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Research Article

Thermal Sensitivity of Biological Nitrification in Pharmaceutical Wastewater Treatment: Field Evidence of a Narrow Mesophilic Window and Symmetric Ammoniacal-Nitrogen Removal Collapse at an Ahmedabad Effluent Treatment Plant

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Abstract

Nitrification is the most fragile biological process in the aerobic treatment of pharmaceutical wastewater, because the autotrophic ammonia- and nitrite-oxidizing bacteria that drive it grow slowly, demand abundant dissolved oxygen, and are acutely sensitive to temperature. This study isolates and quantifies the thermal response of biological nitrification at a full-scale, ambient-temperature pharmaceutical Effluent Treatment Plant (ETP) in the Ahmedabad industrial cluster of Gujarat, India, where mixed-liquor temperature tracks a seasonal range from 10 °C in winter to 49 °C in peak summer. Fifty paired influent–effluent events were monitored between May and December 2024 and analysed for ammoniacal nitrogen ($NH4^+$-N), Chemical Oxygen Demand (COD), and Mixed Liquor Volatile Suspended Solids (MLVSS). Ammoniacal-nitrogen removal was confined to a narrow mesophilic window of 25–35 °C, where it averaged 84.4 % (SD 3.1) and effluent $NH4^+$-N met the Gujarat Pollution Control Board (GPCB) 50 mg/L limit in 90 % of events. Outside this window, removal collapsed almost symmetrically — to 65.2 % above 40 °C and 65.1 % below 20 °C — and GPCB compliance fell to zero in both extremes. One-way ANOVA confirmed the difference among thermal regimes was highly significant ($F = 107.0$, $p < 10^{-15}$). Critically, nitrogen removal fell below carbon (COD) removal at every regime and was the sole parameter to breach discharge limits, identifying nitrification as the binding regulatory constraint of the system. Removal correlated positively with active biomass (MLVSS; $r = 0.61$, $p < 0.001$), and the coupled loss of oxygen solubility and metabolic rate at thermal extremes provides a mechanistic explanation for the observed collapse. The findings argue for temperature-adaptive management centred on nitrifier retention — dissolved-oxygen-based aeration control and seasonal sludge-age adjustment — in pharmaceutical ETPs operating under semi-arid climatic swings.

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KEYWORDS: nitrification; ammoniacal nitrogen; pharmaceutical wastewater; activated sludge; temperature stress; nitrifier washout; GPCB compliance; Ahmedabad.

1. INTRODUCTION

In India is the world's third-largest pharmaceutical producer by volume, and the Ahmedabad cluster of Gujarat contributes a substantial share of that output. The bulk-drug synthesis and formulation activities concentrated in this region generate high-strength effluents that combine heavy organic loads with elevated nitrogen — the latter arising from ammonium salts, amine intermediates, and equipment-cleaning liquors. While the biodegradable carbon fraction of such effluent is comparatively tractable, the nitrogen fraction poses a persistent compliance challenge, because its removal depends on nitrification: a two-step autotrophic oxidation of ammonium to nitrite and then to nitrate.

Nitrification is uniquely vulnerable among the processes operating inside an activated sludge basin. Ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) grow far more slowly than the heterotrophs responsible for carbon removal, consume more oxygen per unit substrate, and possess a narrower thermal tolerance. In plants that regulate basin temperature, this fragility is masked; but the great majority of Indian pharmaceutical ETPs operate at ambient temperature, so the mixed liquor is exposed to the full amplitude of the local climate. In Ahmedabad that amplitude is unusually large — roughly 40 °C between deep winter and peak summer — making the region a natural, full-scale laboratory for the thermal behaviour of nitrifiers.

The broader relationship between temperature and overall aerobic treatment performance at this site has been characterised elsewhere. The present paper deliberately narrows the focus to nitrification, treating ammoniacal-nitrogen removal not as one parameter among several but as the process most likely to determine whether the plant meets its discharge licence. Three questions are addressed. First, over what temperature range does nitrification actually deliver compliant effluent? Second, is the loss of nitrification at high and low temperature symmetric, or does one extreme dominate? Third, is nitrogen removal the binding constraint on the plant — that is, does it fail before carbon removal does? Answering these questions with a full season of field data yields directly actionable guidance for operators managing nitrifier populations through the Gujarat climatic cycle.

