

Simultaneous removal of dissolved nitrogen species and hexavalent chromium by indigenous *Achromobacter* sp. CSND-B12 from tannery activated sludge

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ABSTRACT

Tannery wastewater may contain nitrogen compounds and chromium simultaneously, creating challenges for treatment systems designed to address individual contaminants. This study aimed to isolate and characterize bacteria from activated sludge at a tannery wastewater treatment plant that can simultaneously remove dissolved nitrogen species and hexavalent chromium [Cr(VI)] under aerobic conditions. We recovered twelve isolates from the sludge through four enrichment strategies and screened them for growth under 100 mg L⁻¹ Cr(VI) exposure in nutrient-rich medium and three nutrient-limited media containing ammonium (MM-ACr), nitrate (MM-NCr), and combined ammonium and nitrate (MM-ANCr). All isolates grew, although growth and functional performance varied among the media tested. Based on screening results from the three nutrient-limited media, CSND-B12 consistently showed high biomass accumulation and apparent removal of dissolved nitrogen species and Cr(VI) across the tested media, with significant differences among isolates after 72 h of incubation ($P < 0.05$). CSND-B12 achieved an OD₆₀₀ of 0.543, with 66.80% apparent ammonium removal and 50.42% Cr(VI) removal in MM-ACr; an OD₆₀₀ of 0.475, with 96.10% apparent nitrate removal and 47.76% Cr(VI) removal in MM-NCr; and an OD₆₀₀ of 0.552, with 54.69% apparent removal of the combined ammonium and nitrate pool and 21.52% Cr(VI) removal in MM-ANCr. 16S rRNA gene sequencing showed that CSND-B12 had 99.93% sequence identity to several closely related *Achromobacter* reference sequences, while phylogenetic analysis placed the isolate within the *Achromobacter xylosoxidans* clade. This isolate maintained measurable decreases in dissolved nitrogen species alongside Cr(VI) removal across all three nitrogen regimes. These findings identify CSND-B12 as a strain warranting further evaluation of its bioaugmentation potential in contained treatment systems receiving wastewater containing co-occurring nitrogen and chromium.

Keywords: *Achromobacter* sp. CSND-B12, indigenous bacteria, apparent nitrogen removal, Cr(VI) removal, complex wastewater, aerobic bioremediation.

INTRODUCTION

Industrial wastewater treatment is increasingly challenged by multiple contaminants that require different removal mechanisms within a single treatment process (Tripathy and Prakash, 2025). Tannery wastewater is a representative example because it contains high concentrations of inorganic nitrogen, chromium, and other contaminants generated during leather processing

(Devi et al., 2023). Raw tannery wastewater from different regions has been reported to contain total nitrogen concentrations up to 490 mg L⁻¹ (Tejada-Meza et al., 2023), ammonium concentrations of 32–174 mg L⁻¹ (Faye and Sibali, 2025), and hexavalent chromium concentrations of up to 85 mg L⁻¹ (Fito et al., 2023). The coexistence of these contaminants complicates treatment because they differ in their chemical properties, biological effects, and removal requirements.

Conventional treatment approaches often address nitrogen and heavy metals through separate treatment processes, which can increase process complexity and operational requirements (Singh et al., 2024). The development of biological treatment strategies capable of addressing co-occurring nitrogen compounds and chromium within a common treatment system is of considerable interest for industrial wastewater management.

Chromium, particularly in hexavalent form [Cr(VI)], is highly soluble, mobile, and toxic, making it an important contaminant of concern in industrial wastewater (Harshan Gandhi et al., 2025; Zhao et al., 2022). In parallel, elevated concentrations of inorganic nitrogen, including ammonium, nitrite, and nitrate, can contribute to nutrient pollution, deterioration of water quality, and eutrophication in receiving water bodies (Bhardwaj et al., 2023; Essien et al., 2022). The simultaneous occurrence of chromium and nitrogen may further complicate biological treatment because heavy-metal exposure can inhibit microorganisms involved in biological nitrogen removal and consequently affect treatment stability (Mpongwana et al., 2022). This co-occurrence highlights the need to consider whether nitrogen transformation can be maintained under chromium stress during biological treatment.

Biological nitrogen removal has increasingly focused on heterotrophic nitrification and aerobic denitrification (HN-AD), in which some bacteria can carry out different nitrogen transformations under aerobic conditions. This characteristic may reduce the need for spatially separated nitrification and denitrification zones and offers potential advantages for simplifying biological nitrogen treatment (Xi et al., 2022). Recent studies have highlighted the diversity of HN-AD microorganisms and how environmental factors such as dissolved oxygen, carbon availability, pH, and C/N ratio influence their nitrogen-transformation performance. At the same time, the detailed mechanisms governing HN-AD remain incompletely resolved (Hu et al., 2023). Researchers have also isolated individual HN-AD bacteria from wastewater-related environments, including *Achromobacter* sp., demonstrating the relevance of this bacterial group to biological nitrogen treatment (Liu et al., 2023). These characteristics make HN-AD bacteria potential candidates for treatment systems that must maintain nitrogen transformation under additional environmental stresses.

