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Enrichment of high ammonia tolerant methanogenic culture

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Ammonia is the major toxicant in full scale anaerobic digesters of animal wastes which are rich in proteins and/or urea, such as pig or poultry wastes. Ammonia inhibition decreases methane production rates, increases volatile fatty acids concentration and leads to economic losses for the biogas plants. The methods used today to counteract ammonia inhibition are slow and cost-expensive. A new biological approach to avoid or counteract ammonia inhibition by using ammonia tolerant methanogens, could provide a sustainable solution for cost-effective digestion of abundant ammonia-rich wastes. The aim of the current study was to isolate and identify methanogenic cultures tolerant to high ammonia concentrations. A mixed methanogenic population was stepwise exposed to ammonia concentrations (1 to 9.26 g NH₄⁺-N L⁻¹) during an enrichment process with successive batch cultivations. The methanogenic population was derived from a full scale biogas reactor (Hashøj, Denmark), fed with 75% animal manure and 25% food industries organic waste. Basal anaerobic medium was used for the enrichment along with sodium acetate (1 g HAc L⁻¹) as a carbon source. Fluorescence *in-situ* hybridization (FISH) was used to determine microbial community composition. The outcome of the enrichment process was a mesophilic aceticlastic methanogenic enriched culture able to withstand high ammonia loads and utilize acetate and form methane stoichiometrically. FISH analysis showed that the methanogens of the enriched culture belonged exclusively to strict aceticlastic methanogens. Results obtained in this study, demonstrated for the first time that strictly aceticlastic methanogens, derived from an enriched culture, can efficiently produce methane under high ammonia levels.