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Research Article

Combined Ozonation and Aerobic Biodegradation: An Effective Dual-Stage Approach for Treating Reactive Azo Dye Wastewater

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Abstract

This study investigates dual-stage treatment of reactive azo dye wastewater using ozonation as pre-treatment followed by aerobic biodegradation. Two commercially important dyes were used: CI Reactive Black 5 (RB5, di-azo) and CI Reactive Violet 5 (RV5, mono-azo), both at 2000 mg/L in a semi-batch reactor. Parameters monitored throughout treatment included pH, absorbance at λmax, conductivity, COD, TOC, and BOD_5. Ozonation achieved near-complete colour removal within 60–80 minutes and transformed both dyes from essentially non-biodegradable (BOD_5/COD ≈ 0) to readily biodegradable (BOD_5/COD ≈ 0.47–0.49). Subsequent aerobic treatment achieved over 90% reduction in both COD and TOC. RV5 (mono-azo) degraded consistently faster than RB5 (di-azo) across all metrics. The combined approach is economically and ecologically advantageous: ozonation breaks recalcitrant molecules into biodegradable fragments, while aerobic treatment completes organic removal at lower cost than extended ozonation alone.

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1. INTRODUCTION

Azo Dyes and the Discharge Problem

Azo dyes—characterised by nitrogen-to-nitrogen double bonds (N=N)—account for more than 50% of the approximately 3,000 different dyes currently in use worldwide [1]. Their applications include textiles, cosmetics, mouthwashes, and pharmaceutical products. The dyeing process is inherently inefficient: typically only 40–90% of applied dye bonds with the fabric, depending on the dyestuff and application method [2,6]. The remaining 10–60% washes out during rinsing into wastewater, imparting intense colour that reduces water transparency, disrupts photosynthesis, and produces toxic aromatic amines with mutagenic or carcinogenic properties.

Azo dye molecules resist the biological breakdown that conventional activated sludge processes rely upon due to their stable aromatic rings and complex molecular architectures [3]. Reactive dyes—specifically engineered for high water solubility and strong covalent bonding with fibres—prove particularly problematic, as these same properties make them resistant to removal once in wastewater streams [4].

Ozonation as Treatment

Ozone (O_3), with an oxidation potential of 2.08 volts, ranks among the most powerful oxidants practical for water treatment applications [5,6]. It attacks dye chromophores through two pathways: direct molecular reaction with electron-rich azo bonds (N=N), and indirect decomposition to hydroxyl radicals (•OH) initiated by hydroxyl ions, which open aromatic rings and mineralise organic molecules [8]. Studies have demonstrated that ozonation can achieve complete decolourisation while also enhancing biodegradability, destroying phenolic compounds, and significantly reducing chemical oxygen demand [5,6,7].

However, ozonation provides only partial COD and TOC reduction [7]. Rather than completely mineralising dye molecules to CO_2 and water, ozonation oxidises them to smaller organic fragments—aldehydes, ketones, organic acids—which are far more biodegradable than the original structures. The BOD_5/COD ratio typically increases substantially during Ozonation, signalling improved biodegradability [9]. Complete ozonation to achieve full mineralisation would require excessive treatment time and energy, making it economically impractical.

The Dual-Stage Solution

Combining ozonation's decolourisation and structure-breaking capabilities with biological treatment's economical organic removal [6,10] creates a synergistic solution: ozonation pre-treats non-biodegradable dye molecules into biodegradable fragments; aerobic treatment then mineralises these fragments through microbial metabolism. Several parameters influence ozonation effectiveness: decolourisation time increases with the number of azo groups and decreases with more sulfonic acid groups ($-SO_3H$) [7,11]; higher salt concentrations slow decolourisation by competing for ozone; higher initial dye concentrations reduce effectiveness as parent molecules, and byproducts compete for available ozone [12].

