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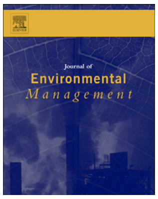

Research article

Full-scale agricultural biogas plant metal content and process parameters in relation to bacterial and archaeal microbial communities over 2.5 year span

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ABSTRACT

A start-up of 4 MW agricultural biogas plant in Vučja vas, Slovenia, was monitored from 2011 to 2014. The start-up was carried out in 3 weeks with the intake of biomass from three operating full-scale 1–2 MW donor agricultural biogas plants. The samples were taken from donor digesters and from two serial digesters during the start-up over the course of 2.5 years. Bacterial and Archaeal microbial communities progressively diverged from the composition of donor digesters during the start-up phase. The rate of change of Bacterial community decreased exponentially over the first 2.5 years as dynamics within the first 70 days was comparable to that of the next 1.5 years, whereas approximately constant rate was observed for Archaea. Despite rearrangements, the microbial communities remained functionally stable and produced biogas throughout the whole 2.5 years of observation. All systems parameters measured were ordered according to their Kernel density (Gaussian function) ranging from the most dispersed (substrate categories used as cosubstrates, quantities of each cosubstrate, substate dry and volatile matter, process parameters) towards progressively least dispersed (trace metal and ion profiles, aromatic-polyphenolic compounds, biogas plant functional output (energy)). No deficiency was detected in trace metal content as the distribution of metals and elements fluctuated within the suggested limits for biogas over 2.5 year observation. In contrast to the recorded process variables, Bacterial and Archaeal microbial communities exhibited directed changes oriented in time. Variation partitioning showed that a large fraction of variability in the Bacterial and Archaeal microbial communities (55% and 61%, respectively) remained unexplained despite numerous measured variables ($n = 44$) and stable biogas production. Our results show that the observed reorganization of microbial communities was not directly associated with impact on the full-scale biogas reactor performance. Novel parameters need to be determined to elucidate the variables directly associated with the reorganization of microbial communities and those relevant for sustained function such as the more in-depth interaction between TSOC, trace metal profiles, aromatic-polyphenolic compounds and ionic strength (e.g. electrical conductivity).

1. Introduction

The anaerobic digestion of organic waste has become the dominant process in alternative waste removal due to amounts of biogas produced and its economic relevance. Biogas production is a result of anaerobic processes that are driven by complex microbial communities of Bacteria and Archaea, next to Protozoa and

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Fungi, capable of adaptation to the parameters of anaerobic digestion (Kolbl et al., 2017; Kunwar et al., 2017; Li et al., 2017). Anaerobic digestion is generally 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) as the performance of an anaerobic digestion reactor is closely linked to the structure and dynamics of its microbial community (Demirel and Scherer, 2008), prone to replacement, immigration and extinction events. For this purpose, the start-up phase of an anaerobic reactor, aiming at developing an active microbial biomass that reaches a satisfactory biogas production performance (Escudié et al., 2011), is considered as a critical point (Ike et al., 2010). However, even the in-depth analysis of microbial communities at amplicon and metagenomics levels has not identified the main drivers of microbial functional processes in relation to energy output in anaerobic digestion (Abendroth et al., 2015; Azman et al., 2017; Goux et al., 2015; Sun et al., 2016).

The functional performance of anaerobic digestion process is in general closely linked to environmental parameters such as digestion temperature, organic loading rate, mixing regime, pH, alkalinity and the substrate type, however, also the dynamics of Bacterial and Archaeal microbial communities (Goux et al., 2016), trace metal status (Fermoso et al., 2015) and polyphenolic compounds (Azman et al., 2017) contribute to fluctuations in the process efficiency.

Polyphenolic compounds such as humic acids are inhibitory compounds produced from substrates of plant origin in anaerobic digestion as their very complex and polyvalent chemical structure has the capacity to influence the chemistry of the microbial environment (Davies et al., 2001) and their effects seem to be case specific depending on bioreactor and feedstock used (Azman et al., 2017). In addition, polyphenolic compounds interact with trace metals in anaerobic digestion influencing the extent of trace metal availability for microbial processes. Consequently, anaerobic fermentation process, microbial growth and dynamics of microbial communities are dependent on the availability and/or optimal supply of nutrients (Fermoso et al., 2015) from the initial start-up of the reactor.

