Bioresource Technology 212 (2016) 217–226

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Start-up phase of an anaerobic full-scale farm reactor – Appearance of mesophilic anaerobic conditions and establishment of the methanogenic microbial community

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HIGHLIGHTS

- Mesophilic biogas farm reactor can be initiated using psychrophilic seeding material.
- pH, alkalinity, free-NH_3, TS and O_2 were the main drivers of the microbial dynamics.
- *Firmicutes* and *Methanosarcina* were the dominant groups established at steady state.
- Interactions between eukaryotes, bacteria and especially archaea were evidenced.

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 8 April 2016
Accepted 10 April 2016
Available online 12 April 2016

Keywords:
Anaerobic digestion
Bacteria
Archaea
Eukaryotes
Microbial ecology
16S/18S high-throughput amplicon sequencing

ABSTRACT

The goal of this study was to investigate how the microbial community structure establishes during the start-up phase of a full-scale farm anaerobic reactor inoculated with stale and cold cattle slurry. The 16S/18S high-throughput amplicon sequencing results showed an increase of the bacterial, archaeal and eukaryotic diversity, evenness and richness during the settlement of the mesophilic anaerobic conditions. When a steady performing digestion process was reached, the microbial diversity, evenness and richness decreased, indicating the establishment of a few dominant microbial populations, best adapted to biogas production. Interestingly, among the environmental parameters, the temperature, alkalinity, free-NH_3, total solids and O_2 content were found to be the main drivers of microbial dynamics. Interactions between eukaryotes, characterized by a high number of unknown organisms, and the bacterial and archaeal communities were also evidenced, suggesting that eukaryotes might play important roles in the anaerobic digestion process.

1. Introduction

In view of a holistic approach towards organic waste management, renewable energy production, environment conservation and nutrients recovery, an increasing number of farmers invest in anaerobic treatment of organic leftovers available on their farms such as wastewater, manure, animal slurries, and crop residues. Furthermore, contrary to solar and wind energies, biomass is regarded as one of the future most prominent renewable energy resources, since a continuous energy generation and storage can be guaranteed (Appels et al., 2011).

Anaerobic digestion (AD, syn. biomethanation) of biomass is regarded as a promising source of green energy because it also generates additional environmental benefits to the society, *e.g.* reduction of greenhouse gas emissions, nutrients recovery, and organic soil conditioners. Indeed, the digestion residue, called digestate, is used in agriculture as nutrient-rich fertilizer and/or organic amendment. Furthermore, the AD process generates other non-profit benefits such as odour reduction and inactivation of microbial pathogens and weed seeds (Engeli et al., 1993; Yiridoe et al., 2009).

The AD process involves different microbial groups that interact together in the absence of oxygen to decompose organic matter into biogas, which is a mixture of methane (CH_4), carbon dioxide (CO_2) and trace gases such as hydrogen sulphide (H_2S), ammoniac (NH_3) and hydrogen (H_2). Biogas can be valorised in a combined

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http://dx.doi.org/10.1016/j.biortech.2016.04.040
0960-8524/

heat and power (CHP) unit to produce electricity and heat. Alternatively, biogas can be upgraded to biomethane to reach the purity of natural gas and be injected into the municipal gas grid or be used as transportation fuel. The AD process is divided into four main stages (hydrolysis, acidogenesis, acetogenesis and methanogenesis), each involving different microbial communities (Weiland, 2010). These microbial groups are in perpetual interactions and the complexity of their associations and functioning is far from being well understood (Carballa et al., 2015). Moreover, according to some authors, the performance of an AD reactor is closely linked to the structure and dynamics of its microbial community (Demirel and Scherer, 2008). For this purpose, the start-up phase of an anaerobic reactor, aiming at developing an active microbial biomass that reaches a satisfactory treatment performance (Escudié et al., 2011), is considered as a critical point (Ike et al., 2010). Especially methanogenic archaea are considered a rate-limiting key-players of the AD process due to their slow growth rates and high sensitivity to different environmental conditions (Weiland, 2010). Thus, process inhibition can often be encountered due to an imbalance between the VFAs and other precursor-producing bacteria and methanogenic archaea (Ahring et al., 1995). Moreover, many environmental parameters such as the digestion temperature (Luo et al., 2015), the organic loading rate (OLR) (Goux et al., 2015) or even the substrate type (Westerholm et al., 2016) greatly influence the microbial community development and structure. In consequence, knowledge on the dynamics of microbial communities is required to prevent process imbalance, to better understand performance and stability of a reactor, but also to shorten the start-up phase of a new reactor and thus to improve the economic competitiveness of the AD process.