Microbial remediation of Cr(VI) has likewise attracted increasing interest because chromium-resistant bacteria may interact with Cr(VI) through several processes, including biosorption, bioaccumulation, cellular efflux, and enzymatic reduction (Dubey et al., 2024). Recent studies have demonstrated that the extent of Cr(VI) removal by chromium-resistant bacteria depends on the microorganism and environmental conditions (Shi et al., 2023). Importantly, the simultaneous presence of Cr(VI) and nitrogen compounds may impose interacting metabolic constraints rather than representing two independent treatment targets. Recent experimental evidence has shown that increasing Cr(VI) exposure can affect simultaneous nitrification and denitrification performance and alter microbial metabolic pathways associated with nitrogen transformation and chromium reduction (Niu et al., 2025). These findings indicate that microorganisms used for simultaneous treatment must remain functionally active under the combined pressure of nitrogen availability and chromium toxicity.

Despite these advances, studies combining different nitrogen regimes with Cr(VI) exposure remain relatively limited. Previous reports of simultaneous nitrogen transformation and Cr(VI) removal have often used individual nitrogen sources and relatively low Cr(VI) concentrations, such as approximately 10 mg L⁻¹ in several reported systems (An et al., 2020; Zhang et al., 2020). Consequently, less is known about the functional performance of indigenous bacteria originating from full-scale tannery activated sludge when exposed to different nitrogen regimes under comparatively high Cr(VI) concentrations. This gap is particularly relevant because tannery activated sludge originates from a treatment environment exposed to complex mixtures of organic matter, nitrogen compounds, salts, and chromium, providing a relevant source for isolating microorganisms capable of maintaining activity under combined environmental stress. However, the functional characteristics of indigenous isolates from such environments across different nitrogen regimes remain insufficiently characterized. Therefore, this study aimed to identify indigenous bacterial isolates that can simultaneously remove dissolved nitrogen species and Cr(VI) under defined aerobic conditions. We applied a sequential enrichment and functional screening strategy to activated sludge from a full-scale tannery wastewater treatment plant. The

isolates were evaluated for growth and removal of dissolved nitrogen species and Cr(VI) under ammonium, nitrate, and mixed-nitrogen regimes containing 100 mg L⁻¹ Cr(VI) under laboratory conditions. We subsequently characterized selected isolates using 16S rRNA gene sequencing to provide taxonomic information.

MATERIALS AND METHODS

Sampling and physicochemical profiling

In this study, we collected activated sludge samples from a local tannery that processes approximately 1.3 tons of sheep and goat hides per day. The treatment system operates under continuous aeration, with a hydraulic retention time of 121 h and an average influent flow rate of 61.83 m³ day⁻¹. Activated sludge was collected from the aeration pond of a full-scale tannery wastewater treatment plant located in Bantul, Yogyakarta, Indonesia (7.8563° S, 110.4370° E). The sludge was randomly collected from several locations within the aeration pond to obtain a representative sample. The sludge was passed through an 18-mesh stainless steel sieve to remove coarse debris, then mixed with aerated wastewater collected from the same pond at a 1:1 (v/v) ratio. Approximately 5 L of homogenized suspension was transferred into sterile containers, transported to the laboratory under cooled conditions, and processed immediately for physicochemical profiling and bacterial isolation. The physicochemical properties of the sludge mixture were analyzed following the Standard Methods for the Examination of Water and Wastewater (APHA, 2017). The measured parameters of the sludge suspension including pH, temperature, biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), organic matter, total kjeldahl nitrogen (Total-N), ammonium (N-NH₄), nitrite (N-NO₂), nitrate (N-NO₃), sulphide (S), phosphate (PO₄), potassium (K), total chromium (Cr), Cr(VI), iron (Fe), zinc (Zn), lead (Pb), manganese (Mn), magnesium (Mg), and chloride (Cl⁻).

Culture media

This study used three categories of culture media: Enrichment media (EM), screening media (SM), and characterization media (CM). We

prepared these media for bacterial enrichment, isolation, growth characterization, and functional evaluation, modified from previous studies (Kallola and Desai, 2020; Xi et al., 2022). Nutrient broth served as the basal medium; NH₄Cl and KNO₃ as nitrogen sources; trisodium citrate dihydrate as the carbon source; and K₂Cr₂O₇ as the Cr(VI) source. All media were supplemented with a mineral solution, consisting of 7.0 gL⁻¹ K₂HPO₄, 3.0 gL⁻¹ KH₂PO₄, 0.1 gL⁻¹ MgSO₄·7H₂O, and 0.05 gL⁻¹ FeSO₄·7H₂O (Pal et al., 2015). Detailed compositions of all media are presented in Table 1. All media were sterilized by autoclaving at 121 °C for 15 min.