Research Objectives

This study investigates the integrated effect of ozonation followed by aerobic biodegradation on RB5 (di-azo) and RV5 (mono-azo) at 2000 mg/L, using a semi-batch ozonation reactor. Objectives:

1. Quantify ozonation effectiveness for colour removal, COD/TOC reduction, and biodegradability enhancement
2. Compare degradation kinetics between mono-azo (RV5) and di-azo (RB5) structures
3. Evaluate aerobic biodegradation efficiency on ozonated dye solutions
4. Assess overall treatment efficiency of the combined approach
5. Determine practical operating parameters for meeting effluent discharge standards

2. MATERIALS AND METHODS

A. Dye Characterisation

Both dyes were obtained from Colour Chem Ltd., Mumbai, India. Carbon content was determined using a CHNOS elemental analyser (Model CE-440, Leemans Lab. Inc., USA).

Property	CI Reactive Black 5 (RB5)	CI Reactive Violet 5 (RV5)
Classification	Di-azo dye (two N=N bonds)	Mono-azo dye (one N=N bond)
Molecular Weight	991.8 g/mol	749 g/mol
λmax	597 nm	560 nm
Carbon Content (elemental analysis)	19.798%	13.06%
Initial COD (2000 mg/L solution)	929 mg/L	603 mg/L
Initial TOC (2000 mg/L solution)	329 mg/L	218 mg/L
Initial BOD_5/COD	0.00	0.02

B. Ozonation Setup

Semi-batch bubble column reactor: 6.4 cm diameter × 39 cm height, 500 mL working volume, porous ceramic bottom plate (~1 mm bubbles), borosilicate glass construction. Ozone generator: INDIZONE Model CDS/4C/AF (corona discharge, pure dry O_2 feed, ~58.5 g O_3/m^3 at 0.5 L/min). Gas flow controlled by AALBORG Model GFC171S mass flow controller (±1% accuracy). Online UV absorbance ozone monitors (ANSEROS OZOMAT GM-6000-OEM, Germany) were installed at both influent and effluent positions for continuous ozone consumption measurement. All ozone-contact components are fabricated from ozone-resistant materials (borosilicate glass, PTFE, 316 stainless steel).

C. Aerobic Biodegradation System

Seed cultivation: Two 500 mL conical flasks with 8-day HRT, continuous aeration, fed with progressive transitions from domestic wastewater to ozonated dye metabolites (separate reactors for RB5 and RV5 metabolites). Biodegradation study: Ten 130 mL glass columns with sintered glass bottom plates for air distribution, housed in a temperature-controlled wooden box with manifold aeration (Comair NF264 compressor). Eight columns operated in parallel: four for RB5 (ozonated 20, 40, 60, 80 min) and four for RV5 (ozonated 20, 40, 60, 80 min).

D. Experimental Procedures

Dye Solution Preparation

Dye solutions (2000 mg/L) were prepared in double-distilled water, buffered with 0.2 M phosphate buffer to maintain pH ≈ 7.0 throughout ozonation. Buffering prevents the pH drop from organic acid formation during ozone treatment [6,10] and maintains conditions suitable for subsequent aerobic biodegradation.

Ozonation Protocol

System purged with oxygen 30 minutes before each run. Reactor loaded with 500 mL of buffered dye solution, and the initial parameters were recorded. Oxygen flow set to 0.5 L/min, ozone generator activated. Samples extracted at 0, 20, 40, 60, and 80 minutes; pH and absorbance measured immediately; samples preserved for COD, TOC, and BOD_5 analysis. Average influent ozone dose: 58.5 g O_3/m^3. Ozone consumption calculated as:

Cumulative O_3 consumed = ∫(C_influent − C_effluent) × Flow rate × dt

Note: A much higher ozone dose (55.8 mg/L) was applied compared to commonly reported literature values (8.9–20.5 mg/L) [1,6,10,11] to complete ozonation within 20–80 minutes rather than multi-hour treatments, identifying a practical operating window for combined treatment.