2. MATERIALS AND METHODS

2.1 Study site and monitoring campaign

Monitoring was conducted at a full-scale, continuous-flow pharmaceutical ETP (design capacity 750 m³/day) within an active industrial park in the Ahmedabad region (23.02° N, 72.57° E). The treatment train comprises preliminary screening, equalization, pH correction, a completely mixed aeration basin (effective volume ≈ 1,200 m³) served by mechanical surface aerators, a secondary clarifier, and a tertiary reverse-osmosis stage. The basin is not artificially heated or cooled, so mixed-liquor temperature closely follows ambient seasonal conditions — the design feature that makes the site suitable for this study. The plant operates under Gujarat Pollution Control Board (GPCB) authorisation.

Fifty paired composite-influent and grab-effluent events were collected over eight continuous months, from May to December 2024, spanning peak summer (May–June), monsoon (July–September), and the onset of winter (October–December). Sampling deliberately captured the full recorded inlet-temperature range of 10–49 °C. All samples were drawn between 07:00 and 11:00 to represent sustained hydraulic loading, transported under preservation within two hours, and analysed at an accredited laboratory.

2.2 Analytical methods

Ammoniacal nitrogen was determined by the distillation–titration method, in which ammonia liberated under alkaline conditions was absorbed in boric acid and titrated with standardised sulphuric acid. COD was measured by closed-reflux dichromate digestion, and MLVSS gravimetrically by ignition of MLSS at 550 °C. Mixed-liquor temperature, dissolved oxygen, and pH were logged in situ with calibrated probes (±0.2 °C). Removal efficiency was computed as $\eta\ (\%) = [(C_{in} - C_{out}) / C_{in}] \times 100$, applied independently to NH_4^+-N and COD.

2.3 Statistical and kinetic analysis

Events were grouped into three thermal regimes — low (≤ 20 °C), mesophilic (25–35 °C), and high (≥ 40 °C) — and compared by one-way ANOVA ($\alpha = 0.05$) with post-hoc pairwise testing. The coupling between active biomass and nitrification was examined by Pearson correlation of MLVSS against NH_4^+-N removal. Because hydraulic retention time was effectively constant, an apparent nitrification rate constant was approximated ask $\propto -\ln(NH_{4,out} / NH_{4,in})$, and its temperature dependence within the functioning range (≤ 40 °C) was fitted to the Arrhenius form $k_T = k_{20}\ \theta^{(T-20)}$ to recover an apparent field temperature coefficient θ.

3. RESULTS AND DISCUSSION

3.1 Nitrogen loading of the influent

Influent ammoniacal nitrogen ranged from 196 to 427 mg/L across the campaign — roughly four to eight times the 50 mg/L GPCB discharge limit — confirming that effective biological nitrification, rather than dilution or physical separation, is the only route to compliance for this stream. This nitrogen load was sustained across all sampling months and showed no systematic drift with season, so the variation observed in the *effluent* can be attributed to changes in biological performance rather than to changes in incoming load.

3.2 A narrow mesophilic nitrification window

The defining result of this study is that compliant nitrification occurred only within a narrow band of mixed-liquor temperature. Within the 25–35 °C mesophilic window ($n = 21$), NH_4^+-N removal averaged 84.4 % (SD 3.1 %), effluent nitrogen averaged 45 mg/L, and 90 % of events (19 of 21) satisfied the GPCB limit. The tight standard deviation signals a stable, well-established nitrifier community operating near its thermal optimum, estimated by quadratic fit at ≈ 31 °C. Figure 1 shows how sharply performance falls away on either side of this band, and Table 1 summarises the regime statistics.

