



# A comprehensive review of two-phase anaerobic digestion for organic fraction of Municipal Solid Waste

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## Abstract

Managing municipal solid waste (MSW) is becoming a more pressing global issue that requires creative and long-lasting solutions due to population expansion and changing consumption habits. Given its capacity to transform organic waste into useful resources like biogas and fertilizers, anaerobic digestion (AD) presents a viable solution to the urgent problems related to waste management in this context. This study investigates the potential of AD, and more specifically the two-stage anaerobic digestion (TSAD) process, as a game-changing technique for the treatment of organic fraction municipal solid waste (OFMSW). The efficiency of TSAD is thoroughly examined in this study, which takes into account a number of variables including temperature, pH, solid retention time, hydraulic retention time, organic loading rate, and carbon to nitrogen ratio. Though TSAD is a promising approach, there is still a significant knowledge gap about its stability, ideal operating parameters, and widespread application, especially when it comes to MSW management. The study highlights the necessity for more investigation to close this knowledge gap and realize TSAD's full promise for handling the difficulties involved in energy production and municipal waste treatment.

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## 1. Introduction

In 2012, the global annual generation of municipal solid waste (MSW) reached approximately 1300 million metric tons [1]. Projections suggest that this figure is on track to surpass 2000 million metric tons by 2025, with more than 40% of the waste being organic [1], [2]. In the broader context of waste management, a substantial volume of MSWs is generated annually, including organic waste and a waste active sludge (WAS) [3]. This contributes to environmental issues due to the high-water content and rich biodegradable organic matter [3]. The factors driving this surge include population growth, escalating urbanization, industrialization, economic development, and shifts in food habits and consumption patterns [4], [5]. As managing MSW poses a considerable challenge for municipalities, with some struggling to collect the entire volume of waste produced, especially in developing nations. The focus in these re-

gions is often limited to collection, transportation, and disposal, with insufficient attention given to resource recovery [6].

Recovering energy and nutrients from organic waste is a vital economic opportunity and essential for sustainable municipal waste management [7]. Addressing this need, anaerobic digestion (AD) emerges as a cost-effective technology for both renewable energy production and the treatment of MSW especially organic matter [7], [8]. It has also been found that AD for OFMSW is a practical and efficient way to turn the waste material into methane (CH<sub>4</sub>) containing biogas [9]. Furthermore, AD process facilitates clean disposal and serves as a complementary method for energy generation [8]. This dual functionality highlights the potential of AD as a sustainable solution for managing MSW while contributing to renewable energy resources. To be more specific AD is frequently employed for specific waste management purposes, including reducing solids content, mitigating pathogenic risks, deterring vector attraction, and efficiently recovering methane as a sustainable energy source [10].

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Anaerobic digestion consists of mainly two types. i) single-stage anaerobic digestion and ii) two-stage anaerobic digestion (TSAD). AD is commonly performed in a single reactor system where microbial groups do not experience their optimal growth and activity conditions, such as hydraulic retention time (HRT), organic loading rate (OLR), pH, and temperature [11]. This lack of optimization can lead to operational instability [12]. In a single-stage anaerobic digestion system, all digestion stages occur in a single reactor, where the sequential processes of hydrolysis, acidogenesis, acetogenesis, and methanogenesis take place. This simplifies the treatment process compared to multi-stage anaerobic digestion systems. But also, not to forget the facts that the challenges faced by single-stage anaerobic digestion are notable, including issues of decrease in system stability, volatile fatty acids (VFA) accumulation, ammonia inhibition, inadequate buffering capacity, and the formation of harmful intermediates due to the managing of high organic loads [8][10].

Recognizing these challenges, the concept of a TSAD process has been introduced. TSAD operates in two distinct stages: the hydrolytic-acidogenic stage and the methanogenic stage, effectively addressing the limitations of a single-stage system. In the first stage, acidogenic bacteria break down complex organic compounds into simpler molecules, producing organic acids and in the second stage, methanogenic bacteria further metabolize these acids, generating  $\text{CH}_4$  gas and carbon dioxide ( $\text{CO}_2$ ). This configuration allows for more controlled and optimized conditions in each reactor, enhancing overall efficiency in the degradation of organic matter and  $\text{CH}_4$  production which can be considered as promising and effective solution for addressing the complexities associated with municipal waste treatment and energy generation [13].

