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# Influence of thermophilic aerobic digestion as a sludge pre-treatment and solids retention time of mesophilic anaerobic digestion on the methane production, sludge digestion and microbial communities in a sequential digestion process

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ABSTRACT

In this study, the changes in sludge reduction, methane production and microbial community structures in a process involving two-stage thermophilic aerobic digestion (TAD) and mesophilic anaerobic digestion (MAD) under different solid retention times (SRTs) between 10 and 40 days were investigated. The TAD reactor ($R_{TAD}$) was operated with a 1-day SRT and the MAD reactor ($R_{MAD}$) was operated at three different SRTs: 39, 19 and 9 days. For a comparison, control MAD ($R_{CONTROL}$) was operated at three different SRTs of 40, 20 and 10 days. Our results reveal that the sequential TAD–MAD process has about 42% higher methane production rate (MPR) and 15% higher TCOD removal than those of $R_{CONTROL}$ when the SRT decreased from 40 to 20 days. Denaturing gradient gel electrophoresis (DGGE) and real-time PCR results indicate that $R_{MAD}$ maintained a more diverse bacteria and archaea population compared to $R_{CONTROL}$, due to the application of the biological TAD pre-treatment process. In $R_{TAD}$, *Ureibacillus thermophiles* and Bacterium thermus were the major contributors to the increase in soluble organic matter. In contrast, *Methanosaeta concilii*, a strictly aceticlastic methanogen, showed the highest population during the operation of overall SRTs in $R_{MAD}$. Interestingly, as the SRT decreased to 20 days, syntrophic VFA oxidizing bacteria, *Clostridium ultunense* sp., and a hydrogenotrophic methanogen, *Methanobacterium beijingense* were detected in $R_{MAD}$ and $R_{CONTROL}$. Meanwhile, the proportion of archaea to total microbe in $R_{MAD}$ and $R_{CONTROL}$ shows highest values of 10.5 and 6.5% at 20-d SRT operation, respectively. Collectively, these results demonstrate

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that the increased COD removal and methane production at different SRTs in $R_{MAD}$ might be attributed to the increased synergism among microbial species by improving the hydrolysis of the rate limiting step in sludge with the help of the biological TAD pre-treatment.



## 1. Introduction

The activated sludge process is indisputably the most frequently employed technique in the municipal and industrial wastewater treatment plants (WWTPs). However, a significant amount of waste activated sludge (WAS) is produced in the activated sludge process, which poses problems with environment pollution. Most of sludge (~62%) in Korea had generally been discharged to the ocean before it was completely prohibited in 2012 by the London Convention 97 protocol (MOE, 2012). Even though sludge has been currently used for industrial application, co-firing feedstocks, and composting as a fertilizer, a large amount of WAS is still disposed of in landfills and by incineration (MOE, 2012). Disposals costs account for nearly 40–60% of the total WWTP operating costs (Appels et al., 2008). Negative effects of sludge disposal by landfill also include the serious contamination of land environments, with the destruction of habitat leading to subsequent loss of plant and animal species, since WAS generally contains pathogenic organisms, toxic organic substances and heavy metals, and inorganic nutrients such as phosphate and ammonium causing eutrophication (Campbell, 2000). In addition, sludge removal by incineration requires high energy consumption, giving rise to foul odor and generating the toxic chemicals that have a bad effect on human respiration (Keffala et al., 2013). Therefore, effective removal of WAS is a critical environmental challenge, especially with the recent stringency of environmental regulations and associated concerns (Paul et al., 2006).

Anaerobic digestion (AD) is generally perceived as a cost-effective alternative for the WAS treatment, because a large proportion of the organic matter can be converted to biogas (e.g., methane or hydrogen) or valuable products (e.g., organic acids) under anaerobic conditions (Li et al., 2011). Additionally, AD generates relatively low biomass, and the residues have potential uses as fertilizers and soil conditioners (Abubaker et al., 2012). However, without a pre-treatment step, the AD process has an organic removal efficiency of only 30–50% for solids retention time (SRT) of 20–40 days, because most of the WAS is composed of microbial cells enmeshed in extracellular polymeric substance (EPS), which is a sturdy structure against hydrolytic enzyme (Toreci et al., 2009). Hence, many types of pre-treatments have been developed to enhance the sludge solubilization, including mechanical, ultrasonic, chemical, thermal and combined thermo-chemical treatments (Carrère et al., 2010). A positive effect in terms of volatile solid (VS) destruction and methane production has been observed in previous studies that examined these pre-treatment methods. However, mechanical and thermal methods require a substantial amount of energy (Weemaes and Verstraete, 1998). Chemical treatment which is usually conducted with an acid or alkali, require large amount of chemicals to maintain the reaction conditions and neutralization after the reaction (Navia et al., 2002). In addition, inhibitory and biologically non-degradable compounds can be generated after thermal and chemical treatment (Stuckey and McCarty, 1984).

As an alternative treatment method, phase-separated digestion composed of two or more phases (e.g., mesophilic–mesophilic or thermophilic–mesophilic) has attracted attention recently. These types of digestion systems have various advantages compared to single-stage digestion, such as increased reactor stability, methanogen activity, and overall COD removal efficiency, since phase separation provides optimal growth conditions for bacteria and archaea group in each phase (Coelho et al., 2011). Among the digestion systems, temperature-phased anaerobic digestion (TPAD) processes have been widely applied and developed (Ge et al., 2011). Typically, TPAD consists of a thermophilic (45–60 °C) anaerobic process, followed by a mesophilic anaerobic process. The thermophilic phase is operated with a short SRT (<5 d) and a high organic loading rate (OLR) in order to accelerate hydrolysis and acidogenesis (Schmit and Ellis, 2001), while the mesophilic phase is operated with a relatively long SRT (>10 d) to obtain further hydrolysis and methanogenesis (Han et al., 1997). However, in such a phase configuration, one major drawback is the sensitivity of the thermophilic phase to the influent characteristics and OLRs (Song et al., 2004). In addition, operating the reactor in thermophilic anaerobic conditions increases the energy requirements (Ziemba and Peccia, 2011).

Some researchers have suggested combined anaerobic and aerobic processes in order to incorporate the advantages of aerobic and anaerobic digestion. In previous research on combined anaerobic and aerobic processes, with an SRT of 3–9 days, a higher VS reduction was achieved than in single-stage mesophilic or thermophilic anaerobic digestion (Kumar et al., 2006; Tomei et al., 2011). However, little attention has been paid to the applied aerobic step as a first stage in aerobic/anaerobic combined process. Particularly, very few studies have been reported with regard to thermophilic aerobic digestion (TAD) prior to a single mesophilic anaerobic digestion (MAD), which has achieved much higher solid reduction and methane production than single-stage MAD (Hasegawa et al., 2000; Pagilla et al., 2000). Based on these results, TAD might be applicable as the first stage for combined thermophilic aerobic/anaerobic sludge digestion processes, since TAD has a fast degradation rate of sludge leading to an increased amount of soluble organic products which are beneficial to methane production and to the self-heating ability, which can reduce the operational costs (Gomez et al., 2007).

To better understand and collect operating data related to the microbial community structure, numerous approaches have been developed and applied, including cultivation-dependent and independent approaches. PCR-based methods are used extensively, because they can detect both