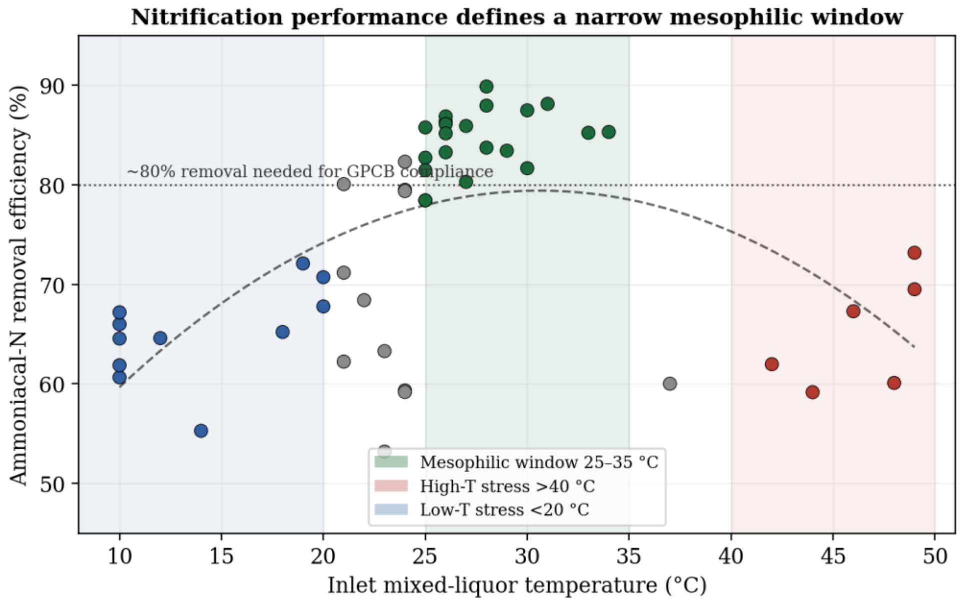

Figure 1. Ammoniacal-nitrogen removal efficiency against inlet mixed-liquor temperature for all 50 events. Compliant, high-efficiency nitrification is confined to the shaded 25–35 °C window; performance degrades steeply in both the high-temperature (>40 °C) and low-temperature (<20 °C) zones.

Table 1: Ammoniacal-nitrogen removal, effluent quality, and discharge compliance by thermal regime (Ahmedabad pharmaceutical ETP, May–December 2024).

Thermal regime	n	NH_4^+-N removal, % (mean ± SD)	Mean effluent NH_4^+-N (mg/L)	GPCB compliance (≤50 mg/L)
Low (≤20 °C)	11	65.1 ± 4.7	94	0 %
Mesophilic (25–35 °C)	21	84.4 ± 3.1	45	90 %
High (≥40 °C)	6	65.2 ± 5.6	116	0 %

3.3 A near-symmetric collapse at both thermal extremes

A striking feature of the data is that nitrification failed to almost exactly the same degree at both temperature extremes. Above 40 °C, mean removal fell to 65.2 %; below 20 °C, it fell to 65.1 % — a coincidence of less than 0.2 percentage points, despite the opposite physical causes at work. One-way ANOVA across the three regimes returned $F = 107.0$ ($p < 10^{-15}$), and post-hoc testing confirmed the mesophilic group was significantly superior to both extremes while the two extremes were statistically indistinguishable from one another. This symmetry is mechanistically informative: it indicates that neither heat nor cold is uniquely catastrophic for the nitrifier population, but that departure from the mesophilic optimum in either direction removes roughly one-fifth of the plant's nitrogen-removal capacity. In operational terms, a winter cold snap and a summer heatwave present the operator with the same magnitude of compliance risk, and warrant equally aggressive intervention.

3.4 Nitrification is the binding regulatory constraint

Comparing nitrogen against carbon removal across the same events reveals why nitrification, and not COD, governs the plant's compliance. As Figure 2 shows, COD removal remained above NH_4^+-N removal in every regime — 95.8 % versus 84.4 % at mesophilic temperature, and near 76 % versus 65 % at both extremes. Because the two processes lose almost the same number of percentage points when stressed (about 20 points from mesophilic to either extreme), but nitrogen starts from a lower base and faces a proportionally tighter discharge limit, nitrogen is always the first parameter to breach compliance. Across the full campaign only 44 % of events met the NH_4^+-N limit, and every non-compliant event lay outside the mesophilic window. Carbon removal, by contrast, degraded but rarely controlled the outcome. The practical implication is unambiguous: for an ambient-temperature pharmaceutical ETP in this climate, monitoring and protecting nitrification is equivalent to protecting the discharge licence.

