Comparing different strategies for start-up of thermophilic anaerobic digestion: Reactor stability and microbial community structure

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Introduction

The effects of varying temperature on the operations of anaerobic digesters have been well reported in existing literature. There are three established temperature ranges at which anaerobic digesters operate at, namely the psychrophilic $(0^{\circ}C - 25^{\circ}C)$, mesophilic $(25^{\circ}C - 40^{\circ}C)$ and thermophilic $(40^{\circ}C - 60^{\circ}C)$ ranges (Cha & Noike, 1997). Today, majority of the full-scale reactors operate at mesophilic conditions as associated operating costs are relatively lower (Kardos, et al., 2011). However, there has been an increasing interest in operating anaerobic digesters at thermophilic conditions as it allows for higher specific gas output, organic loading rates, pathogen reduction rates, efficiency in reducing antibiotic resistant genes and reduced residence time in the digester (Gebreeyessus, et al., 2016). However, the conversion of a mesophilic consortium into a thermophilic one is known to be a difficult and time-consuming process. Experience reported in literature is still evolving and is considered to be relatively insufficient or sometimes even contradictory (Maroun, et al., 2007). This study aims to compare between two known methods of converting a mesophilic colony into a thermophilic colony, namely the step-wise increase and one-step increase of temperatures from mesophilic conditions (35°C) to thermophilic conditions (55°C) to determine which method allows for better start-up of thermophilic AD from a mesophilic digester. The stability of the reactors as well as shifts in microbial community structures throughout the experiment in both thermophilic and mesophilic reactors were investigated.

Material and methods

Food waste used in this study was collected from the canteen located at UTown within the campus of National University of Singapore. A total of 10 kg food waste was collected and it consisted of a mixture of mainly rice and noodles with smaller portions of meat and vegetables. Two different types of mesophilic digester sludge were used as the start-up inoculum: (1) PUB sludge – anaerobic sludge collected from a digester treating activated sludge from a wastewater reclamation plant in Singapore (PUB, Singapore); and (2) FW sludge – anaerobic sludge collected from our laboratory's pilot-scale 1000 L digester treating food waste for more than 3 months at 35°C. The inocula for thermophilic reactors in this study were acclimatized from mesophilic sludge taken from PUB and the pilot-scale 1000 L food waste digester.

This study consists of two phases, with each phase lasting for 30 days. The first phase had a total of 6 reactors (3 L working volume each) fed daily with food waste and operated at 35°C in semi-continuous mode. Phase one acted as the acclimatisation period to reactivate the mesophilic inoculum to steady-state. There were triplicates for each condition in phase 1 of this study. R1-PUB refers to the reactors that contained PUB sludge while R1-FW refers to the reactors that contained FW sludge.

Before the second phase of the experiment, the contents of the three reactors of each condition (R1-PUB and R1-FW) were mixed together before being transferred into twelve 2 L Duran laboratory glass bottle reactors (R2-PUB-a/R2-PUB-b/R2-PUB-c and R2-FW-a/R2-FW-b/R2-FW-c) with working volumes of 1.5 L each. Duplicates were conducted for phase 2 of this study. Three different conditions were investigated. Semi-continuous AD of food waste was carried out at 35°C (R2-PUB-a and R2-FW-a), step-wise increase in temperature at a rate of 1°C per day from 35°C to 55°C (R2-PUB-b and R2-FW-b), and one-step increase in temperature (at the start of phase 2) from 35°C to 55°C (R2-PUB-c and R2-FW-c).

Results and discussion

Biogas from all reactors were measured daily and expressed in mL-biogas/L-reactor/day (mL/L/d). Biogas production rates for both R1-PUB and R1-FW increased gradually at the start of the study. By the end of phase 1, biogas levels fluctuated at between 400 to 800 mL/L/d.

During phase 2 of the study, the reactors at 35° C continued to produce biogas at a steady state. The average biogas production rate for R2-PUB-a and R2-FW-a were 518 ± 149 and 541 ± 121 mL/L/d, respectively. The average biogas production rates for every 5 days are shown in Table 1. When operating temperature increased step-wise from 35° C to 55° C for R2-PUB-b and R2-FW-b, biogas production rates remained similar to that of R2-PUB-a and R2-FW-a, respectively between days 27 to 41, where the operating temperature increased from 35° C to 50° C. When the temperature was raised beyond 50° C, biogas production rates for both R2-PUB-b and R2-FW-b.

b dropped drastically and average values at the end of the study were 130 \pm 27 mL/L/d and 194 \pm 32 mL/L/d, respectively.

When the temperature was raised in one step to 55°C for R2-PUB-c and R2-FW-c at the start of phase 2, both reactors experienced a sudden drop in biogas production. This was likely due to the accumulation of VFA and pH drop for both reactors. However, the pH of R2-FW-c recovered quickly to levels similar to R2-FW-a while that of R2-PUB-c continued to drop till the end of the study. The biogas yield of R2-FW-c quickly recovered to levels similar to R2-FW-a at around day 37 of the study, while that of R2-PUB-c continued to be low.

These findings differ slightly from that in the literature where it was reported that a thermophilic anaerobic digester could be started up from a steady state mesophilic system using this one-step temperature increase strategy. A possible reason for this could be the addition of NaHCO3 to their CSTR set-up immediately after the temperature elevation to prevent digester failure due to a pH drop (Tian, et al., 2015).

	Temp. of R2-	Biogas production rates (mL/L/d)					
Day of study	PUB/FW-b	R2-PUB-a	R2-PUB-b	R2-PUB-c	R2-FW-a	R2-FW-b	R2-FW-c
27-31	36-40	610±165	609±167	318±344	584±122	693±203	447±397
32-36	41-45	615±120	542±101	192±23	619±185	505±132	365±120
37-41	46-50	574±74	502±152	119±34	519±147	576±141	632±203
42-46	51-55	507±91	237±75	143±52	505±87	395±158	511±118
47-53	55	349±87	130±27	193±39	498±33	194±32	413±116

Table 1: Average biogas production rates every 5 days during phase 2 of study

Conclusions

Stable thermophilic AD (biogas yield of more than 500mL/L/d) was achieved within 10 days from a mesophilic digester treating food waste by adopting the one-step start-up strategy. On the other hand, biogas yield of thermophilic AD with the step-wise start-up strategy dropped to less than 200mL/L/d after the temperature was raised beyond 50°C. After the increase of temperature, thermophilic methanogenic community was established, which was characterised by the colonisation of *Thermotogae*, *Methanosarcina*, *Methanomassiliicoccaceae* and *Methanoculleus*. Results from this study demonstrated that the one-step start-up strategy could allow the rapid establishment of the thermophilic anaerobic microbial community.

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