Even though the start-up phase of anaerobic reactors has been studied for lab-scale reactors (Ziganshina et al., 2014; Goberna et al., 2015), only a few studies have dealt with the microbial community dynamics at this stage in full-scale biogas units (Angenent et al., 2002; Ike et al., 2010). Furthermore, none of these studies used the high-throughput sequencing approach to characterize the microbial populations, and to the best knowledge of the authors, the studied reactors were all inoculated with warm and acclimated anaerobic sludge coming from another running reactor, such as a secondary anaerobic digester (Angenent et al., 2002), or thermophilic and mesophilic reactors from cow and pig manure-treating plants (Ike et al., 2010), but never with psychrophilic non-anaerobic materials.

In the present study, the performance and dynamics of microbial consortia, including small anaerobic eukaryotes, were monitored during the start-up phase of a full-scale farm AD reactor inoculated with psychrophilic non-anaerobic materials (run-off water and stale and cold cattle slurry), and the progressive warming and establishment of anaerobic conditions till full operational status was reached.

2. Methods

2.1. Full-scale farm reactor design and operation

The construction of a farm anaerobic reactor of around 500 m^3 working volume, located in the Northeast France, was completed at the end of 2013. The reactor is a completely stirred tank reactor type and consists of a concrete tank of 12 m in diameter and 5 m high. It is equipped with a heating system to reach the mesophilic temperature range (defined as 37–42 °C in this study, temperature commonly used in AD) and a mixer (Fig. 1).

Even though the seeding microorganisms and the inoculum size are considered important factors for the start-up of an AD process (Ike et al., 2010), due to the lack of an adequate inoculum, the reactor was initially partly filled with 250 m^3 of a mixture of run-off water collected on the farm premises and courtyard and cattle slurry available on the farm, and previously stored for a few months in an outdoor open storage tank. This inoculation method is common when no anaerobic seeding sludge is available. Following the reactor filling, the heating system was initiated with the heat provided by the combined heat and power unit (CHP) operating with propane. During 30 days, the reactor was regularly filled with a mix of run-off water, cattle slurry and manure to initiate the digestion process (Supplementary Fig. S1). Following this period, feeding was carefully initiated with plant biomass, including grass, immature rye and maize silages, hay, straw, green grass, and animal effluents, such as solid meat- and dairy-cattle manure and slurry available on the farm at an average loading rate (LR) calculated over the entire monitoring period of 5420 ± 2821 kg d^{-1}. Additional details on the input materials and loading rate are specified in Supplementary Fig. S1. Over a period lasting 152 days, the sludge temperature increased progressively inside the anaerobic reactor from 5.6 °C to around 42 °C, which was the final temperature desired by the plant owner.

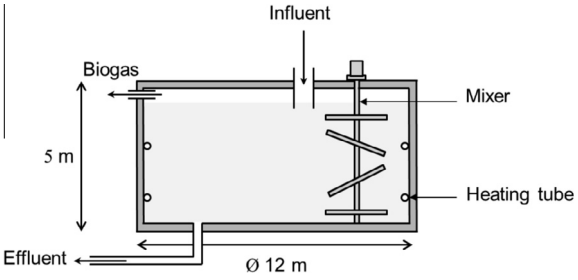

Fig. 1. Schematic of the full-scale anaerobic reactor.

2.2. Samples collection and metabolic parameters analysed

Establishment of anaerobic conditions inside the reactor during the start-up phase was monitored during 175 days. For this purpose, a sludge volume of around 1 L was collected following a thorough reactor mixing. The first sampling was done on the day the heating was initiated (day 1). During the study time, a total of 12 sludge samples were collected according to the biogas plant operator availability at day 1, 6, 14, 21, 30, 35, 42, 57, 70, 96, 152 and 175 and named hereinafter from d1 to d175 in the figures. For each sludge sample, different aliquots were frozen on-site in liquid nitrogen. Back to the lab, aliquots of 200 µL were preserved at −80 °C prior the DNA extraction while aliquots of around 15 mL were stored at −20 °C prior the VFAs concentration measurements. The remaining unfrozen sludge was used to measure the pH, total solids (TS, %) and volatile solids (VS, %) contents, total alkalinity (mg $CaCO_3$ L^{-1}), and ammonium-nitrogen concentration (kg NH_4-N m^{-3}), as described in Goux et al. (2015). In accordance to Emerson et al. (1975) and Körner et al. (2001) the free ammonia content (free-NH_3, kg m^{-3} of sludge) was calculated, taking into account the temperature, pH, and the measured NH_4-N content of the sludge in the reactor at the moment of sampling. The detailed protocol for the VFAs concentration (mg kg^{-1}) measurement is as described in Goux et al. (2015). Total VFAs concentration was expressed in terms of the sum of measured acetate, propionate, isobutyrate, butyrate, isovalerate, valerate and caproate concentrations. The estimated methane production (m^3 CH_4 m^{-3} reactor day^{-1}) was calculated starting from day 54 (*i.e.* when the produced biogas started to be used in the CHP unit to generate heat and electricity), based on an energy content of methane of 35 MJ m^{-3} and the monthly amount of electricity produced by the plant, and the CHP unit electrical efficiency as stated by the