

PROCESS STABILITY AND KINETIC LIMITATIONS OF SINGLE PHASE ANAEROBIC DIGESTION OF SEWAGE SLUDGE UNDER PSYCHROPHILIC CONDITIONS (21°C–24°C)

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ABSTRACT

Anaerobic digestion (AD) of municipal sewage sludge is an essential technology for volume reduction, pathogen destruction, and renewable bioenergy production. However, operating digestion systems in temperate or tropical winter environments without external heating remains a major challenge due to the temperature sensitivity of methanogenic microorganisms. This study evaluates the operational performance, kinetic limitations, and process stability of an unheated, single-phase, bench-scale anaerobic digester (26-liter capacity; 23-liter active volume) treating thickened primary and waste activated sewage sludge under natural psychrophilic-mesophilic boundary conditions (21°C–24°C). The reactor was operated across nine distinct steady-state organic loading rates (OLRs of 0.60 to 2.66 kg VS/m³/day) corresponding to solid retention times (SRTs of 67 to 15 days). Experimental results demonstrate that maximum operational efficiency was achieved at an OLR of 1.33 kg VS/m³/day and an SRT of 30 days at a core temperature of 23°C. Under these optimum parameters, the system achieved a volatile solids (VS) reduction of 35.73% and a maximum specific biogas yield of 0.362 m³/kg VS/day with a peak methane content of 39.42%. Progressive reduction of SRT to 25, 20, and 15 days (OLRs of 1.60, 2.00, and 2.66 kg VS/m³/day) at lower ambient temperatures (21.5°C–21.0°C) triggered severe volatile fatty acid (VFA) accumulation, reaching 1,400 mg/L as acetic acid. This acid accumulation exhausted the system's bicarbonate buffering capacity, causing the VFA/alkalinity ratio to spike from 0.243 to a critical value of 0.56, while the methane content collapsed to just 8.0%. These findings outline the precise biochemical and kinetic boundaries of unheated, low-temperature digestion, providing a design framework for cost-effective, decentralized wastewater treatment infrastructures in developing nations.

KEYWORDS: Anaerobic Digestion; Low-Temperature Digester; Process Stability; Volatile Fatty Acids; Psychrophilic Methanogenesis; Sewage Sludge

INTRODUCTION

Anaerobic digestion (AD) represents a cornerstone of modern municipal wastewater sludge management, offering a sustainable dual pathway for waste volume minimization and renewable energy recovery (George Tchobanoglous et al., 2003). By microbially converting complex organic fractions into energy-rich biogas, wastewater treatment plants (WWTPs) can substantially lower their net carbon footprint and offset operational electricity costs. This technology is increasingly vital in developing nations like India, where aggressive urban growth, expanding municipal sewage infrastructure, and national sanitation programs are generating unprecedented quantities of primary and secondary sewage sludge.

Biochemically, anaerobic digestion proceeds through three highly coordinated, sequential biological stages: (1) hydrolysis, (2) acidogenesis/acetogenesis (the acid phase), and (3) methanogenesis (the methane phase) (Sundarajan et al., 1996). Complex polymers (proteins, lipids, carbohydrates) are first extracellularly broken down during hydrolysis into soluble monomers. Acidogenic and acetogenic bacteria then metabolize these monomers into short-chain volatile fatty acids (VFAs), primarily acetic, propionic, and butyric acids. Finally,

strict anaerobic methanogenic archaea utilize these VFAs (alongside hydrogen and carbon dioxide) to produce methane (CH₄) and carbon dioxide (CO₂). Because methanogenic archaea are slow-growing, highly sensitive to environmental fluctuations, and possess lower thermodynamic energy yields, methanogenesis is widely recognized as the rate-limiting step of the overall digestion process. To prevent microbial washout and process failure, it is essential that the solid retention time (SRT) of the reactor remains well within the duplication limits of these methanogenic populations.