Bacterial isolation and screening of bacterial isolates

Four enrichment media (EM) were prepared to selectively enrich general heterotrophic bacteria (EM-GH), chromium-tolerant heterotrophs (EM-Cr), ammonium-utilizing bacteria under Cr(VI) stress (EM-ACr), and nitrate-utilizing bacteria under Cr(VI) stress (EM-NCr). For each enrichment, 2 mL of a 10⁻¹-diluted sludge suspension was inoculated into 200 mL of the corresponding enrichment medium and incubated aerobically on a rotary shaker at 120 rpm under ambient laboratory conditions for 9 days. Four enrichment strategies (Treatments A, B, C, and D) were applied in parallel to increase the recovery of functionally diverse chromium-tolerant bacteria. Treatment A: EM-GH with periodic addition of NH₄Cl (0.764 g on days 2, 4, 6) and KNO₃ (1.442 g on days 3, 5, 7); Treatment B: EM-Cr with the same nutrient schedule as Treatment A; Treatment C: EM-ACr with serial transfers to EM-NCr and back over every 24 h; Treatment D: EM-GH as in Treatment A, followed by subculture into EM-Cr on day 8. Detailed protocols of these strategies are provided in Supplementary Table S1.

After enrichment at final incubation (9 days), 10 µL aliquots were spread onto four screening media (SM) containing different concentrations of nutrient and Cr(VI) levels including 1% NB and 100 mg L⁻¹ Cr(VI) (SM-1), 10% NB and 100 mg L⁻¹ Cr(VI) (SM-2), 1% NB and 1.000 mg L⁻¹ Cr(VI) (SM-3) and 10% NB and 1.000 mg L⁻¹ Cr(VI) (SM-4). The plates were incubated at 30 °C for up to 12 days. We selected and purified several colonies with distinct morphologies on MPCr agar. Colony morphology, oxidase test, and Gram staining were characterized according to Bergey's

Table 1. Composition and functional roles of culture media employed for the isolation and characterization of indigenous bacteria from tannery sludge in this study

Purposes	Medium	Composition (per L)					
		NB (%)	Agar* (%)	Citrate (g L ⁻¹)	NH ₄ Cl (g L ⁻¹)	KNO ₃ (g L ⁻¹)	K ₂ Cr ₂ O ₇ (g L ⁻¹)
Enrichment media (EM)	EM-GH	50	0	0	0	0	0
	EM-Cr	10	0	0	0	0	0.283
	EM-ACr	1	0	4.085	0.382	0	0.283
	EM-NCr	1	0	4.085	0	0.721	0.283
Screening media (SM)	SM-1	1	1.5	40.85	1.91	3.607	0.283
	SM-2	10	1.5	40.85	1.91	3.607	0.283
	SM-3	1	1.5	40.85	1.91	3.607	2.830
	SM-4	10	1.5	40.85	1.91	3.607	2.830
Characterization media (CM)	MPCr	100	0 and 1.5	0	0	0	0.283
	MM-ACr	1	0 and 1.5	4.085	0.382	0	0.283
	MM-NCr	1	0 and 1.5	4.085	0	0.721	0.283
	MM-ANCr	1	0 and 1.5	4.085	0.382	0.721	0.283

Note: NB – nutrient broth, Agar* – Agar powder used only for agar-based medium preparation, EM-GH – enrichment medium for general heterotrophic, EM-Cr – enrichment medium containing chromium, EM-ACr – enrichment medium containing ammonium and chromium, EM-NCr – enrichment medium containing nitrate and chromium, MPCr – medium preculture containing chromium, MM-ACr – minimal media containing ammonium and chromium, MM-NCr – minimal media containing nitrate and chromium, MM-ANCr – minimal media containing ammonium, nitrate and chromium.

Manual of Determinative Bacteriology (Acharya et al., 2023; Holt et al., 1994). The selected isolates were subsequently subjected to growth characterization and evaluation of simultaneous changes in dissolved nitrogen species and Cr(VI) concentrations under different nitrogen regimes.

Growth characterization under Cr(VI) stress

To evaluate bacterial growth under Cr(VI) stress, we cultivated all CSND isolates on agar and in liquid characterization media (CM) containing 100 mg L⁻¹ Cr(VI) under different nitrogen regimes. The media consisted of nutrient-rich MPCr (100% nutrient broth) and three nutrient-limited media: MM-ACr (100 mg L⁻¹ N-NH₄), MM-NCr (100 mg L⁻¹ N-NO₃), and MM-ANCr (100 mg L⁻¹ N-NH₄ and 100 mg L⁻¹ N-NO₃). The C/N ratio of the nutrient-limited media was adjusted to 10 for single-nitrogen media and 5 for the mixed-nitrogen medium to maintain comparable carbon availability relative to total nitrogen.

