

# Response of a continuous anaerobic digester to temperature transitions: A critical range for restructuring the microbial community structure and function



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## ABSTRACT

Temperature is a crucial factor that significantly influences the microbial activity and so the methanation performance of an anaerobic digestion (AD) process. Therefore, how to control the operating temperature for optimal activity of the microbes involved is a key to stable AD. This study examined the response of a continuous anaerobic reactor to a series of temperature shifts over a wide range of 35–65 °C using a dairy-processing byproduct as model wastewater. During the long-term experiment for approximately 16 months, the reactor was subjected to stepwise temperature increases by 5 °C at a fixed HRT of 15 days. The reactor showed stable performance within the temperature range of 35–45 °C, with the methane production rate and yield being maximum at 45 °C (18% and 26% greater, respectively, than at 35 °C). However, the subsequent increase to 50 °C induced a sudden performance deterioration with a complete cessation of methane recovery, indicating that the temperature range between 45 °C and 50 °C had a critical impact on the transition of the reactor's methanogenic activity from mesophilic to thermophilic. This serious process perturbation was associated with a severe restructuring of the reactor microbial community structure, particularly of methanogens, quantitatively as well as qualitatively. Once restored by interrupted feeding for about two months, the reactor maintained fairly stable performance under thermophilic conditions until it was upset again at 65 °C. Interestingly, in contrast to most previous reports, hydrogenotrophs largely dominated the methanogen community at mesophilic temperatures while acetotrophs emerged as a major group at thermophilic temperature. This implies that the primary methanogenesis route of the reactor shifted from hydrogen- to acetate-utilizing pathways with the temperature shifts from mesophilic to thermophilic temperatures. Our observations suggest that a mesophilic digester may not need to be cooled at up to 45 °C in case of undesired temperature rise, for example, by excessive self-heating, which offers a possibility to reduce operating costs.

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## 1. Introduction

Anaerobic digestion (AD) is an attractive waste/wastewater treatment technology which converts organic pollutants into energy-rich biogas. During AD, complex organic compounds such as carbohydrates, proteins, and lipids are first hydrolyzed and fermented into short-chain volatile fatty acids (VFAs), H<sub>2</sub>, and CO<sub>2</sub>, which are then converted into methane. This multi-stage process is mediated by complex consortia of anaerobic microorganisms with different functions. Providing optimal conditions for the activity of

the involved microbial community is therefore the key to the stable operation of an AD process. Accordingly, great attention has been paid to how the environmental factors influence the microbial community structure and, in turn, the process performance of AD processes.

Many efforts have been made to investigate the effect of operating temperature, among the most critical environmental factors affecting microbial growth, in AD processes. As one of such approaches, many previous studies have compared process performance between AD systems run at different temperatures, primarily mesophilic (30–40 °C) versus thermophilic (50–60 °C) temperatures. Several studies reported that biogas production was proportionally related to temperature (Bouallagui et al., 2004;

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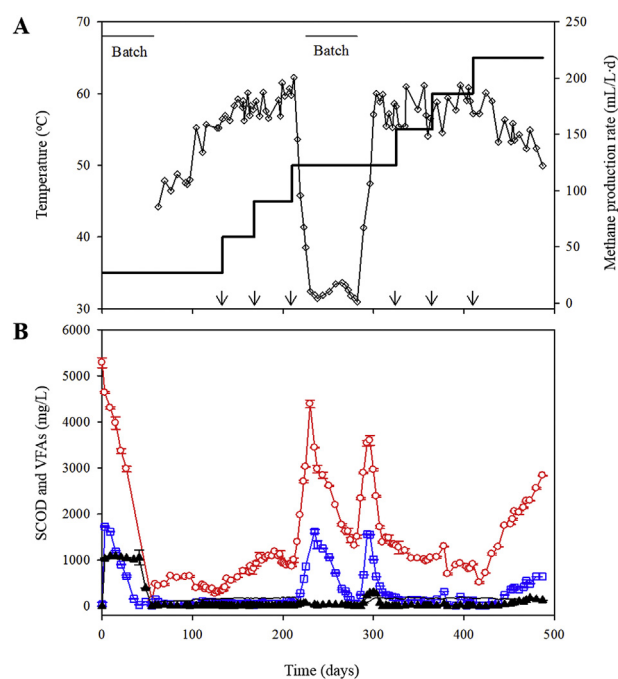
Hobson et al., 1980), while some other studies suggested a negative correlation between temperature and biogas yield due to the decreased solubilization rate and the increased free ammonia inhibition (Angelidaki and Ahring, 1994; Li et al., 2014). Another approach to examine the effect of temperature on AD is to compare different temperature shift strategies, i.e., one-step versus stepwise changes. Such an approach has been applied primarily to the adaptation of mesophilic consortia to thermophilic temperature as thermophilic digesters are often started up with mesophilic sludge as inoculum due to the limited sources of thermophilic sludge available at the field scale. Bouskova et al. (2005) reported that one-step change was much more effective than stepwise shift in adapting a mesophilic (37 °C) sewage sludge digester to 55 °C. Tian et al. (2015) also successfully applied one-step temperature shift from 35 °C to 55 °C for rapid start-up of a thermophilic sewage sludge digester. Meanwhile, Tezel et al. (2014) demonstrated a stable transition of a sewage sludge digester from mesophilic (36 °C) to thermophilic (53.3 °C) temperatures with a gradual temperature increase at a rate of 3 °C/day. Temperature fluctuation, due often to daily and seasonal temperature variations or mechanical malfunctioning, is a common problem in the operation of AD plants. The influence of temperature shock and fluctuation is therefore of great interest, and many studies have been made to address the problem. A previous study on the anaerobic treatment of pulping pressate in a membrane bioreactor showed that the system was resistant to 5–10 °C temperature shocks at 37 °C but sensitive at 45 °C (Gao et al., 2011). Regueiro et al. (2014) examined the response of a mesophilic (37 °C) digester to gradual and abrupt perturbations in temperature down to 17 °C. They reported that, regardless of the mode of temperature perturbation, the reactor recovered a stable performance without altering the operating conditions once the temperature was restored. Chae et al. (2008) observed in batch AD of swine manure that a temperature drop from 35 °C to 30 °C and a subsequent increase to 32 °C each caused a decrease in biogas production rate. Overall, results from previous studies support that temperature has a significant influence on the performance of AD processes.