A critical parameter governing the kinetics and thermodynamic viability of AD is temperature. Traditional anaerobic reactors are designed to operate under mesophilic (35°C–40°C) or thermophilic (55°C–60°C) temperature regimes to optimize microbial growth rates, hydraulic retention times, and methane yields (Fischer and Greene, 1945). However, maintaining such elevated temperatures requires continuous, energy-intensive external heating, which represents a massive operational cost and is often technically unviable for decentralized treatment facilities in low-income or semi-urban regions. Consequently, operating digestion systems under unheated, ambient psychrophilic conditions (<25°C) has emerged as an attractive, low-cost alternative. Historical studies have demonstrated a clear

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relationship between digesting temperature and biogas yields. Rudolphs (1927) reported a specific gas yield of 0.32 L/g VS added using sewage sludge in a batch reactor operating at 22.5°C over an extended period of 108 days. Fair and Moore (1934) established that complete digestion at a mesophilic boundary of 25°C required a detention time of approximately 27.5 days. Furthermore, Maly and Fadrus (1971) observed that specific gas production ranged from 0.35 L/g to 0.65 L/g VS added at 22.5°C, rising to 0.40–0.71 L/g VS added at 25°C, with volatile solids reduction peaking at 46% only after 100 days of batch digestion.

Despite these early foundational insights, modern literature lacks systematic empirical studies that define the precise operational boundaries and buffering capacities of single-phase, unheated anaerobic reactors operating in the critical psychrophilic-mesophilic transition range (21°C–24°C) under high-strength loading conditions. Most contemporary studies focus either on highly controlled, heated systems or on pre-treated, synthetic feedstocks. To bridge this gap, this study examines the performance of a 26-liter bench-scale single-phase reactor treating thickened, high-solids primary and waste activated municipal sewage sludge under fluctuating winter temperatures (21°C–24°C) in Vadodara, India, without active external heating. By systematically varying the organic loading rate (OLR

from 0.60 to 2.66 kg VS/m³/day) and monitoring key chemical parameters—including VFA accumulation, alkalinity, and volatile solids destruction—this study establishes the practical limits of low-temperature digestion, providing a realistic framework for unheated decentralized municipal AD application.

MATERIALS AND METHODS

Sludge Source and Characterization

The municipal sewage sludge used as the biological feedstock in this study was sourced directly from the gravity thickener unit of the municipal sewage treatment plant (STP) located in Tarsali, Vadodara (Gujarat, India). This facility treats municipal domestic wastewater using an activated sludge process (ASP). The sludge collected represented a thick, combined mixture of primary settling sludge and waste activated sludge (WAS). This raw thickened sludge was characterized by a highly concentrated solids profile and moderate acidity, with a pH ranging between 5.6 and 5.9. The high concentration of raw volatile fatty acids (1,600 to 2,000 mg/L as acetic acid) and low alkalinity (620 to 900 mg/L) reflected an active, ongoing acidogenic pre-fermentation within the sewage collection and thickening systems. The comprehensive initial chemical characteristics of the raw thickened sludge feed are summarized in Table 1.

Table 1: Physical and Chemical Characteristics of the Thickened Raw Sewage Sludge Feed (Tarsali STP, Vadodara)

Sr. No.	Parameter Analyzed	Measured Concentration / Value Range
1	pH	5.6 – 5.9
2	Total Solids (TS)	71,500 – 75,000 mg/L
3	Volatile Solids (VS)	38,800 – 41,200 mg/L
4	Alkalinity (as CaCO ₃)	620 – 900 mg/L
5	Volatile Fatty Acids (VFA, as Acetic Acid)	1,600 – 2,000 mg/L

Bioreactor Design and Setup

A bench-scale, single-phase anaerobic digester with a total volume of 26.0 L was fabricated for this study. To provide headspace for biogas collection and prevent sludge foam carryover, the operating liquid sludge volume was maintained strictly at 23.0 L. To ensure complete mixing and eliminate dead zones, the reactor was equipped with a manual overhead mechanical stirrer (1.0 meter in length) fitted with multi-level paddles designed to provide gentle vertical and radial mixing of the high-viscosity sludge. Stirring was performed manually five times a day for approximately 2 minutes per session. Feeding was carried out through a dedicated, vertical PVC inlet pipe (0.5 meters in length) that

extended deeply below the liquid sludge surface to create a natural hydraulic seal and prevent the escape of pressurized biogas during feed additions. Sludge withdrawal was controlled via a heavy-duty brass gate valve installed at the lowermost conical point of the reactor, ensuring the representative extraction of digested settled solids.