Pre-culture preparation

A loopful of each purified isolate was inoculated into 100 mL liquid MPCr and incubated

aerobically on a rotary shaker at 120 rpm under ambient laboratory conditions. Optical density at 600 nm (OD₆₀₀) was measured every 3 h for 36 h. We used the average time required for isolates to reach the exponential phase to determine the pre-culture incubation period for subsequent experiments.

Initial qualitative growth assessment

Agar-based CM media were used to qualitatively compare bacterial growth under Cr(VI) stress across different nitrogen regimes. Ten microliters of each pre-culture (24 h) were spot-inoculated onto agar plates of MPCr (control), MM-ACr, MM-NCr, and MM-ANCr, incubated at 30 °C for up to 72 h. We visually assessed and compared colony development across media.

Growth and functional assessment

We inoculated a 2 mL aliquot of each pre-culture into 100 mL liquid CM medium (MM-ACr, MM-NCr, and MM-ANCr), and incubated the cultures aerobically on a rotary shaker at 120 rpm under ambient laboratory conditions for 72 h. For each treatment condition, three independently inoculated 250 mL Erlenmeyer flasks were prepared

as biological replicates ($n = 3$), with each flask representing one experimental unit. Uninoculated flasks containing the same medium were prepared in parallel as abiotic controls and subjected to the same incubation and sampling conditions. Each flask was sampled at 24-h intervals during the 72-h incubation period to determine OD_{600} , medium pH, dissolved nitrogen species, and Cr(VI) concentrations. Measurements at 0, 24, 48, and 72 h represented repeated observations from the same experimental units. At each sampling interval, 5 mL culture samples were centrifuged at 10,000 rpm for 15 min to obtain supernatants for analysis of dissolved nitrogen species and Cr(VI). Concentrations of ammonium, nitrite, nitrate, and Cr(VI) were determined using standard UV–Vis spectrophotometric methods (APHA, 2017), including *Nesslerization* for ammonium, *N-(1-naphthyl)ethylenediamine dihydrochloride* for nitrite, *Brucine* for nitrate, and *Diphenylcarbazide* for Cr(VI). Removal efficiency (%) was calculated as described by Yang et al. (2021).

$$\text{Removal efficiency (\%)} = \frac{(C_i - C_f)}{C_i} \times 100 \quad (1)$$

where: C_i and C_f represent the initial concentration at 0 h and final concentration at 72 h, respectively. For statistical analysis, we used the OD_{600} and pH values at 72 h and the 72-h removal efficiencies of dissolved nitrogen species and Cr(VI) as endpoint response variables.

Endpoint data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) to determine significant differences among isolates at $P < 0.05$, using IBM SPSS Statistics 23. Data are presented as mean $\pm$ standard error of the mean (SEM). Based on the screening results, selected representative isolates were subjected to 16S rRNA gene sequencing for initial taxonomic identification.

Molecular identification of selected isolates

Genomic DNA was extracted using the Quick-DNA MagBead Plus Kit (Zymo Research, USA). We amplified the 16S rRNA gene with universal primers 27F and 1492R under the following PCR conditions: 95 °C for 60 s, then 35 cycles of 95 °C for 10 s, 52 °C for 15 s, and 72 °C for 15 s.

Products were verified on an agarose gel (0.8%) and sequenced by Apical Scientific (1st BASE, Malaysia). Sequences were compared against NCBI GenBank using BLASTN, aligned in MEGA 11, and phylogenetic trees were constructed by Neighbour-Joining and Maximum Likelihood methods using the Tamura–Nei model with 1000 bootstrap replicates (Tamura et al., 2021).

RESULTS AND DISCUSSION

Site characteristics and physicochemical properties

Physicochemical characterization of the collected sludge sample showed a pH of 7.64, temperature of 29 °C, BOD_5 of 662.2 mg L⁻¹, COD of 3.275 mg L⁻¹, TSS of 7.240 mg L⁻¹, TDS of 3.935 mg L⁻¹, organic matter of 149.97 mg L⁻¹, total-N of 67.6 mg L⁻¹, ammonium (N-NH₄) of 37.95 mg L⁻¹, nitrite (N-NO₂) below the detection limit (<0.01 mg L⁻¹), nitrate (N-NO₃) of 3.98 mg L⁻¹, sulfide of 1.596 mg L⁻¹, phosphate of 0.043 mg L⁻¹, potassium of 54.26 mg L⁻¹, total Cr of 17.3 mg L⁻¹, Cr(VI) below the detection limit (<0.01 mg L⁻¹), iron (Fe) of 0.398 mg L⁻¹, zinc (Zn) of 0.11 mg L⁻¹, lead (Pb) below the detection limit (<0.01 mg L⁻¹), manganese (Mn) of 0.617 mg L⁻¹, magnesium (Mg) of 56.1 mg L⁻¹, and chloride of 1,737.1 mg L⁻¹.

