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# Microbial community adaptability to altered temperature conditions determines the potential for process optimisation in biogas production

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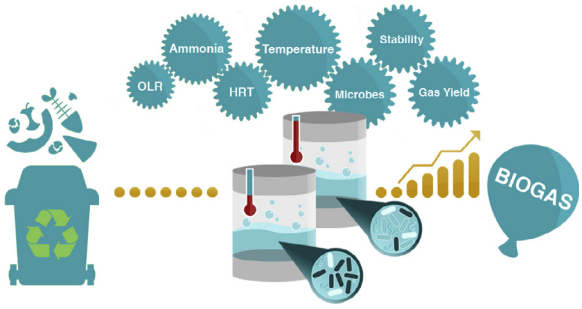
## HIGHLIGHTS

- Changing from mesophilic to thermophilic AD and vice versa is feasible.
- Thermophilic-to-mesophilic changes gave lower resistance to operative stress.
- Increased loading rate showed potential for optimisation of biogas production.
- The thermophilic community had low adaptability to mesophilic temperature condition.
- Low resilience of key microbial populations was a possible cause of process failure.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The operating temperature in anaerobic digestion strongly affects biogas yield, process stability and the potential for process optimisation. However, many questions remain on how to manage process operation for optimized microbial community adaptation following temperature changes. A long-term anaerobic digestion experiment was conducted to determine temperature-related issues in operative full-scale biogas plants and to evaluate optimisation potential and links to microbial community structure and responses. Four digesters fed household and slaughterhouse waste were operated in sets of two, at 37 °C or 52 °C, followed by a gradual increase or decrease in temperature in one digester in each set. Stability and flexibility of the digesters were then assessed by step-wise increases in organic loading rate (OLR) from 3 to 7 g VS/(L day), concurrently with decreased hydraulic retention time from 33–40 days to 14–17 days. Transition of operating temperature regime was possible, irrespective of starting temperature. However, slight temporary instability occurred at 42–44 °C and for the thermophilic to mesophilic process a period of adaptation was required to overcome this imbalance. The digesters with constant temperature and the mesophilic-to-thermophilic digester remained stable at the target OLR, demonstrating considerable optimisation potential for the large-scale biogas plants investigated. However, the digester that was changed from thermophilic to mesophilic conditions failed at 6 g VS/(L day). Comparisons of biological and chemical parameters suggested that this failure was caused by a lag in resilience of the acetate and propionate-degrading populations inherited from the community shaped by initial operation in thermophilic conditions. Taken together, these results demonstrate that the existing biogas plants are operating below capacity and that, depending on temperature, the annual energy production could be increased from 26–28 to

*Abbreviations:* AD, anaerobic degradation; OLR, organic loading rate; HRT, hydraulic retention time; SAO, syntrophic acetate oxidation; SAOB, syntrophic acetate-oxidising bacteria; VS, volatile solids; TS, total solids; VFA, volatile fatty acid; GC, gas chromatography; HPLC, high performance liquid chromatography; DADA2, divisive amplicon denoising algorithm 2; RSVs, ribosomal sequence variants; NMDS, non-metric multidimensional scaling; PERMANOVA, permutational multivariate analysis of variance; BLAST, Basic Local Alignment Search Tool; NCBI, National Center for Biotechnology Information; SRA, Sequence Read Archive; qPCR, quantitative PCR

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59–65 GWh through increasing OLR from 3 to 7 g VS/(L day). However, the results also highlight the importance of careful management and the risks when applying strategies not fully evaluated for the specific system. To our knowledge, this is the first study to demonstrate process performance, optimisation potential and microbial community adaptability to temperature changes in continuously fed anaerobic digesters under both increasing and decreasing operating temperature. The results could be used to guide operation management under temperature changes and increasing OLR in industrial-scale biogas processes.

## 1. Introduction

Biogas produced through anaerobic degradation (AD) from organic material is now a significant contributor within the European renewable energy sector [1]. Biogas technology is also a sustainable process for waste treatment, as it contributes to nutrient recycling and increases the security of energy supply [2]. It is estimated to have considerable potential for expansion [3], but to achieve this the economic benefits of commercial biogas production must be increased. Applied research on biogas technology must therefore aim at increasing energy recovery through more efficient use of digester volume, by increased organic load and/or increased methane yield per unit organic matter added. Improved robustness to stress factors, such as altered operating conditions and inhibitory compounds, is another valuable target for biogas producers, since it reduces the risk of costly process failure. Temperature strongly affects the performance of biogas digesters [4–9] and can be adjusted to optimise energy efficiency, process productivity and stability. However, the profound influence of temperature changes on process performance also poses major risks of process disturbance and failure when performed on a large scale. Changing AD processes from mesophilic to thermophilic conditions has been studied previously [8,10,11], but the influence of changing from thermophilic to mesophilic has been less investigated. Furthermore, many questions remain on how to manage process operation in order to optimise microbial community adaptation following decreases or increases in temperature. In this aspect, the role of deterministic (microbial competition and environment) and stochastic (birth-death immigration) factors may be helpful to interpret the shaping of microbial communities [12,13].