To evaluate the system under realistic, low-cost decentralized conditions, no active electrical or chemical heating systems were installed. Instead, a simple, low-cost passive thermal insulation mechanism—consisting of multi-layered, thick gunny sacks securely wrapped around the external reactor shell—was utilized to shield the reactor from rapid ambient temperature fluctuations.

The reactor was operated in an unheated laboratory environment during the winter season (spanning from October to January), with digested sludge core

temperatures naturally fluctuating between 21.0°C and 24.5°C depending on daily ambient weather patterns.

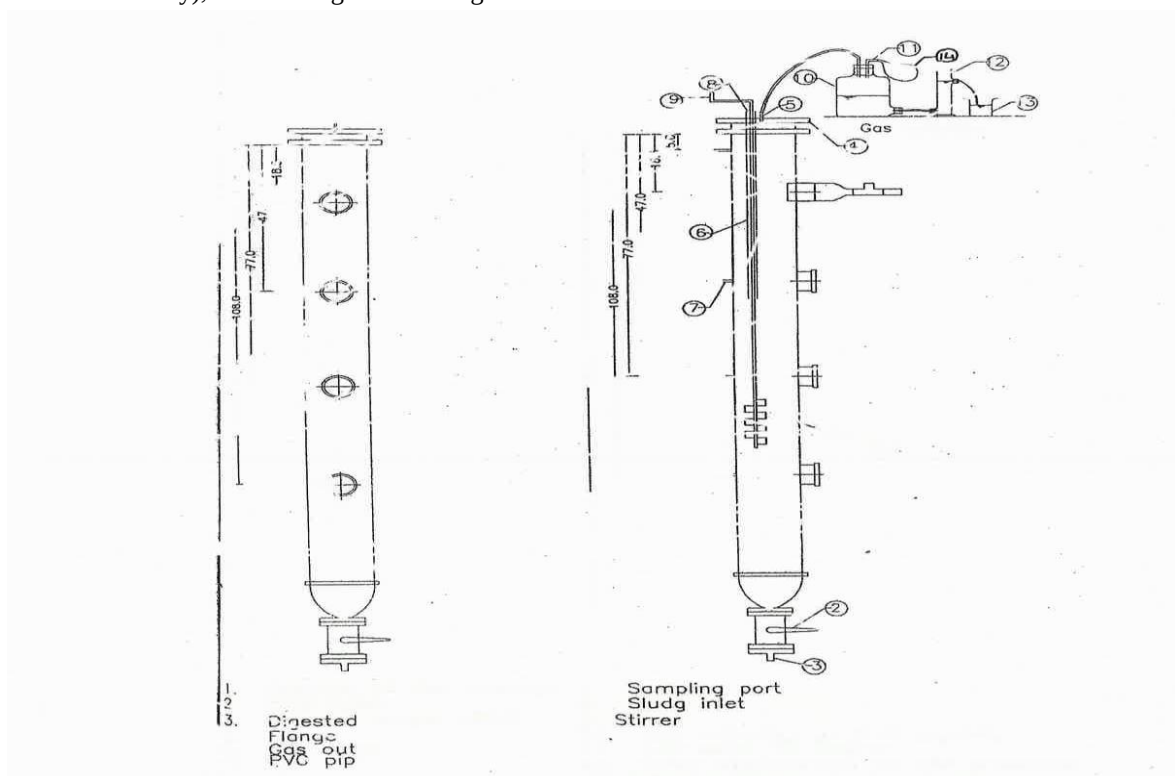


Figure 1: Schematic Diagram of Anaerobic Digester



Figure 2: Working Model of Anaerobic Digester

Bioreactor Startup and Operational Protocol

Reactor startup was initiated by seeding the reactor with active anaerobic digested sludge to establish a robust microbial community. The reactor was filled with 70% of its working volume (16.1 L) using active, well-digested sludge obtained from an operational anaerobic digester. The remaining 30% of the working volume (6.9 L) was filled with thickened raw sludge containing 7.5% total solids. This seed-to-feed ratio provided an abundant microbial population while maintaining sufficient organic feedstock for start-up acclimatization. During the initial 10-day start-up phase, the reactor was operated under a highly conservative "light feeding" regime of 0.20 kg VS/m³/day. For the subsequent 10 days, this organic loading rate (OLR) was increased to 0.40 kg VS/m³/day. Successful start-up and the establishment of basic anaerobic syntrophy were confirmed when steady, measurable biogas production was observed through the gas collection system after 20 days of operation.

