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Start-up strategies in manure-fed biogas reactors: Process parameters and methanogenic communities

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ABSTRACT

Four start-up strategies were assayed in 75 dm^3 continuous stirred-tank reactors to select the optimal conditions for the start-up of the demonstration BIO4GAS® plant. Two reactors were completely filled with manure and their temperature increased from 20 to 37 °C at either a slow or fast rate. The other two reactors were started at 37 °C with a seed sludge from a stably operating plant and their load (cattle manure) increased at a low or high rate. Reactor performance was monitored for 35 days. The composition and abundance of the methanogenic communities was determined using a phylogenetic microarray and quantitative PCR. All reactors performed successfully in terms of biogas production and experienced a steady start-up, with pH values above 7.3 and VFA/alkalinity ratios below 0.3, denoting stability. Similar methanogenic loads (averaging 5×10^7 g^{-1} of 16S rRNA gene copies) were detected in the cattle manure and seed sludge, however the methanogenic diversity was higher in the manure with dominance of *Methanosarcina* and subdominance of *Methanocorpusculum* and *Methanobrevibacter*. Starting-up the reactors with an initial full load of manure and progressively increasing the temperature entailed less changes in the sludge chemical environment and in the dynamics of the dominant methanogens. Using a rate of temperature increase of 0.61 K d^{-1} proved better than1.21 K d^{-1} with a methane yield of 103.8 dm^3 kg^{-1} VS and a COD mass removal efficiency of 28.3%. The diverse methanogenic community in the manure easily adapted to reactor upsets due to forced overload conditions.

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1. Introduction

The anaerobic digestion of animal manures provides various advantages compared to their conventional treatment (e.g. tank storage or land spreading). Anaerobic digestion produces biogas that can be converted to electrical and thermal energy, and reduces the biological oxygen demand in the end product, its levels of pathogens, weed seeds and flies, and the spread of malodours [1–4]. The start-up phase of anaerobic digestion is critical for the proper operation of an anaerobic digester [5], as it influences the microbial communities involved in the degradation of the organic wastes. During the start-up phase dramatic changes occur in the sludge environment that are related to the inoculum, the organic loading rate and the hydraulic retention time (HRT) [6]. For this reason reactors need to be started-up by gradually increasing substrate loadings in order to avoid an accumulation of reduced metabolites and a drop in pH [7]. Such conditions can inhibit methanogenesis, the most sensitive step to process imbalances in engineered environments [7,8]. This is partly due to the inherently low functional diversity of methanogenic communities [9]. A successful start-up and a stable methane production not only requires that methanogenic communities are diverse but also abundant, and this is partly determined by the communities present in the initial loading material [5,10]. To accelerate the start-up process an anaerobically digested sludge from a stably operating plant is frequently used as an inoculum to supply a microbial community which is already acclimatised to the reactor operation conditions [7]. The use of a seed sludge, however, does not necessarily guarantee that the microbial community is sufficiently diverse [11–13] and able to resist, and recover from, the sudden environmental changes expected during the start-up period. An alternative approach could be to use fresh cattle manure in the start-up phase. The rumen of cattle contain abundant and diverse methanogenic communities [14,15], and we hypothesize that cattle manure itself might be an appropriate source to provide the reactor with the microbiota that will allow a successful start-up.

This study was designed in order to select the most appropriate strategy to start up a four-chamber digestion system (BIO4GAS® GmbH, Innsbruck, Austria) which has already been implemented in more than 60 plants in Germany, France, Italy and South Africa. This technology was developed to increase the efficiency of biogas production through the wet digestion of various materials, usually by co-digestion with cattle and pig manure [16]. It incorporates two major technical innovations in its two inner reactor chambers. Firstly, a hydraulic stirring of the sludge, based on the equalisation of the gaseous pressure in the two chambers, and secondly, the inclusion of a Thermo-Gas-Lift® which provides heating, additional mixing and avoids H_2S formation [17]. The two outer reactor chambers help increase the sludge retention time, and serve as storage tanks if the input load is low, which is the case for the demonstration full-scale BIO4GAS® plant in Rotholz (Tyrol, Austria). The plant was constructed in 2008 and prior to its start-up, four strategies were assayed at the laboratory scale. The objectives of this study were: i) to compare two types of start-up strategies, firstly, using an inoculum from a stably operating reactor and progressively rising the loading rate of cattle manure, and secondly, fully loading the reactor with cattle manure and progressively increasing the temperature, and ii) to define an optimal rate of feeding or temperature increase in the aforementioned strategies to guarantee a stable start-up. Reactor performance and the properties of the effluent sludge were monitored for 35 days. During the same period, the methanogenic communities were investigated by combining two 16S rRNA gene based techniques: an initial screening of the community composition using a phylogenetic oligonucleotide microarray (ANAEROCHIP) followed by the quantification of specific targets by real-time PCR with genus-specific primers [18].

2. Materials and methods

2.1. *Reactor operation*

Anaerobic sludge obtained from an operating biogas reactor treating animal manure, biowaste and grass clippings located in Pertisau, Tyrol, Austria (47° 26′ 00″ N, 11° 42′ 00″ E) and fresh cattle manure, obtained from the agricultural school in Rotholz, Tyrol, Austria (47° 23′ 00″ N, 11° 48′ 00″ E) were used for the initial loading of the reactors. To prevent clogging of reactor pipes, both materials were sieved (<5 mm) immediately after sampling and stored at 4 °C. The physical and chemical properties of both materials, which will be hereafter referred to as “seed” and “manure” respectively, are given in Table 1.

Four continuously stirred tank reactors (CSTRs; 75 dm^3 working volume, height 1.4 m, diameter 0.36 m) were started-up under different conditions (Table 2). Two reactors were filled with 75 dm^3 of manure and subjected to a temperature increase from 20 °C to 37 °C: reactor manure_slow at 0.61 $K\ d^{-1}$ and reactor manure_fast at 1.21 $K\ d^{-1}$. From day 29 on, reactor manure_slow was fed with cattle manure at a customary loading rate of 6 $dm^3\ d^{-1}$ corresponding to a HRT of 12.5 d. Reactor manure_fast was fed from day 29 on at a rate simulating overload conditions (24 $dm^3\ d^{-1}$; HRT = 3.1 d). The other two digesters were loaded with 15 dm^3 of seed sludge and 60 dm^3 tap water. Both reactors were operated at 37 °C from the beginning and fed with manure at an increasing loading rate: reactor seed_low from 0.75 to 6 $dm^3\ d^{-1}$ (HRT decreasing from 100 to 12.5 d) and reactor seed_high from 0.75 to 24 $dm^3\ d^{-1}$ (HRT from 100 to 3.1 d). The reactors were operated

Table 1 – Physical and chemical properties of the initial loading materials.

Parameters[a]	Seed sluge	Cattle manure
TS (%)	4.9	4.1
VS (w/w of % TS)	66.9	68.6
pH	8.7	8.4
Total COD ($kg\ m^{-3}$)	49.7	36.7
Soluble COD ($kg\ m^{-3}$)	11.8	12.4
NH_4^+-N ($kg\ m^{-3}$)	2.2	1.0
Alkalinity H^+ equ ($mol\ m^{-3}$)	214	143

[a] TS: Total Solids; VS: Volatile Solids: COD: Chemical Oxygen Demand.