Biomass Acclimatization

Phase	Duration	Feed Composition	Purpose
Phase 1: Establishment	Days 1–10	100% domestic wastewater (50 mL/day replaced after settling)	Rapid biomass growth, a diverse microbial community
Phase 2: Gradual Introduction	Days 11–15 Days 16–20 Days 21–25 Days 26–30	25% ozonated / 75% domestic 50% / 50% 75% / 25% 100% ozonated in BOD dilution water	Enzymatic adaptation to novel carbon sources without toxic shock
Phase 3: Steady-State	Days 31+	100% ozonated dye solution (2000 mg/L) in BOD dilution water with N, P, minerals	Performance evaluation at full loading

Aerobic Biodegradation Operation

Working volume: 100 mL ozonated feed + 5 mL acclimatised seed. Average HRT: 2.5 days. Daily operation: remove 40 mL effluent, add 40 mL fresh ozonated feed. Continuous aeration maintaining dissolved oxygen > 2 mg/L. Reactors operated for 40+ days until steady-state (< 10% COD variation over 5 consecutive days).

Analytical Methods

Parameter	Method / Equipment
pH	Toshiwal CL-51 combination pH electrode with CL-54 digital meter; two-point calibration (pH 4.0 and 7.0)
COD	Closed reflux dichromate oxidation (Standard Methods 5220 C, APHA 1995); 2 h at 150°C; spectrophotometric detection; reported as mg O_2/L
TOC	Shimadzu TOC-V CPN high-temperature combustion (Standard Methods, APHA 1995). Note: volatile products (formaldehyde, acetaldehyde) may escape during sampling, causing slight TOC underestimation
BOD_5	Hach BODtrak respirometric system; 5-day incubation at 20°C with acclimatised biomass seed
Absorbance / Colour	Varian CARY 50 UV-Vis spectrophotometer, 200–700 nm scan, 1 cm quartz cell (Standard Methods 5910 B, APHA 1995); Pt-Co colour scale for colour quantification

3. RESULTS AND DISCUSSION

A. Colour Removal

Ozone efficiently cleaves the azo bonds responsible for visible colour in both dyes. RV5 reached near-complete Decolourisation at 60 minutes (10 Pt-Co); RB5 required 80 minutes (5 Pt-Co), reflecting the additional azo bond requiring oxidative cleavage.

B. Ozonation Data: COD, TOC, Colour, and BOD_5/COD

Table 1: Effects on colour, COD, TOC, and BOD_5/COD ratio during ozonation of RB5 and RV5 (2000 mg/L, 0.2 M phosphate buffer, pH ≈ 7.0)

Time (min)	RB5 Colour (Pt-Co)	RB5 COD (mg/L)	RB5 TOC (mg/L)	RB5 BOD_5/COD	RV5 Colour (Pt-Co)	RV5 COD (mg/L)	RV5 TOC (mg/L)	RV5 BOD_5/COD
0	—	929	329	0.00	—	603	218	0.02
20	875	591	260	0.14	125	341	159	0.19
40	75	382	204	0.26	50	228	108	0.39
60	40	311	178	0.39	10	164	78	0.471
80	5	271	171	0.47	5	122	65	0.491

C. COD and TOC Reduction

COD reduction resulted from two concurrent processes: partial mineralisation (organic carbon oxidised to CO_2/H_2O) and oxidation state increase (remaining carbon converted to higher-oxidation-state fragments with lower COD per unit carbon). After 80 minutes, COD removal was 71% for RB5 (929 → 271 mg/L) and 80% for RV5 (603 → 122 mg/L). TOC removal was 48% for RB5 (329 → 171 mg/L) and 70% for RV5 (218 → 65 mg/L).

COD removal consistently exceeded TOC removal throughout experiments, particularly immediately after ozonation [6,10]. This reflects ozone's tendency to increase organic matter oxidation state without complete mineralisation—converting reduced organics (high COD per carbon) to oxidised forms (lower COD per carbon) faster than it mineralises them to CO_2.