Near-neutral pH and mesophilic temperature under continuous aeration generally favor microbial growth and biological treatment (Henze et al., 2008; Z. Wang et al., 2023). High organic load (BOD_5 , COD, and organic matter) and elevated solids (TSS and TDS) point to abundant carbon substrates, while nitrogen, phosphate, potassium, and trace elements (Fe, Mn, Zn, and Mg) indicate a nutrient profile capable of sustaining diverse microbial metabolic activity (Dixit et al., 2015; Sodhi et al., 2021). The presence of ammonium and nitrate suggests ongoing nitrogen transformation tied to organic matter degradation and microbial nitrogen cycling (Fu et al., 2022; Lejri et al., 2022). Elevated total chromium and chloride, together with measurable sulfide, reflect the saline, metal-impacted conditions typical of tannery wastewater treatment systems (Bhardwaj et al., 2023; Faye and Sibali, 2025).

The concentration of Cr(VI) was below the detection limit (<0.01 mg L⁻¹). At the same time, total chromium remained detectable, indicating that chromium in the aeration pond was

predominantly present in forms other than dissolved Cr(VI). These physicochemical characteristics indicate that the activated sludge represented a complex, metal-impacted environment in which microorganisms were exposed to nitrogen compounds and chromium simultaneously. Activated sludge typically harbors a diverse consortium of microorganisms, with bacteria as the largest component (Xia et al., 2018). Continuous exposure likely imposed long-term selective pressure on microorganisms that can maintain growth under metal stress. This activated sludge represents a suitable ecological niche for isolating bacteria with potential applications in simultaneous nitrogen and chromium bioremediation.

Isolation and screening of bacteria

In chromium-contaminated habitats such as activated sludge, chromate-resistant bacteria play an important ecological role by maintaining microbial community stability under metal stress while contributing to Cr(VI) removal (Singhal et al., 2026). The coexistence of organic matter, inorganic nitrogen species, and chromium in tannery wastewater is expected to impose selective pressure favoring microorganisms capable of tolerating chromium while maintaining active nitrogen metabolism. We implemented four enrichment strategies (A to D) in parallel to recover bacterial populations adapted to these combined constraints. All four produced colonies that grew under combined selective pressure, indicating successful recovery of heterotrophic bacteria with potential chromium tolerance (Table 2).

All enrichment cultures grew on SM-1 and SM-2 containing 100 mg L⁻¹ Cr(VI), with visible colonies appearing within 3–5 d. Growth at 1000 mg L⁻¹ Cr(VI) occurred only on the nutrient-rich SM-4 medium (10% nutrient broth), requiring 9–12 d for visible colony formation. These

results indicate that nutrient availability strongly influenced bacterial growth under elevated Cr(VI) stress. This observation is consistent with previous studies showing that sufficient nutrient supply enhances bacterial tolerance to chromium toxicity by supporting cellular maintenance and stress responses (An, Deng, et al., 2020). Notably, only cultures derived from enrichment strategies B and C grew on SM-4 containing 1000 mg L⁻¹ Cr(VI), indicating that these enrichment strategies were more effective at recovering bacterial populations capable of growth under the higher Cr(VI) exposure tested. This observation agrees with previous findings that prolonged chromium exposure enriches chromium-resistant microorganisms in contaminated environments (Boros-Lajszner et al., 2025).

Based on distinct colony morphology, tolerance to Cr(VI)-containing media, and growth under nutrient-limited conditions, twelve representative isolates were selected and designated CSND-B01 to CSND-B12 (Figure 1). The selected isolates displayed considerable phenotypic diversity, indicating that multiple bacterial taxa survived the enrichment process. All isolates were oxidase-positive, suggesting an aerobic respiratory metabolism consistent with the aerated conditions of the activated sludge. The presence of both Gram-positive cocci and Gram-negative rods indicates phenotypic diversity among the recovered isolates. Table 3 provides detailed colony morphology, biochemical characteristics, and enrichment origin. This phenotypic variation provided a diverse set of isolates for subsequent evaluation of growth and functional performance under simultaneous nitrogen and Cr(VI) exposure.

Growth characteristics under Cr(VI) stress

All isolates grew in nutrient-rich MPCr medium containing 100 mg L⁻¹ Cr(VI), although growth performance varied among isolates

Table 2. Time required for visible colony formation on selective screening media containing different nutrient and Cr(VI) concentrations

Culture sample	Media			
	SM-1	SM-2	SM-3	SM-4
ES-A	5 d	3 d	No Growth	No Growth
ES-B	5 d	3 d	9 d	9 d
ES-C	5 d	3 d	9 d	12 d
ES-D	5 d	3 d	No Growth	No Growth

Note: ES – enrichment strategy; SM – screening media.