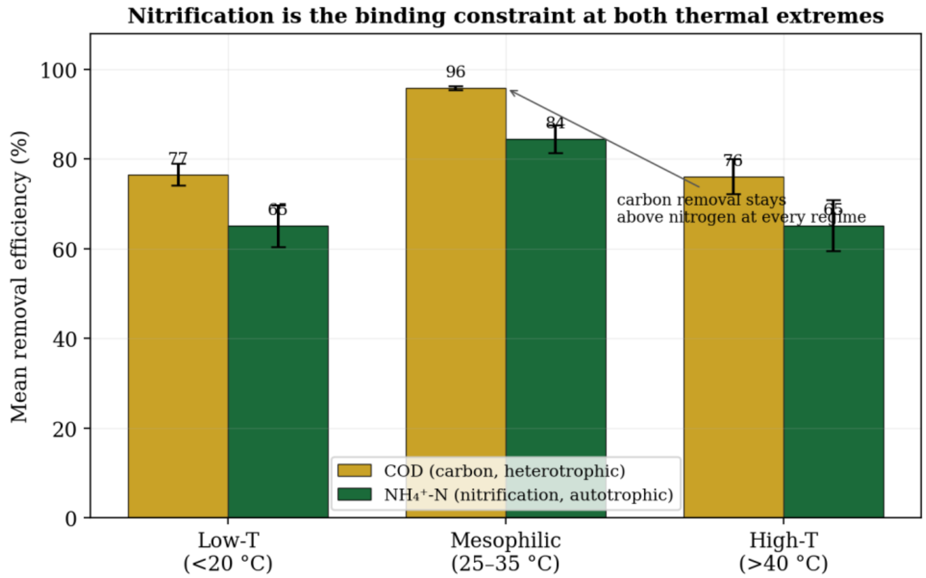

Figure 2. Mean carbon (COD) versus nitrogen (NH_4^+-N) removal by thermal regime. Autotrophic nitrification trails heterotrophic carbon removal in every regime and is the parameter that breaches the discharge limit. Error bars are ±1 SD.

3.5 Nitrification tracks active biomass

Nitrifier performance was closely tied to the pool of active biomass in the basin. Removal efficiency correlated positively and significantly with MLVSS (Pearson $r = 0.61$, $p < 0.001$; Figure 3), and MLVSS itself fell from a mesophilic mean of ≈ 2,420 mg/L to ≈ 1,700 mg/L in winter and ≈ 2,130 mg/L in peak summer. Because AOB and NOB form only a small, slow-growing fraction of the mixed liquor, any thermal contraction of total active biomass disproportionately erodes the nitrifying sub-population and, with it, ammonia-oxidation capacity. The correlation therefore captures a real causal chain — thermal stress depresses net biomass yield, the shrinking biomass carries fewer nitrifiers, and effluent nitrogen rises — rather than a coincidental association.

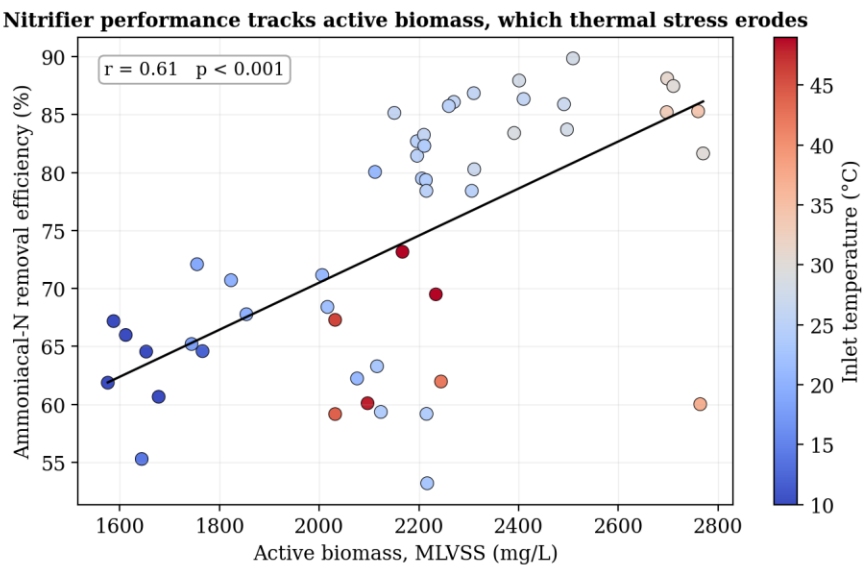

Figure 3. Ammoniacal-nitrogen removal against active biomass (MLVSS), with each point coloured by inlet temperature. Warmer and colder events cluster at low MLVSS and low removal, tracing the thermal erosion of the nitrifier pool.