Following start-up, the reactor was transitioned into a semi-continuous, daily draw-and-fill operational mode. The experimental design systematically evaluated nine distinct steady-state OLRs: 0.60, 0.70, 0.80, 0.90, 1.143, 1.33, 1.60, 2.00, and 2.66 kg VS/m³/day. These OLRs corresponded directly to target solid retention times (SRTs) of 67, 57, 50, 45, 35, 30, 25, 20, and 15 days, respectively. Each OLR stage was maintained for at least two to three weeks until the reactor reached a stable, steady-state condition, defined as less than 5% variance in daily pH, VFA levels, and biogas production over five consecutive days. Daily operational steps involved withdrawing a predetermined volume of digested sludge from the bottom gate valve, followed by the immediate gravity feeding of an identical volume of raw thickened sludge from the top PVC inlet. No supernatant separation or recycling was practiced, ensuring that the SRT and hydraulic retention time (HRT) remained identical.

Analytical Methods

To comprehensively assess the reactor's performance, physical and chemical parameters were monitored at strict, predefined intervals. Sludge temperature was recorded daily using a calibrated mercury-in-glass thermometer. The pH of the influent and effluent was monitored daily using a digital benchtop pH meter. Volatile fatty acids (VFA) and total alkalinity were measured daily via standard titration methods (APHA, 2012), with VFA concentrations expressed as milligrams of equivalent acetic acid per liter (mg/L) and alkalinity expressed as milligrams of calcium carbonate equivalent per liter (mg/L). Thrice a week, total solids (TS), volatile

solids (VS), ammonium nitrogen (NH₃-N), soluble calcium (Ca²⁺), soluble magnesium (Mg²⁺), and dissolved sulphides were determined in accordance with the standard analytical protocols outlined in Standard Methods for the Examination of Water and Wastewater (APHA, 2012).

Daily biogas production volumes were quantified using a custom-fabricated water displacement collection system, with gas volumes normalized to standard temperature and pressure (STP) conditions. Biogas was collected in robust, gas-impermeable bladders. The precise composition of the collected biogas—specifically the fractions of methane (CH₄) and carbon dioxide (CO₂)—was determined using a Shimadzu GC-14B gas chromatograph equipped with a thermal conductivity detector (TCD) and a packed column, operating with helium as the carrier gas.

RESULTS AND DISCUSSION

The complete steady-state chemical and biological performance of the bench-scale unheated anaerobic digester across all nine OLR and SRT stages is documented in Table 2. This multi-parameter dataset provides a highly detailed map of the system's metabolic shifts and operational transitions as organic load was increased and retention time was compressed under cold ambient winter temperatures (21.0°C–24.5°C).

Organic Loading Capacity and Volatile Solids Destruction Kinetics

A critical indicator of an anaerobic digester's metabolic efficiency is the percentage reduction of volatile solids (VS), which reflects the portion of organic macromolecules converted to gaseous endproducts. As illustrated by the experimental data, the percentage of VS reduction exhibits a clear parabolic trend with respect to OLR and SRT. In the initial low-loading stages (OLRs of 0.60 to 0.90 kg VS/m³/day), the VS reduction rose progressively from 10.35% (at a highly conservative SRT of 67 days) to 23.68% (at an SRT of 45 days). This positive correlation indicates that as the organic feed rate was slowly increased, the active biomass under-acclimatized at very long retention times gradually matured, improving substrate-microbe contact within the unheated vessel.

The peak performance of the digestion process was observed at an organic loading rate of 1.33 kg VS/m³/day and a solids retention time of 30 days, where the volatile solids reduction reached its absolute maximum of 35.73%. Under these conditions, the digested effluent solids concentration was reduced to 25,900 mg/L from an inlet concentration of 40,300 mg/L.

This VS reduction is exceptionally high for an unheated psychrophilic system operating at a temperature of 23.0°C, and is highly comparable to the efficiencies typically reported for heated mesophilic systems. This suggests that at a 30-day SRT, the rate of extracellular enzymatic hydrolysis was perfectly synchronized with acidogenesis and methanogenic consumption, facilitating complete carbon conversion.