Even though the start-up of anaerobic reactors has been studied on a lab-scale (Goberna et al., 2015; Ziganshina et al., 2014) only a few studies have dealt with a start-up of a large full-scale biogas reactors (Angenent et al., 2002; Azman et al., 2017; Ike et al., 2010). However, none of these studies integrated Bacterial and Archaeal microbial community dynamics over prolonged time frame of observation and the process parameters, trace metal and polyphenol content in relation to the actual reactor functional output of parallel full scale reactors.

In this study, a start-up of 4 MW biogas plant (Kolbl et al., 2014) was monitored throughout the initial three-week phase of reactor set-up with donor biomass from the three mesophilic donor full-scale biogas plants in vicinity until its full functional performance. The study was divided into three phases: (i) 70 days of start-up, (ii) 30 day span at 1.5 years and (iii) 30 day span at 2.5 years after the start-up. A number of variables ($n = 44$) ranging from substrate characteristics ($n_{substrate} = 5$), industrial process parameters ($n_{process} = 15$), XRF analysis of trace metal content ($n_{metals} = 15$), spectral deconvolution of polyphenolic compounds ($n_{polyphenols} = 6$) next to the dynamics of microbial communities at the level of Bacteria and Archaea were monitored in reactors F1 and F2 and used to contrast microbial community dynamics and functional performance of the 4 MW biogas plant ($n_{function} = 3$).

2. Materials and methods

2.1. Start-up schedule and substrate loading in biogas plant Vučja vas

The start-up of the 4 MW agricultural biogas plant Vučja (Figure S1) started in July 2011. The biogas plant Vucja vas consists of six reactors (F1–F6; each with volume of 3931 m^3), two post fermentors (PF1-PF2; each with volume of 10,700 m^3) with the total volume of 34,286 m^3. Each gasholder mounted on the top of reactor and post-fermentors has a volume of 1490 m^3. Homogenization of feeding material in each reactor is enabled by adjustable height agitator and two fixed-mounted slow rotating agitators. Digestate is further separated to liquid and solid fraction. Liquid fraction is stored in digestate tank with volume of 1356 m^3, while solid fraction is occasionally dried. Both digestates are used as fertilizer. Part of liquid fraction is also sold to the local farmers. Prior to the final use and according to the Slovenian regulations the concentration of trace metals is measured in order to guide the correct disposal of digestate with critically high trace metal concentration (Kolbl et al., 2014).

Complete biogas plant is controlled and regulated via SPS control using SCADA system (Figure S2), which is located at the area of electro-technical chambers of biogas plant. Electricity is produced by 4 gas motors (MWM, Germany), each with 1 MW_{el} capacity range. Online gas sensors for all four reactors and post-reactors monitored biogas level. Gas analysis was monitored by online gas analyzing device (ADOS, Germany).

Inocula from three fully operational donor biogas plant in vicinity were delivered for the start-up procedure from Logarovci, Jurša and Ginjevec on daily basis, transported by tractor and three axle tanks with capacity up to 20.000 L per ride (Figure S1). All donor biogas plants were operating on mixture of manure, sorghum and maize silage. All donor biogas plants operated in constant substrate supply and each contributed 333 m^3 of biomass per day to biogas plant Vučja vas that sums up 1000 m^3 of biomass transported per day. Online monitoring of the anaerobic process regarding biogas composition, biogas production was established. First two days only inocula were pumped into anaerobic reactors. Before dosing to mixing tanks (each with 235 m^3 volume; diameter 10 m, height 3 m) substrates were weighted by industry weight plate together with Excavator-loader to determine exact mass of substrates. Water from nearby water tanks was pumped to the four mixing tanks and mixed with substrates to reach the desired moisture. Concomitant with the reactor filling, the heating system was initiated with the heat provided by the combined heat and power unit (CHP) operating on electricity or propane. From the third day on biogas plant Vučja vas continuously received corn silage. The start-up of dosing of inocula, corn silage and cow manure is presented in Table S1. After the completion of dosing of inocula and substrate, the organic loading rate steadied between 9 and 10 t VS/m^3 day (Kolbl et al., 2014).

2.2. Long term monitoring of biogas plant Vučja vas

The biogas plant Vučja vas received corn silage and cow manure on daily basis since the start-up procedure in July 2011 until November 2011. Detailed monitoring of the process and substrates was conducted for the period of substrate diversification taking place from November 2011 to April 2014. The substrates (corn silage, cow manure, wet corn meal, olive pomace, molasses, sorghum and dry corn meal) were transported to the mixing chambers where water from water tanks was added to reach desired moisture. Afterward the mixture was heated to the 30 °C and pumped to the anaerobic reactors. The samples were obtained from the full

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