While the TSAD process is not a novel approach, a significant gap exists in our understanding of its process stability, optimal operational parameters, full-scale implementation, particularly in the management of MSW [14]. The renewed interest in adopting TSAD is motivated by efficiency on organic matters destruction which meets Class-A biosolids requirement, addressing the growing concerns surrounding waste management [14][15].

## 2. Overview of TSAD process

It was in 1904 that Travis first introduced the concept of a two-stage digestion process [16]. The TSAD process is a sophisticated and highly effective method designed for the management of organic waste in a controlled, oxygen-free environment [17]. This intricate process unfolds through several distinct phases, each character-

ized by specific bacterial groups [17][18]. The success of the overall process hinges on achieving a delicate balance in the growth and metabolism of these bacterial groups, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis[17][18]. In this system's particular scenario, the acid and methane phases undergo separation within a pair of sequentially arranged vessels. Whether to prioritize hydrolysis/acidogenesis or acetogenesis/methanogenesis in each stage is a critical decision. This separation is typically determined by factors such as digester temperature, SRT, and pH, each linked with OLR [14].

The acidogenesis operates with a short hydraulic retention time (HRT) and thrives at a pH range of 5.0–6.0, ensuring rapid processing [14]. Conversely, the methanogenesis, which lasts 20–30 days, supports the proliferation of slow-growing methanogens and is optimized at a pH range of 6.0–8.0 [19][20]. Additionally, temperature can influence separation, accounting for the distinct physiochemical requirements of microorganisms involved in different phases [21]. The staging based on digester temperature introduces a range of combinations, including thermophilic-mesophilic (T-M), thermophilic-thermophilic (T-T), mesophilic-thermophilic (M-T), mesophilic-mesophilic (M-M), hyperthermophilic-thermophilic (H-T), and hyperthermophilic-mesophilic (H-M) [14]. This flexibility underscores the adaptability of the TSAD process in optimizing the treatment of diverse organic substrates. Research consistently highlights the advantages of TSAD systems over their single-staged counterparts [16]. Higher  $\text{CH}_4$  production, increased resistance to higher loading rates, effective AD content degradation, better effluent quality, increased VS and COD reduction efficiency, improved pH management, and overall system resilience are some of these advantages [14][21].

For instance, extruded lignocellulosic biomass exhibits heightened energy yields by 18-33% in both solid and liquid phases within the TSAD framework rather than single-stage [20]. Furthermore, removal efficiencies are notably improved in TSAD, evident in a 16% increase in COD removal efficiency during the methanogenic phase [22]. This system also exhibits enhanced VS removal efficiency, especially in waste types like vinasse and food waste [22]. Additionally, biogas production sees a significant boost in TSAD. Pilot-scale comparisons reveal 1.17 times increase in the specific gas production rate and a 17% rise in overall specific gas production, emphasizing the system's efficacy [23][24].

## 3. Factors affecting TSAD process

In the TSAD process, several pivotal factors profoundly influence its efficiency and success. Among these, bal-

ance in the Carbon-to-Nitrogen (C/N) ratio, and temperature conditions, pH, HRT and SRT along with OLR play roles in shaping microbial activity and overall degradation rates of MSW [25].

### 3.1. Carbon-to-Nitrogen ratio

The C/N ratio provides insights into the composition and characteristics of the solid organic waste introduced into the AD system. The implications of the C/N ratio are multifaceted. A low C/N ratio signifies a higher concentration of ammonia (NH<sub>3</sub>-N) and a higher pH within the AD system [26]. Conversely, a high C/N ratio leads to elevated concentrations of VFAs, impacting the alkalinity by neutralizing ions such as bicarbonate, carbonate, and acetate [27]. Optimal C/N ratios vary for different types of OFMSW. The optimal C/N ratio for food waste (FW) is between 25:1 and 30:1 for biodegradable carbon to nitrogen and between 30:1 and 40:1 for total carbon to nitrogen [28]. Studies on the OFMSW propose optimal C/N ratios ranging from 25:1 to 36:1 for efficient microbial functioning [29].

However, challenges arise with fluctuating C/N ratios, especially in AD systems dedicated solely to stabilizing FW [26]. Because of the quick breakdown of carbohydrates, high C/N ratios might cause permanent acidification and overall process instability [26]. Additionally, higher C/N ratios indicate lower nutrient availability, particularly nitrogen, which is crucial for methanogens-microbes responsible for methane production [26]. These difficulties become more noticeable when significant AD process stages happen in a single chamber.