Crucially, however, any further compression of the SRT beyond 30 days resulted in a catastrophic decline in VS destruction. At an OLR of 1.60 kg VS/m³/day

(SRT of 25 days), VS reduction dropped to 25.70%, and as the loading was aggressively increased to 2.00 and 2.66 kg VS/m³/day (SRTs of 20 and 15 days), the VS reduction collapsed to 17.70% and 12.12%, respectively. Under these short SRT regimes, the hydraulic flow rate was increased up to 1.53×10^{-3} m³/day, effectively purging the slow-growing methanogenic archaea from the reactor. The hydraulic wash-out of these crucial syntrophic partners halted the anaerobic chain, leaving complex organic polymers completely undegraded and causing raw suspended solids to pass through the digester virtually untouched.

Table 2: Steady-State Analytical Profile of the Unheated Single-Phase Anaerobic Digester Across Nine OLR and SRT Stages

Parameter \ OLR (kg VS/m ³ /d)	0.60	0.70	0.80	0.90	1.143	1.33 (Opt)	1.60	2.00	2.66
SRT (days)	67	57	50	45	35	30	25	20	15
Daily Flow Rate (m ³ /day × 10 ⁻⁴)	3.43	4.04	4.60	5.11	6.57	7.67	9.20	11.5	15.3
pH	7.20	7.17	7.15	7.15	7.15	7.17	7.12	7.11	7.10
Sludge Core Temperature (°C)	24.5	24.0	23.5	22.0	22.5	23.0	21.5	21.0	21.0
Total Solids - Inlet (mg/L)	72,500	74,000	73,500	73,100	74,200	74,500	73,250	74,200	73,800
Total Solids - Outlet (mg/L)	69,500	70,600	68,650	66,800	63,800	62,700	64,100	67,000	70,700
Volatile Solids - Inlet (mg/L)	39,600	38,750	39,200	40,750	40,500	40,300	39,700	45,500	39,600
Volatile Solids - Outlet (mg/L)	35,500	33,200	32,000	31,100	27,600	25,900	29,500	33,300	34,800
Volatile Solids Reduction (%)	10.35	14.32	18.37	23.68	31.85	35.73	25.70	17.70	12.12
Volatile Fatty Acids (mg/L as Ac)	720	880	970	840	800	730	1,050	1,250	1,400
Total Alkalinity (mg/L as CaCO ₃)	3,500	3,100	2,800	2,850	2,900	3,000	2,750	2,550	2,500
VFA/Alkalinity Ratio	0.20	0.283	0.346	0.294	0.275	0.243	0.381	0.490	0.560
Ammonium Nitrogen (NH ₃ -N, mg/L)	560	540	520	500	520	530	490	485	465
Calcium (Ca ²⁺ , mg/L)	380	375	372	392	395	392	405	410	430
Magnesium (Mg ²⁺ , mg/L)	175	180	188	193	190	195	205	200	200
Dissolved Sulphides (mg/L)	8.0	12.0	33.0	26.0	20.0	29.0	25.0	21.0	23.0
Daily Gas Production (L/day)	3.65	4.90	6.10	7.12	9.40	11.1	7.20	4.85	3.90
Specific Biogas Yield (m ³ /kg VS/d)	0.264	0.304	0.331	0.344	0.357	0.362	0.195	0.105	0.063
Methane Content (%)	21.00	22.64	31.50	34.53	38.16	39.42	28.30	21.00	8.00

Volatile Fatty Acids, Alkalinity, and Process Buffering Capacity

Anaerobic digestion is highly dynamic, and stability depends on a delicate equilibrium between fast-growing acid-producing bacteria (acidogens) and slow-growing acid-consuming archaea (methanogens). Since methanogens are highly sensitive to low temperatures, monitoring the concentrations of intermediate volatile fatty acids (VFAs) and the total alkalinity (TA) provides a rapid and highly accurate snapshot of the reactor's health. In a well-buffered, stable anaerobic digester, the VFA/alkalinity ratio should be maintained strictly below the critical threshold of 0.35. A ratio exceeding 0.35 indicates that volatile organic acids are being produced

faster than methanogens can metabolize them, threatening the system's bicarbonate buffering capacity.

