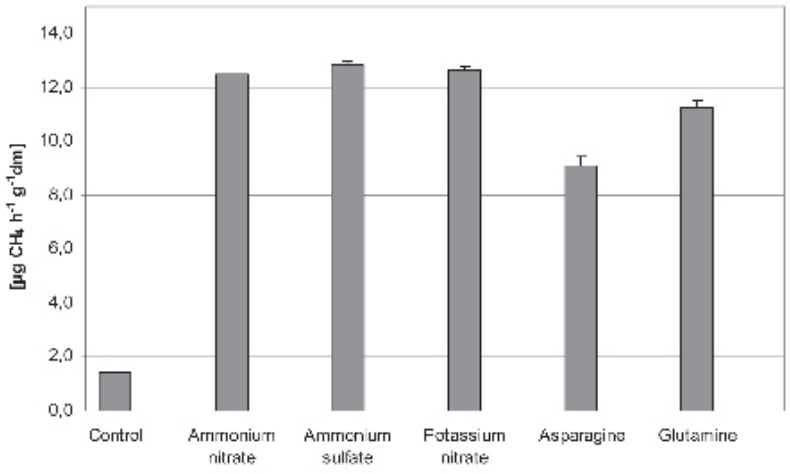


Fig. 3: Methane oxidation rates under the impact of mineral and organic nitrogen compounds (Soil moisture content: 68% of the WHC; Testing substance concentration: 0,25 mole N per mole supplied CH_4)

amino acids. All amino acids evidently showed a stimulating effect on the degradation activity. Compared with the control variants the oxidation rates lay between 6 and 8 times higher (Control variant: 1.5 μg CH_4 $h^{-1}g^{-1}$dm; Alanine: 9.2 μg CH_4 $h^{-1}g^{-1}$dm and Glycine: 12.2 μg CH_4 $h^{-1}g^{-1}$dm).

In order to investigate in more detail the role of nitrogen in the testing substances, further experiments were carried out which compared the impact of mineral and organic compounds. Anthony (1982) stated that 0,25 moles of N are required for every mole of assimilated carbon. Figure 3 shows the results of the testing variants with a dosage of 0.25 moles N per mole of supplied methane. Ammonium nitrate, ammonium sulfate and potassium nitrate were used as mineral compounds. All N-containing substances showed a stimulating effect, while the mineral compounds (ca. 12.5 μg CH_4 $h^{-1}g^{-1}$dm) seem to have a higher impact than asparagine and glutamine (9.1 and 11.3 μg CH_4 $h^{-1}g^{-1}$dm, respectively).

4 Conclusion

- The impact of selected biochemicals on the microbial methane oxidation could be shown. Thus, allelopathic interactions between plants and methanotrophic communities in landfill biocovers have to be expected.
- Most of the organic acids showed an inhibiting impact. This might be due to the drop of the pH. More plausible seems to be a competition of methanotrophic with heterotrophic bacteria in the biofilm. The stimulating effect of nicotinic acid might be attributed to nitrogen release during the degradation of the pyridine compound through heterotrophic bacteria.
- The positive result of N-containing biochemicals could be repeated with amino acids. All tested compounds showed a clear increase of the oxidation rates. The stimulation by mineral nitrogen compounds was even higher. This confirms the hypothesis that the nitrogen in the biochemicals does not become available until the degradation of the substance through heterotrophic communities. Furthermore, the nitrogen impact might be supported by its relatively low background concentration in the soil substrate (see Table 1).
- Further research should be done to understand these effects. The identification of specific plant species showing stimulating impacts on the methane oxidation in landfill covers might be an applicable long-term objective.

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