To overcome these challenges, solutions include stage separation or co-digestion. In instances where co-digestion is not feasible due to the unavailability of diverse substrates, two-stage digestion emerges as the only viable solution [26]. In a TSAD system, separation of the methanogenesis phase from the fermentative phases prevents rapid acidification in the hydrolytic-acidogenic chamber from affecting methanogens in the secondary chamber [30]. This allows for the dilution of excess volatile acids before entering the methanogenesis chamber, maintaining optimal conditions for biogas generation [30].

Moreover, TSAD systems offer flexibility in adjusting nutrient concentrations suitable for methanogenesis [31]. Nutrients may be added in the form of trace elements specific to the needs of different methanogen species or as cow-dung slurry, which is readily available and aids in spontaneous pH adjustment due to its excellent buffering ability [32].

Solid organic wastes with high nitrogen content pose challenges in the context of C/N ratios, leading to reduced C/N ratios in AD systems [33]. Consequently,

this results in an increment in ammonia production, with inhibitory effects observed on methanogenesis [34]. Managing ammonia concentrations is crucial, necessitating the separation of the methanogenic stage to achieve stable digester operations [31]. Overall, understanding the intricate dynamics of C/N ratios, ammonia production, and microbial interactions is essential for optimizing AD processes and ensuring their efficiency and stability.

### 3.2. Temperature

Temperature is identified as a critical factor influencing the rate of substrate and nutrient diffusion in microorganisms within AD systems. The categorization of the process based on temperature includes psychrophilic (below 20°C), mesophilic (20–45°C), and thermophilic (55–70°C) phases, each exerting a distinct impact on the overall anaerobic digestion process [35][36]. This detailed breakdown provides a foundational understanding of the thermal conditions crucial for optimal microbial activity. The initial stage of TSAD systems typically operates at high temperatures, termed thermophilic conditions, encompassing both mesophilic-thermophilic (M-T) and thermophilic-mesophilic (T-M) AD systems [37]. Extreme thermophilic (65–79°C) and hyper thermophilic (>80°C) temperatures are also observed in the acid phase of certain systems [38][39].

The effectiveness of higher temperatures is discussed through contrasting findings. A study by Jensen et al. [37] indicates that operating the first stage at 65°C and 70°C did not significantly enhance biodegradability, and the latter temperature consumed more energy. In contrast, another study Lin et al. [40] indicated a reduction in daily methane and overall biogas production at 55°C compared to 50°C, suggesting that the optimal temperature varies depending on the specific AD system.

Different temperature ranges are highlighted for the acid phase of mesophilic-thermophilic (M-T) and mesophilic-mesophilic (M-M) TSADs, where lower temperatures (35–37°C) are employed [40]. The application of mesophilic temperatures is especially prevalent in scenarios where elevated methane levels are desired, either on a process-wide scale or during specific digestion phases [41]. It can be said that the optimal temperature can differ based on the specific AD system and its operational phase, and that higher temperatures do not universally guarantee improved biodegradability or gas production, sometimes also resulting in increased energy consumption.

Typically, OFMSW anaerobic digestion is commonly implemented in both thermophilic and mesophilic environments [42]. Indeed, thermophilic conditions are considered a more dependable choice when compared to mesophilic operation [42]. The mesophilic



methanogenic process is noted for its superior process stability [42]. According to a review by Hartmann & Ahring [43], the thermophilic process effectively eliminates pathogens in a shorter operational time, enhancing hygienization. Additionally, Lv et al. [44] demonstrated that thermophilic conditions exhibit superior startup performance and result in a twofold increase in biogas production compared to mesophilic conditions for OFMSW.

### 3.3. pH and Alkalinity

Maintaining an optimal pH range is crucial, as extremely low values inhibit methanogenic microorganisms, while alkaline conditions can generate toxic compounds [45]. For overall digestion in OFMSW without stage division, the recommended pH range is close to neutrality, approximately 6.5 to 7.4 [46][47][48]. Nevertheless, specific microorganisms, like acidogenic ones focused on hydrogen production, thrive in slightly acidic conditions, around 5 to 6 [49]. It's noteworthy that a pH lower than 4 can impede crucial steps such as hydrolysis and acidogenesis. In the early phases of AD, organic acids, particularly volatile fatty acids, are produced, serving as essential precursors for methane [50]. The presence of acetic acid exceeding 2000 mg/L and total volatile acid content surpassing 8000 mg/L has a substantial effect on the methanogenesis phase [51].