However, previous approaches have mostly been confined to the analysis of changes in physicochemical parameters while paying little attention to the underlying microbial ecology. Hence, very limited information is available to bridge between the performance variation and the microbial community dynamics in response to temperature shifts. This missing link is to be addressed given that the performance of a bioprocess basically depends on the functioning of the microorganisms involved. Therefore, this study aimed to investigate the response of an AD process, in terms of community structure as well as process performance, to temperature shifts over a wide range from 35 °C to 65 °C. A continuous anaerobic reactor fed with cheese-processing wastewater served as the model system for this study, and the dynamics of reactor microbial community was monitored by a combination of molecular and statistical analyses. To provide greater insight than previous studies, a long-term temperature shift-up experiment with a stepwise increment of 5 °C was performed for a 16-month period while allowing a sufficient time for acclimation (at least 2–3 turnovers of the hydraulic retention time (HRT)) after every temperature change. Most previous studies evaluated the process response within a relatively short period (less than 1–2 turnovers) after a temperature change, which may be too short to assess the stabilized performance (i.e., steady-state data). Particular attention was given to the sudden, serious deterioration of AD performance induced by a temperature shift from 45 °C to 50 °C, which has also been observed in previous studies but not investigated for the associated change in microbial community (Bouskova et al., 2005; Tezel et al., 2014).

## 2. Materials and methods

### 2.1. Bioreactor operation

A continuously stirred tank reactor (CSTR) with a working volume of 5 L was operated with whey permeate (WP), a high-strength organic wastewater from cheese manufacturing, at varying temperatures for 487 days. The reactor was initially filled with equal volumes of WP, diluted to 10 g/L as soluble chemical oxygen demand (COD), and anaerobic sludge from a mesophilic co-digester treating sewage sludge and food waste. The soluble to total COD ratio of the WP feed was 93.2%, indicating that most of the organic matter in the feed is soluble. Carbohydrate (mostly lactose) is the major organic compound that contributed 86.5% of the substrate soluble COD. More detailed characteristics of the WP feed are summarized in Table S1. The WP feed was freshly prepared everyday by dissolving dried WP powder from Samik Dairy & Food Co. (Korea) in distilled water to a desired SCOD concentration. The prepared feed was continuously fed to the reactor using a peristaltic pump while being kept in a fridge at 4 °C to prevent spoilage. To minimize possible variations in the feed characteristics during the continuous feeding, the feed was prepared using one single bag of WP powder throughout the experiment which was also stored at 4 °C. WP was chosen as the substrate because it contains most of the essential nutrients for microbial growth and has widely been treated anaerobically with no supplements added. The total and soluble COD concentrations of the anaerobic seed sludge were 41.2 and 7.5 g/L, respectively. The reactor was run in batch mode during the first 57 days for start-up until biogas production ceased and then fed with the WP feed at an HRT of 25 days until day 98. The HRT was shortened to 15 days on day 98 and maintained unchanged until the end of the reactor experiment. At a fixed HRT of 15 days, the reactor was subjected to temperature shifts from 35 °C to 65 °C with a stepwise increment of 5 °C (i.e., Phases 1–7 in Fig. 1 and Table S2). Each temperature tested was run for a period of at



**Fig. 1.** Changes in the reactor performance during the temperature shifts: (A) temperature (—), methane production rate (◇); (B) SCOD (○), acetate (□), and propionate (▲) concentration. Arrows indicate the points where community DNA was collected for molecular analyses.

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