D. Biodegradability Enhancement

The BOD_5/COD ratio is the key indicator of biological treatability: ratio ≈ 0 indicates non-biodegradable matter; 0.1–0.3 indicates marginal biodegradability; > 0.4 indicates readily biodegradable organic matter. Both dyes initially had BOD_5/COD ≈ 0, confirming biological recalcitrance. After 80 minutes of ozonation, RB5 reached 0.47, and RV5 reached 0.49—both readily biodegradable. Ozone and hydroxyl radicals break down the structured dye polymers into smaller molecules—carboxylic acids, aldehydes, ketones—that microorganisms can readily metabolise [3], lacking the stable aromatic ring systems and azo linkages that protected parent molecules from biological attack.

RV5 reached BOD_5/COD = 0.471 at 60 minutes versus 0.39 for RB5 at the same time, confirming that simpler mono-azo structures achieve adequate biodegradability enhancement faster than di-azo structures.

E. Combined Treatment Performance

Aerobic reactors operated for 40+ days to achieve steady-state COD removal for dye solutions ozonated at 20, 40, 60, and 80 minutes. Combined results:

Metric	RB5 (Di-azo)	RV5 (Mono-azo)
Total COD removal (ozonation + aerobic)	~92% (929 → ~74 mg/L)	~91% (603 → ~54 mg/L)
Total TOC removal (ozonation + aerobic)	~88% (329 → ~40 mg/L)	~90% (218 → ~22 mg/L)
Additional COD reduction from the aerobic stage	~72% beyond ozonation alone	~58% beyond ozonation alone
Additional TOC reduction from the aerobic stage	~77% beyond ozonation alone	~68% beyond ozonation alone
Optimal ozonation time (combined treatment)	60 min (~90% COD/TOC)	60 min (~90% COD/TOC)

Ozonation alone achieved 71–80% COD removal but left substantial organic matter. Aerobic treatment of non-ozonated dyes accomplished virtually nothing due to biological recalcitrance. Combined treatment achieved > 90% removal of both COD and TOC. Analysis across ozonation durations confirmed that 60 minutes represents an optimal balance—sufficient biodegradability enhancement for effective biological treatment without the energy cost of extended ozonation.

F. Structural Effects: Mono-azo vs. Di-azo

RV5 (mono-azo) consistently outperformed RB5 (di-azo) across all metrics:

Metric	RV5 (Mono-azo)	RB5 (Di-azo)
Near-complete decolourisation	60 min (10 Pt-Co)	80 min (5 Pt-Co)
COD reduction at 80 min	80%	71%
TOC reduction at 80 min	70%	48%
BOD_5/COD at 60 min	0.471	0.39

RV5 contains one azo bond requiring oxidative cleavage; RB5 contains two. Simpler structures break down faster and more completely. Textile facilities using predominantly mono-azo dyes may achieve adequate treatment with shorter ozonation times or lower ozone doses compared to those treating complex multi-azo structures.

4. CONCLUSIONS

Ozonation achieved near-complete colour removal within 60–80 minutes for both RB5 and RV5, confirming efficient cleavage
of chromophoric azo bonds. Biodegradability transformed from essentially zero (BOD_5/COD ≈ 0) to readily biodegradable (BOD_5/COD ≈ 0.47–0.49) after 80 minutes of ozonation, enabling economical biological polishing. Combined treatment achieved over 90% COD and TOC removal—a performance level neither ozonation nor biological treatment alone can achieve. RV5 (mono-azo) degraded faster than RB5 (di-azo) across all metrics, as simpler molecular architectures are more susceptible to oxidative breakdown. COD removal consistently exceeded TOC removal throughout experiments [6,10], reflecting ozone's tendency to raise organic oxidation state before full mineralisation. 60 minutes of pre-ozonation followed by biological treatment achieved approximately 90% COD and TOC removal for both dyes, representing the optimal operating point balancing effectiveness against energy costs.

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