To counter pH fluctuations and prevent inhibition of methanogenic organisms, alkalinity is crucial, sourced either from the substrate or additives like sodium bicarbonate ( $\text{NaHCO}_3$ ) and sodium hydroxide ( $\text{NaOH}$ ) [52], with the recommended range being 1500 to 3000 mg/L [53]. When the anaerobic digestion feedstock exhibits extremely high or low pH levels, neutralization becomes essential prior to introducing it to the plant [54]. If minimal acidification occurs during the AD process, the pH can be chemically enhanced by introducing a base, such as lime, into the reactor [55],[56]. The incorporation of biochar has the potential to play a significant role in maintaining pH stability, particularly during the stage of volatile fatty acids (VFAs) accumulation in two-stage anaerobic digestion (TSAD) [57].

Considering potential optimizations, an intriguing substitute is the idea of performing AD with phase separation. This approach aims to tailor reaction conditions for distinct microorganism groups, minimizing the impact of high organic loads. In TSAD systems, pH continues to exert a profound influence on the microbial community and product formation in both acidogenic and methanogenic phases. Acidogenic and acetogenic microorganisms display resilience in slightly acidic conditions, ranging from pH 5 to 6, with a shift towards solvent production below pH 5. Notably, researchers [37] have reported superior degradability (33–48%) within

the pH range of 6–7 for the first stage in TSAD, in contrast to degradability rates of 21–42% at lower pH values of 4–5. Additionally, alkaline conditions within the range of pH 8–11 are tolerated by acidogens and acetogens, leading to the chemical disintegration of EPS during AD. However, given the accompanying cost, more research is necessary to determine whether it is economically feasible to operate acid phase digesters under alkaline conditions. [39][58][59][60].

### 3.4. HRT/SRT

These parameters play important roles from the production of biogas in OFMSW to the breakdown of complex substrates [26]. SRT, representing the average time biomass is retained, must consistently surpass HRT, the average time substrate is retained, for stability and efficiency [61]. Notably, HRTs and SRTs vary significantly across hydrolysis, acidogenesis, and methanogenesis phases, each demanding specific time durations [26]. The significance of SRT is emphasized as an operational parameter imposing stress on bacterial communities [62]. It influences the composition of microbial populations by selecting organisms based on their generation times. On the other hand, HRT, defined as the duration for which substrate stays in contact with microbes, differs for each phase [63]. Hydrolysis, requiring longer HRTs, is critical during the initial stages of a two-stage AD system dedicated to OFMSW stabilization [63].

Decoupling hydrolysis and acidogenesis is identified as a beneficial strategy. Operational changes causing this separation enable more efficient solubilization in the hydrolytic chamber and rapid conversion in the acidogenic chamber [64]. This is particularly advantageous, preventing the inhibition of the acidogenic phase by certain particulate matter and overcoming mass transfer limitations [26][64]. Long SRTs are acknowledged as vital for the development of organisms with extended generation times [65]. However, the separation of important phases becomes imperative to avoid potential impediments in the AD process. Studies indicate that lipid degradation enhancement occurs at SRTs of 4 days or longer, while the conversion of fatty acids may happen at shorter SRTs [66]. Also, the interplay between SRT and dissimilation of proteins is explored [26]. Longer SRTs favor greater dissimilation, particularly impacting the solubilization of proteins [67]. Separation of hydrolytic and acidogenic phases provides flexibility in optimizing SRT for each phase, addressing the varied demands of hydrolysis and acidogenesis [26].

HRT's influence on methanogenesis is highlighted, with prolonged HRTs encouraging the initiation of methanogenesis in high hydraulic rate anaerobic bioreactors [64][66]. Careful adjustment of HRTs is essential to prevent premature methanogenesis and ensure stable

digestion [66][68].