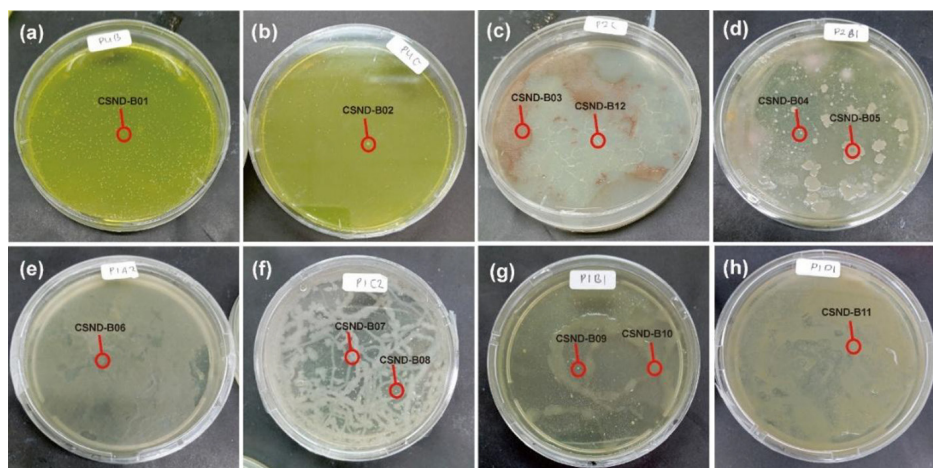


Figure 1. Twelve isolates CSND-B were selected based on growth adaptability to Cr(VI) at SM-4 (a) (b), growth morphology uniqueness (c) (d), and growth adaptability on minimal media at SM-1 (e) (f) (g) (h)

Table 3. Morphological and biochemical characteristics of bacterial isolates (CSND strains) obtained from tannery sludge after enrichment and Cr(VI) stress screening

Sources	Isolate	Color	Size	Shape	Oxidase	Form	Gram staining
ES-B:SM-4	CSND-B01	White-cream	Small	Round	Positive	Coccus	Positive
ES-C:SM-4	CSND-B02	White	Small	Round	Positive	Coccus	Positive
ES-C:SM-2	CSND-B03	Brown	Small	Round	Positive	Rod	Negative
ES-B:SM-2	CSND-B04	Yellow-white	Small	Round	Positive	Coccus	Positive
ES-B:SM-2	CSND-B05	Yellow-white	Small	Round	Positive	Coccus	Positive
ES-A:SM-1	CSND-B06	Yellow-white	Moderate	Rhizoid	Positive	Rod	Positive
ES-C:SM-1	CSND-B07	White	Small	Round	Positive	Rod	Negative
ES-C:SM-1	CSND-B08	Yellow-white	Small	Irregular	Positive	Coccus	Positive
ES-B:SM-1	CSND-B09	Yellow-white	Small	Round	Positive	Coccus	Positive
ES-B:SM-1	CSND-B10	White	Small	Round	Positive	Rod	Negative
ES-D:SM-1	CSND-B11	White	Small	Round	Positive	Rod	Negative
ES-C:SM-2	CSND-B12	White	Small	Round	Positive	Rod	Negative

Note: ES – enrichment strategies; SM – screening media.

(Figure 2). CSND-B07 exhibited the shortest lag phase (approximately 3 h), whereas most isolates required approximately 6 h before entering exponential growth and reached the stationary phase between 24 and 36 h. At the end of incubation, CSND-B04 achieved the highest biomass accumulation ($OD_{600} = 1.820$), whereas CSND-B03 exhibited the lowest biomass ($OD_{600} = 0.764$). These differences indicate variation in growth performance among isolates under 100 mg L^{-1} Cr(VI) exposure. These observations align with previous reports of chromium-resistant bacteria isolated from tannery-associated environments, where some strains remain capable of growth at Cr(VI) concentrations exceeding 100 mg L^{-1} (Ashfaq et al., 2026; Karim et al., 2020).

Qualitative observations on agar-based media further confirmed that the CSND isolates could initiate growth directly under nutrient limitation rather than only in the nutrient-rich MPCr (Figure 3). All CSND colonies developed within 24 h on nutrient-rich MPCr. In contrast, visible colony formation on minimal media containing ammonium (MM-ACr), nitrate (MM-NCr), and mixed nitrogen (MM-ANCr) required about 72 h, with growth varying among isolates. The delayed colony development indicates that nutrient limitation likely prolonged the adaptation period before active growth could occur. This observation is consistent with previous studies showing that adequate carbon and energy availability support biomass accumulation