The experimental results in Table 2 demonstrate that the system maintained excellent stability from OLR 0.60 up to the optimum OLR of 1.33 kg VS/m³/day. At OLR 1.33 (SRT of 30 days and 23.0°C), the VFA concentration reached its lowest steady-state value of 730 mg/L (as acetic acid) while the alkalinity was maintained at a robust 3,000 mg/L (as CaCO₃), resulting in a highly stable VFA/alkalinity ratio of 0.243. This high alkalinity was generated by the biological degradation of proteins and organic nitrogen, releasing ammonia which reacted with dissolved carbon dioxide to form ammonium bicarbonate, the primary buffering agent in anaerobic

reactors. The strong buffering capacity maintained the pH at a highly favorable, near-neutral level of 7.17, optimizing methanogenic enzyme activity.

However, when the OLR was increased to 1.60 kg VS/m³/day (SRT of 25 days) and the temperature decreased to 21.5°C, the VFA concentration immediately surged to 1,050 mg/L while the alkalinity dropped to 2,750 mg/L, driving the VFA/alkalinity ratio up to 0.381. This marked a critical operational threshold, indicating the onset of process instability. As the system was loaded further at OLRs of 2.00 and 2.66 kg VS/m³/day (SRTs of 20 and 15 days), the low temperature of 21.0°C severely inhibited the metabolic activity of psychrophilic methanogens. In contrast, the robust acidogenic bacterial populations continued to hydrolyze and ferment incoming organics at high rates. This kinetic mismatch caused a massive accumulation of VFAs, which peaked at 1,400 mg/L. This acid surge rapidly consumed the bicarbonate buffer, driving the alkalinity down to 2,500 mg/L and pushing the VFA/alkalinity ratio to a highly critical value of 0.560. This severe imbalance is known as "sour digester syndrome," wherein the accumulation of toxic undissociated organic acids permanently damages the methanogenic population.

Biogas Yield and Methane Content Sensitivities to Low Temperatures

The ultimate goal of anaerobic digestion is the conversion of organic waste into renewable methane gas. In this study, both daily biogas production and specific methane content showed extreme sensitivity to temperature and OLR. In the stable operational region (OLRs of 0.60 to 1.33 kg VS/m³/day), the daily biogas production rose linearly from 3.65 L/day to a maximum of 11.10 L/day, corresponding to an optimal specific biogas yield of 0.362 m³/kg VS/day. Concurrently, the methane content of the biogas increased from 21.00% to a peak of 39.42% at OLR 1.33. This high methane percentage indicates that the methanogenic archaea were highly active, converting acetic acid and carbon dioxide into fuel-grade biogas.

This exceptional biogas production is particularly impressive given that the digester operated under ambient winter conditions (23.0°C) without external heating. It demonstrates that wrapping the reactor in simple, low-cost gunny bags successfully minimized heat losses, allowing the exothermic reactions within the biological slurry to maintain a core temperature of 23.0°C even when ambient night temperatures dropped significantly. This passive insulation strategy represents a highly scalable and cost-

effective approach for decentralized applications in developing nations.

However, when the OLR was increased beyond 1.33 kg VS/m³/day and the core temperature dropped to 21.0°C, the biogas production collapsed. Daily gas production plummeted to 7.20 L/day (at OLR 1.60), 4.85 L/day (at OLR 2.00), and finally 3.90 L/day (at OLR 2.66). The specific biogas yield crashed by over 82%, dropping to a negligible 0.063 m³/kg VS/day. Most dramatically, the methane content of the gas collapsed from its peak of 39.42% to 28.30%, 21.00%, and finally a mere 8.00% at OLR 2.66. This complete failure of methanogenesis is a direct result of low-temperature kinetic limitations. At temperatures below 22°C, the biochemical activation energy required for methanogenesis increases exponentially, making the conversion of acetate to methane thermodynamically unfavourable. This temperature-induced inhibition, combined with the physical wash-out of methanogens at short SRTs, caused the system to produce a low-quality, carbon dioxide-dominated biogas that is entirely unusable for energy recovery.

Nutrient and Trace Mineral Characteristics

To ensure a comprehensive analysis, nutrient and mineral concentrations were monitored across all operational stages. Ammonium nitrogen (NH₃-N) levels remained highly stable, fluctuating within a narrow range between 465 mg/L and 560 mg/L. In anaerobic digestion, ammonium nitrogen is a double-edged sword: while it is an essential nutrient for microbial growth and provides vital bicarbonate buffering capacity, high concentrations of free ammonia (>1,500 mg/L) are highly toxic and can cause severe process inhibition (APHA, 2012). In this study, NH₃-N levels remained well below the inhibitory threshold, indicating that the digested sludge maintained a healthy nutrient balance.