### 3.5. Organic loading rate

Organic Loading Rate (OLR) also serves as an crucial element in the domain of TSAD systems, for the OFMSW. Operating as a crucial parameter, OLR quantifies the amount of organic waste introduced per unit time and reactor volume, [69] directly influencing biogas production efficiency, particularly  $\text{CH}_4$  [9]. While elevated OLR levels exhibit the potential to enhance VFA production due to increased substrate availability, [70] the absence of a unanimous consensus on the optimal OLR underscores the nuanced interplay with factors like retention time, temperature, and substrate composition. This lack of consensus prompts a critical examination of the contextual variables at play, emphasizing the need for precision in determining the OLR to avoid challenges such as , VFA accumulation, hydraulic short circuits, and inhibition of methanogenesis [71]. The range of OLR in organic digestion is 1.2–12 kg of VS/m<sup>3</sup>/day or 2.2–33.7 kg of COD/m<sup>3</sup>/day [72][73]. By optimizing the OLR higher, or around 6 kg VS/m<sup>3</sup>/day, Hartmann et al.[74] stated that the high biogas level for the digestion of OFMSW could be attained at 0.3 to 0.5 m<sup>3</sup>/kg VS. Microorganism malnourishment and detrimental effects on AD can result from insufficient OLRs [75]. A comprehensive understanding of OLR's influence is essential for tailored optimization, offering pathways for the degradation of fats, oils, and glycerol and suggesting potential avenues for bioaugmentation or OLR manipulation to refine the TSAD process [76].

In navigating these factors, TSAD systems offer a promising solution for MSW management, providing flexibility and efficiency through phase separation and nutrient adjustments. The intricate dynamics of these factors necessitate a tailored approach, considering the specific characteristics of the waste stream and the desired outcomes of the AD process. Continuous research and optimization efforts are crucial for advancing the effectiveness and sustainability of TSAD systems in addressing the challenges posed by diverse organic waste streams.

## 4. Current challenges

The full-scale implementation of AD involving the OFMSW has shown a mix of advantages and challenges. Positive results have been documented in Denmark and Slovenia, where 22 full scale centralized biogas facilities digesting organic wastes have demonstrated enhanced energy production and increased efficiency in waste degradation in 2001 [77]. Despite being studied in labs for around half a century, TSAD systems have not yet made the leap to the industrial scale, even though they could be included into future energy systems. [78] and

the broader adoption of anaerobic digestion presents several challenges. Anaerobic digestion plant installations have decreased from 23% between 1990 and 1995 to 5% between 2006 and 2010 [79]. Key bottlenecks include a lack of design and operating experience, insufficient understanding of downstream processing impacts, inappropriate waste collection and handling, and inadequate financial incentives [80]. Additionally, the research landscape calls for substantial attention to various domains, involving execution of experiments at the pilot scale, examination of efficiency of mass and heat transfer, execution of techno-economic evaluations, participation in trade-off analyses, exploration of life cycle assessments, resolution of debottlenecking challenges, investigation of solid-liquid separation methods, and improvement of energy efficiency [16]. These aspects collectively underscore the multifaceted nature of the hurdles and research imperatives essential for advancements in the field.

## 5. Future consideration and conclusion

The evolution from single-stage to two-stage AD systems presents a promising avenue for overcoming existing challenges and propelling MSW treatment forward. TSAD approach, characterized by the separation of hydrolytic and acidogenic phases, unlocks opportunities to optimize organic breakdown [26]. This separation also facilitates the maintenance of pH ranges conducive to methane and bio-hydrogen production. Leveraging genetic engineering technologies, as synthetic biology, metagenome technologies, and gene sequencing, holds immense potential for enhancing specific microbial activities vital to the AD process.

A crucial future direction involves addressing gaps in comprehensive post-treatment and enhancing biofertilizer production. A synergistic approach, integrating technologies from various enhancement techniques, stands out as a promising strategy to significantly improve the overall AD process. Exploring integrated conceptual design models that encompass pre-treatments, digestion process designs, and product recovery is essential for identifying the most effective strategies.

While promising results emerge from laboratory-scale experiments, the transition to full-scale implementation presents unique challenges. Future research should delve into the scalability of TSAD systems, considering factors like reactor design, cost-effectiveness, and integration with existing waste management infrastructure. Case studies of successful large-scale TSAD projects will offer valuable insights into overcoming implementation hurdles.

Temperature optimization emerges as a key consider-

ation within TSAD systems, offering the separation of fermentation phases and operation under distinct temperature regimes. This feature facilitates the efficient breakdown of solid fractions and allows for higher organic loading rates without compromising the pH ranges suitable for each phase. The potential for solid digestate valorization, including pyrolysis and enzymatic breakdown, adds to the attractiveness of Two stage systems for comprehensive waste treatment.

In conclusion, addressing challenges and embracing future considerations in the context of TSAD systems for OFMSW treatment necessitates a nuanced and multidisciplinary scientific approach. Advancements in system design, microbial engineering, and comprehensive analysis methodologies play a pivotal role in realizing the full potential of AD in municipal solid waste management. This forward-looking approach is crucial for establishing sustainable and effective waste treatment solutions.

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