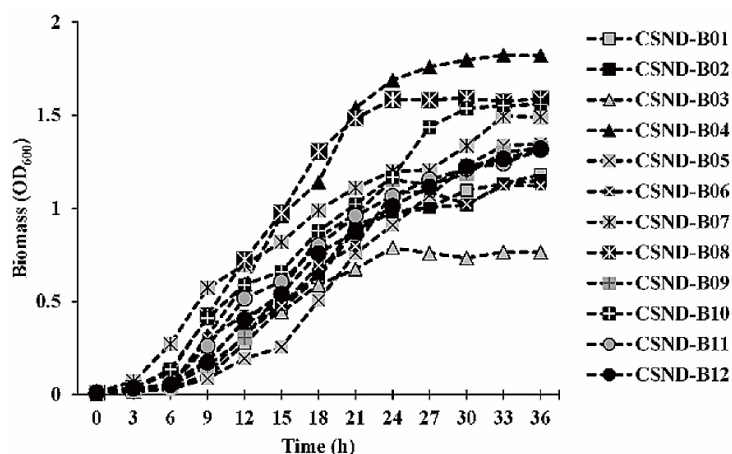


Figure 2. Growth curves (OD_{600}) of CSND isolates cultured in MPCr medium over 36 hours of incubation

and enhance bacterial tolerance under metal stress (An et al., 2020; Dubey et al., 2024). Nevertheless, variation was observed on minimal media containing ammonium, nitrate, or mixed nitrogen together with $100 \text{ mg L}^{-1} \text{ Cr(VI)}$, indicating that nutrient limitation slowed growth rather than eliminating chromium tolerance and suggesting variation in growth responses under nutrient limitation and Cr(VI) exposure through strain-dependent nitrogen utilization.

Growth characteristics at different nitrogen regimes under Cr(VI) stress

Growth in liquid minimal media showed that all isolates grew under combined nutrient limitation and Cr(VI) exposure across nitrogen regimes throughout the 72-h incubation (Figure 4–6). Biomass accumulation varied among nitrogen regimes, but every isolate showed a measurable increase in OD_{600} (Figure 4a, 5a and 6a). Among

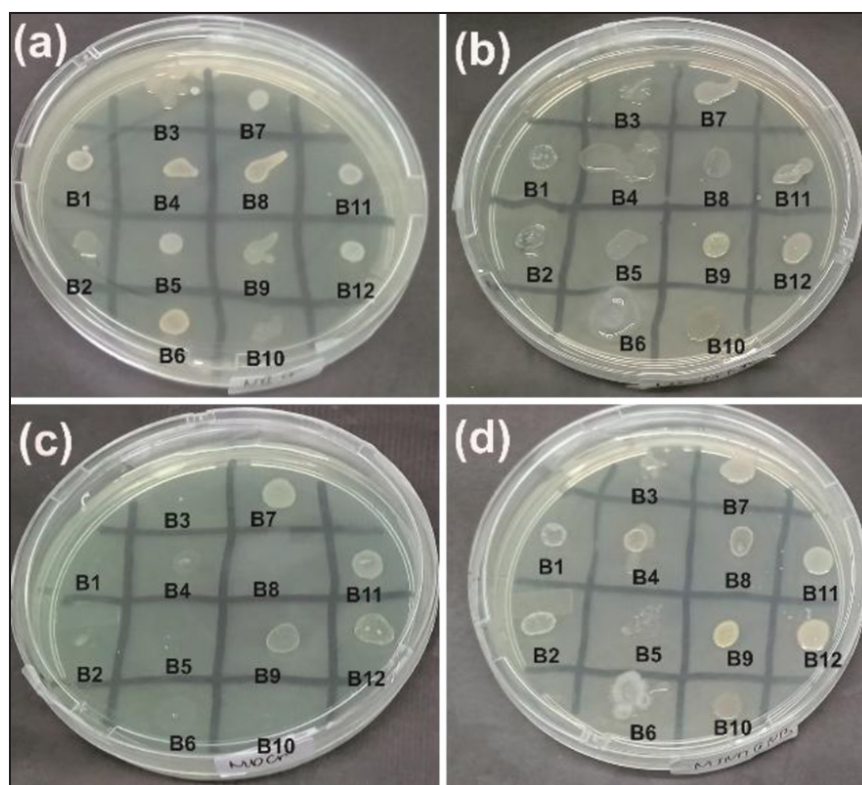


Figure 3. Qualitative growth characteristics of isolates on agar media after incubation for 24 h, reflecting their nitrogen utilization and Cr(VI) tolerance in MPCr (a) and 72 h at MM-ACr (b), MM-NCr (c), and MM-ANCr (d)

twelve isolates, CSND-B12 achieved the highest biomass in all three media tested, including MM-ACr ($OD_{600} = 0.543$), MM-NCr ($OD_{600} = 0.475$), and MM-ANCr ($OD_{600} = 0.552$). In the single-nitrogen systems, CSND-B09 ranked second, reaching $OD_{600} = 0.377$ in MM-ACr and $OD_{600} = 0.435$ in MM-NCr. In the mixed-nitrogen system MM-ANCr, CSND-B01, CSND-B02, and CSND-B05 grew to the next-highest levels below CSND-B12. The uninoculated controls showed negligible OD_{600} values throughout the experiment, confirming that microbial activity drove biomass accumulation. These differences indicate isolate-specific growth responses under nutrient limitation and Cr(VI) exposure (Ghasemian-Dastjerdi et al., 2026).