Temperature accelerates metabolic rates and biochemical processes, thermophilic processes (50–70 °C) have been shown to enable higher degradation rates and biogas yields from a wide variety of substrates than mesophilic anaerobic digestion (30–42 °C) [14–17]. Operation under thermophilic conditions also reduces the need for external sanitation and decreases the viscosity, reducing the need for energy for stirring [18,19]. Furthermore, due to the faster degradation rate, thermophilic AD can enable higher organic loading rate (OLR) and shorter hydraulic retention time (HRT). However, thermophilic digesters are considered to be more susceptible to inhibition and sudden environmental changes [7,20]. In AD of protein-rich material, an increased ratio of toxic ammonia ($NH_3$) to ammonium ($NH_4^+$) is a plausible causal factor in this instability at higher temperatures [21]. Ammonia can interfere and decelerate overall microbial activity and has been shown to be particularly inhibiting for aceticlastic methanogens, which are commonly responsible for the methane-producing step in low-ammonia AD processes [22]. Microbial adaptation towards dominance of the syntrophic acetate oxidation (SAO) pathway, involving activity of syntrophic acetate-oxidising bacteria (SAOB) and hydrogen-utilising methanogens, can prevent break-down of the process, even though process operating problems still persist [21].

In the present study, AD operation was set to mimic two operative full-scale commercial biogas plants planning changes related to process temperature. One plant running at 52 °C sought to improve process stability by decreasing the temperature to mesophilic conditions, with aim of lowering ammonia inhibition. The other plant operated at 37 °C but was interested in integrated thermophilic sanitation, which would significantly reduce the heat energy demand through removal of the additional pasteurisation step required by EU legislation [18]. Previous studies have shown microbial adaptability to increasing temperature [8,10,23–25]. However, effects on process performance and microbial community by changing from thermophilic to mesophilic temperature conditions in continuously operated digesters are less documented and understood. An experiment was thus designed to investigate how a change in temperature would influence the operating stability, the potential for process optimisation and the microbial community structure. This involved operating two laboratory-scale digesters at 37 °C and two at 52 °C. The operating temperature was thereafter gradually increased in one of the mesophilic digesters and decreased in one of the thermophilic digesters. Process performance and stability were monitored during the temperature transition and the robustness and optimisation potential were thereafter assessed by step-wise increases in OLR concurrently with decreased HRT. Increases in OLR and decreases in HRT (i.e. shock loading) can cause instability and therefore are often used for evaluating the robustness of AD processes [20,26].

AD performance is outmost dependent on the microbial community activity and a good understanding of factors influencing microbial ecology, in terms of community dynamics and diversity, provides opportunities to develop management tools for process operation [27]. This requires increased knowledge of the interlinked microbial processes (hydrolysis, fermentation, acetogenesis, methanogenesis) and the parallel metabolic pathways and syntrophic associations that are essential for maintaining process stability and productivity [28]. Thus, in the present study, collection of process data from the laboratory-scale digesters was complemented with amplification of 16S rRNA gene sequences to investigate links between taxonomic diversity and biogas digester performance. We expected instability and low optimisation potential in the digester with increasing temperature, due to the need for microbial adaptation to higher ammonia levels. Similarly, we expected the reduced ammonia inhibition in processes with decreased temperature to improve stability and increase the optimisation potential through operation at higher OLR.

## 2. Material and methods

### 2.1. Digester operation

Anaerobic digestion was conducted in four identical laboratory-scale continuously stirred tank digesters (Belach Bioteknik, Stockholm, Sweden) with a working volume of 5 L. The digesters (D) were operated in sets of two ($D_{37}$ and $D_{inc}$; $D_{52}$ and $D_{dec}$), where $D_{37}$ and $D_{52}$ served as reference digesters and $D_{inc}$ and $D_{dec}$ as experimental digesters (the subscripts *37* and *52* indicate operating temperature 37 °C and 52 °C, respectively, and *inc* and *dec* indicate increased and decreased operating temperature in period 1, respectively). $D_{37}$ and $D_{inc}$ were inoculated with slurry from a mesophilic biogas plant (37 °C, Simsholmen, Jönköping, Sweden; Table S1) and $D_{52}$ and $D_{dec}$ with sludge from a thermophilic biogas plant (52 °C, Kungsängens gård, Uppsala, Sweden [18]; Table S1). To mimic operation of the two industrial-scale biogas plants, the laboratory-scale digesters were operated with average daily OLR 2.5–3.0 g volatile solids (VS) per litre and day and HRT 33–40 days (Table S2). Feeding was performed semi-continuously (daily batch feeding six days a week) and the substrate was taken from the same biogas plant as the inoculum. The substrate was thus taken from two different sources for $D_{37}/D_{inc}$ and for $D_{52}/D_{dec}$. However, the substrate mixes consisted of similar amounts of household