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Ammonia effect on hydrogenotrophic methanogens and syntrophic acetate oxidizing bacteria

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Introduction

Substrates that contain high ammonia levels can cause inhibition on the anaerobic digestion process and suboptimal biogas production. Aceticlastic methanogenic pathway has been proven to be more sensitive to ammonia toxicity effect compared to hydrogenotrophic methanogenic coupled by syntrophic acetate oxidation (SAO) pathway. Specifically, there are two steps in SAO pathway:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 4H_2 + H^+ \quad \Delta G^{0'} = +104.6\ kJ \cdot mol^{-1}$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad \Delta G^{0'} = -135.6\ kJ \cdot mol^{-1}$$

Aim & objectives

So far, information about the effect of different ammonia levels on the syntrophic cultivation of SAOB and hydrogenotrophic methanogens are still lacking. Therefore, the aim of the current study was to assess the effects of different ammonia levels on pure strains of SAOB and hydrogenotrophic methanogens.

Materials & methods

Two hydrogenotrophic methanogens (*Methanoculleus bourgensis* MS2: mesophilic *Methanoculleus thermophilus* UCLA: thermophilic) and two SAO bacteria (*Tepidanaerobacter acetatoxydans*: mesophilic, *Thermacetogenium phaeum* strain PB: thermophilic) were used as inocula in the experiments.

Materials & methods

Hydrogenotrophs SAOB Incubation

All hydrogenotrophic methanogens and SAO bacteria were cultivated under **four different ammonia and free ammonia concentrations**.

A **gas mixture of H_2 and CO_2** (2 bar, 80%/20%) was introduced into the batch bottles headspace as substrate for the **hydrogenotrophic methanogens.**

Glucose (1.8 g L^{-1}) and **methanol** (3 g L^{-1}) were used as carbon source for ***T. acetatoxydans* and *T. phaeum***, respectively.

Results

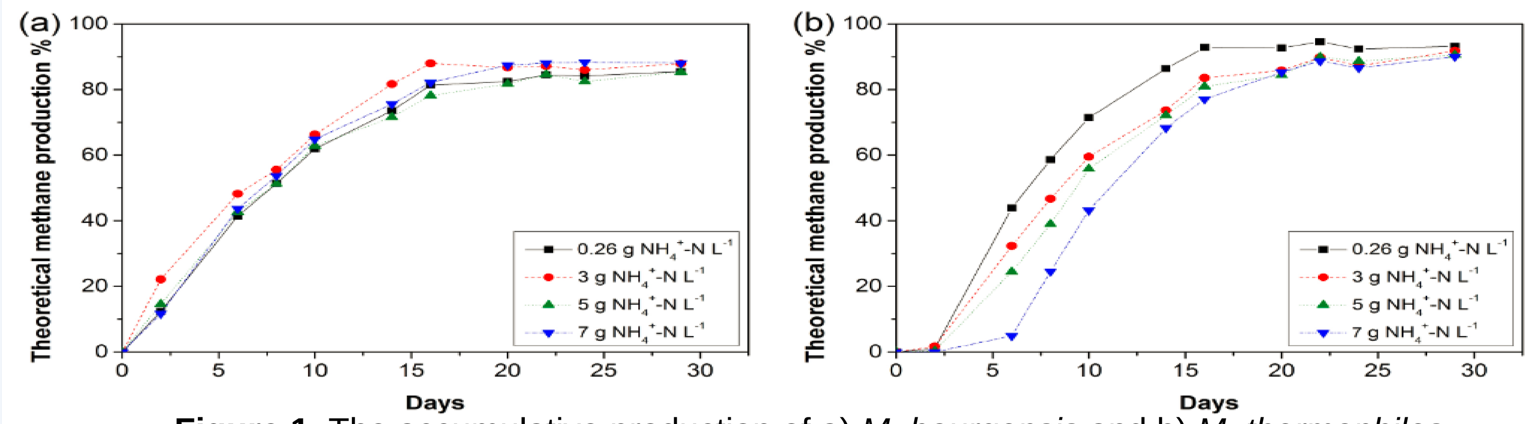

Figure 1. The accumulative production of a) *M. bourgensis* and b) *M. thermophiles*

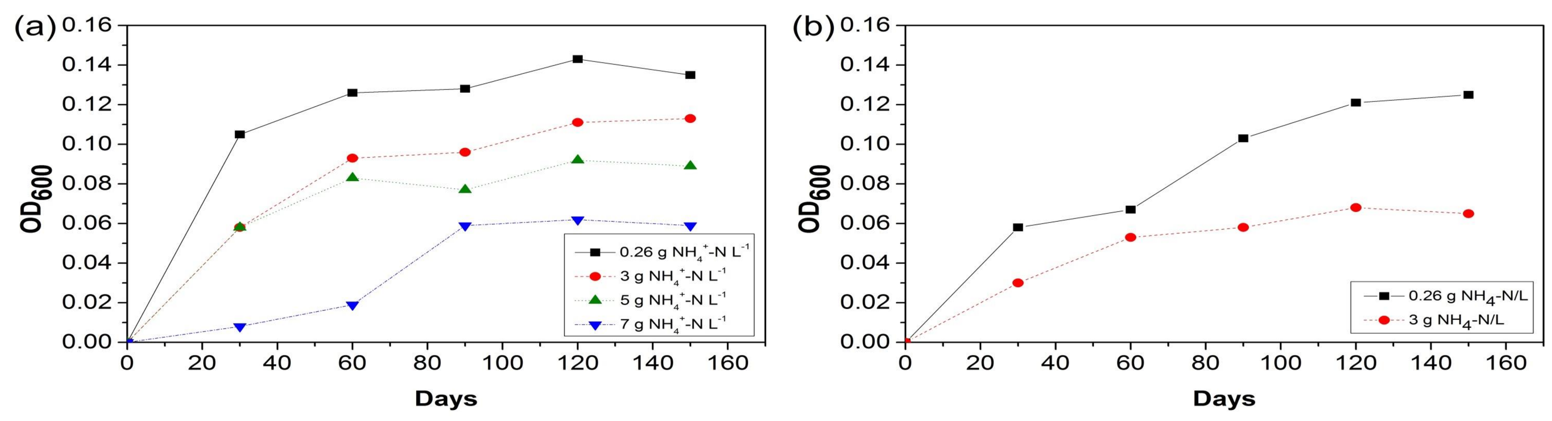

Figure 2. The OD_{600} of a) *T. acetatoxydans* and b) *T. phaeum*

Results

The methane production for *M. bourgensis* was not significantly ($p>0.05$) affected by the increased ammonia levels (Figure 1a). On contrary, methane production of *M. thermophilus* was only slightly but significantly ($p<0.05$) decreased, while the ammonia levels were increased (Figure 1b). This small inhibitory effect could be attributed to the higher free ammonia concentrations .

The growth of SAO bacteria suffered a significant ($p<0.05$) inhibition as the ammonia concentrations were increased (Figure 2), although *T. acetatoxydans* was able to grow at 7 g NH_4^+-N/L although at much reduced rate. On contrary, the OD_{600} of *T. phaeum* was below detection limits at 5 and 7 g NH_4^+-N/L (Figure 2b) indicating a complete inhibition most probably caused by the high free ammonia levels .

Conclusions

Hydrogenotrophic methanogens could be equally, if not more, tolerant to high ammonia concentrations compared to SAO bacteria.

Thus, **it seems that hydrogenotrophic methanogens and not the SAO microorganisms are microorganisms determining whether biomethanation would be possible under high ammonia** concentrations.

Acknowledgments

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