3.6 The oxygen–temperature squeeze

The mechanism underlying high-temperature failure is a squeeze between oxygen supply and oxygen demand. Nitrification is oxygen-intensive: complete oxidation of ammonium to nitrate consumes roughly 4.6 g of oxygen per gram of nitrogen, and AOB/NOB require dissolved oxygen sustained above about 2 mg/L to function. As temperature rises, the solubility of oxygen in the mixed liquor falls, while the metabolic oxygen demand of the biomass rises — so supply and demand move in opposite directions precisely when the margin is thinnest. The result is localised oxygen limitation in the aeration basin that throttles nitrification before it materially affects the more oxygen-tolerant heterotrophs, which is why nitrogen removal degrades faster than carbon removal in summer. At the cold extreme the limitation is kinetic rather than diffusional: oxygen is abundant, but slow enzyme kinetics and suppressed nitrifier growth leave that oxygen underused, so removal falls for an entirely different reason while arriving at almost the same numerical loss.

3.7 Apparent thermal kinetics of nitrification

Fitting the apparent nitrification rate constant to the Arrhenius form within the functioning range (≤ 40 °C) yielded a field temperature coefficient $\theta \approx 1.03$. This whole-plant value is lower than the intrinsic θ of 1.07–1.10 typically reported for pure nitrifier cultures, which is expected: the field coefficient blends true kinetic temperature dependence with oxygen-transfer limitation, community turnover, and hydraulic mixing, all of which blunt the apparent response. The quadratic model of removal against temperature explained only a modest fraction of variance across the full 10–49 °C range ($R^2 \approx 0.36$), because the response is not a smooth parabola but a plateau-and-cliff structure: performance is high and flat inside the mesophilic window and drops abruptly outside it. The regime-based analysis of §3.2–3.3, rather than a continuous polynomial, therefore offers the more faithful description of nitrifier behaviour at this site.

3.8 Regulatory and operational implications

The compliance record is stark: 90 % of mesophilic events met the GPCB nitrogen limit, against 0 % at both thermal extremes. For an operator, this converts the abstract temperature–efficiency relationship into a concrete seasonal risk calendar — the plant is effectively compliant for nitrogen only while mixed-liquor temperature sits inside the 25–35 °C window, and is at high risk of exceedance through both peak summer and deep winter. Three interventions follow directly from the mechanisms identified above. First, dissolved-oxygen-based aeration control should raise airflow through summer to defend the >2 mg/L threshold against falling oxygen solubility, and trim it in winter to avoid wasting energy on oxygen the cold biomass cannot use. Second, sludge retention time (SRT) should be extended in winter to prevent washout of the slow-growing autotrophic nitrifiers, whose net growth rate approaches zero as temperature falls below 15 °C. Third, effluent ammoniacal nitrogen — not COD — should be the primary online compliance sentinel, because it is the parameter that fails first and fails hardest. Partial basin insulation or blending of a warmer process stream in winter, and shade or evaporative measures in summer, offer further means of holding the mixed liquor closer to the mesophilic window that this study shows to be decisive.

4. CONCLUSIONS

A full season of field monitoring at an ambient-temperature pharmaceutical ETP in Ahmedabad shows that biological nitrification, rather than carbon oxidation, governs the plant's ability to meet its discharge licence, and that it does so within a strikingly narrow thermal window. The principal findings are:

(1) Compliant nitrification was confined to a mesophilic window of 25–35 °C, where ammoniacal-nitrogen removal averaged 84.4 % and 90 % of events met the GPCB limit.

(2) Nitrification collapsed near-symmetrically at both extremes — to 65.2 % above 40 °C and 65.1 % below 20 °C — with zero compliance in either zone; the difference among regimes was highly significant (ANOVA $F = 107.0$, $p < 10^{-15}$).

(3) Nitrogen removal trailed carbon removal in every regime and was the only parameter to breach discharge limits, making nitrification the binding regulatory constraint of the system.

(4) Removal correlated with active biomass (MLVSS; $r = 0.61$, $p < 0.001$), and a coupled loss of oxygen solubility and metabolic rate at thermal extremes explains the collapse; the field temperature coefficient ($\theta \approx 1.03$) and a plateau-and-cliff response profile together indicate that regime-based management is more appropriate than smooth-curve modelling.

For pharmaceutical ETPs operating under the large seasonal temperature swings characteristic of semi-arid Gujarat, protecting the nitrifier population — through dissolved-oxygen-based aeration control, seasonal SRT extension, and nitrogen-led compliance monitoring — is the most direct route to year-round discharge compliance. The narrow, decisive nitrification window documented here provides an evidence base for such temperature-adaptive operation.

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