Soluble calcium (Ca²⁺) and magnesium (Mg²⁺) concentrations were also monitored, with Ca²⁺ ranging from 372 mg/L to 430 mg/L and Mg²⁺ ranging from 175 mg/L to 205 mg/L. These divalent cations are crucial co-factors for metabolic enzymes and contribute to the structural integrity of anaerobic sludge flocs. However, their high concentration also presents a technical risk: under alkaline pH conditions, dissolved calcium, magnesium, ammonium, and phosphate ions can precipitate to form hard mineral scales such as struvite (MgNH₄PO₄·6H₂O) and calcium carbonate (CaCO₃). These scales can cause severe clogging of digester piping and pump impellers (Water Environment Federation, 1995). The slight increase in soluble Ca²⁺ (up to 430 mg/L) and Mg²⁺ (up to 200 mg/L) observed at the high-

loading, acidified stages (OLRs of 2.00 to 2.66 kg VS/m³/day) was caused by the lower pH, which dissolved existing mineral precipitates back into their soluble ionic forms.

Dissolved sulphide levels fluctuated between 8.0 mg/L and 33.0 mg/L, with a value of 29.0 mg/L at the optimum OLR of 1.33 kg VS/m³/day. These low concentrations are highly favorable, as dissolved sulphides are required in trace amounts for the synthesis of key methanogenic enzymes. Crucially, they remained far below the toxic threshold of 100 to 200 mg/L, ensuring that sulphide-induced inhibition of methanogenesis did not occur during the experimental campaign.

CONCLUSIONS AND PRACTICAL IMPLICATIONS

This study successfully defines the precise biochemical, kinetic, and operational boundaries of a single-phase, unheated anaerobic digester treating high-solids municipal sewage sludge under low-temperature psychrophilic-mesophilic winter conditions (21°C–24°C). The key conclusions and practical engineering implications of this work are summarized below:

- **Optimal Operational Parameters:** The optimum performance of the unheated digester was achieved at a steady-state organic loading rate (OLR) of 1.33 kg VS/m³/day and a solids retention time (SRT) of 30 days under an operating core temperature of 23.0°C. Under these parameters, the system achieved a maximum volatile solids (VS) reduction of 35.73%, an optimal specific biogas yield of 0.362 m³/kg VS/day, and a peak methane content of 39.42%.
- **Souring and Kinetic Limitations:** Aggressive increase of the organic load beyond the optimum threshold (OLR $\geq$ 1.60 kg VS/m³/day; SRT $\leq$ 25 days) at low ambient temperatures (21.5°C–21.0°C) triggered severe process instability. The slow-growing methanogenic archaea were physically washed out of the reactor faster than they could reproduce. This created a major kinetic mismatch with the more robust acidogens, leading to a massive accumulation of volatile fatty acids (VFAs) up to 1,400 mg/L.
- **Buffering Capacity Exhaustion:** The rapid accumulation of VFAs overwhelmed the reactor's bicarbonate buffering capacity, causing the VFA/alkalinity ratio to spike from a stable 0.243 to a highly critical value of 0.560. This acid accumulation neutralized the buffering capacity and led to digester acidification ("sour digester syndrome"), causing the methane content of the biogas to collapse to a negligible 8.0%.

- **Trace Nutrient and Mineral Balance:** The anaerobic slurry maintained a highly favorable chemical balance. Ammonium nitrogen (NH₃-N) concentrations remained between 465 mg/L and 560 mg/L, providing vital buffering capacity without reaching toxic thresholds. Dissolved sulphides (8.0 to 33.0 mg/L) also remained well below inhibitory levels.
- **Engineering and Economic Viability:** Operating anaerobic digesters under unheated, ambient conditions using low-cost passive insulation (such as wrapping with simple gunny bags) is highly viable and effective during temperate winter seasons. This configuration maintained a stable core temperature of 23.0°C, supporting robust biological activity and energy recovery. This passive insulation strategy provides a highly scalable, low-cost solution for decentralized municipal sludge management in developing nations, eliminating the need for expensive, energy-intensive external heating systems.

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