Beyond biomass accumulation, pH trajectories in MM-ACr, MM-NCr, and MM-ANCr provided further evidence of isolate-specific responses to Cr(VI) stress across the three media. Some cultures showed slight acidification, whereas others became more alkaline, suggesting differences in metabolic responses among isolates under the tested nitrogen and Cr(VI) conditions. Similar pH shifts have been associated with ammonium and nitrate utilization in other microorganisms

(Carneiro Fidélis Silva et al., 2019; Xi et al., 2022), and Cr(VI) stress can further accentuate these trajectories (Q. Wang et al., 2023). Changes in inorganic nitrogen species during incubation indicated dynamic changes in the measured dissolved nitrogen pool under aerobic conditions. Low nitrate accumulation during ammonium decrease in MM-ACr, ammonium accumulation during nitrate decrease in MM-NCr, and transient nitrite production in MM-ANCr may reflect nitrogen transformation during aerobic incubation (Niu et al., 2025; Xi et al., 2022). These results indicate that several isolates grew under Cr(VI) exposure while showing measurable changes in dissolved nitrogen species. However, these concentration changes alone do not establish the specific metabolic pathways responsible.

Functional potency of simultaneous N and Cr(VI) removal by CSND isolates

In addition to growth, all CSND isolates showed measurable decreases in dissolved nitrogen species and Cr(VI) concentration across the three minimal media over 72 h of incubation

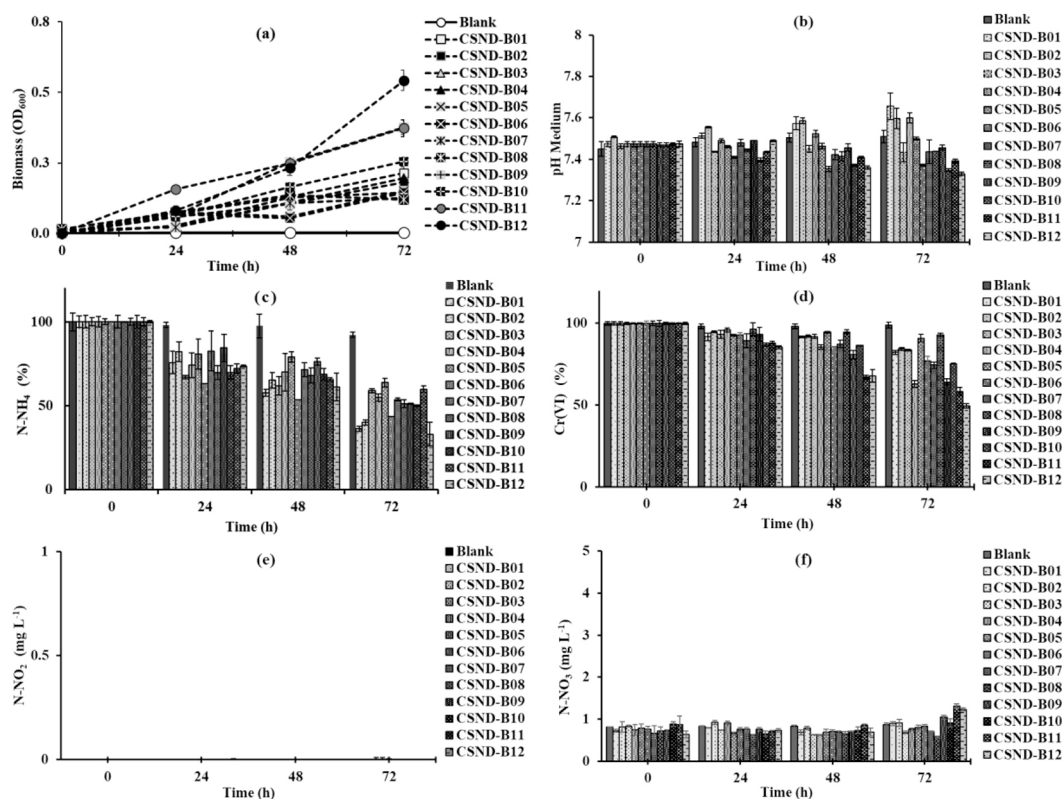


Figure 4. Growth, pH, and dissolved nitrogen and Cr(VI) profiles of the twelve CSND isolates in MM-ACr containing 100 mg L^{-1} initial ammonium and Cr(VI) over 72 h: OD_{600} (a), pH (b), ammonium (c), Cr(VI) (d), nitrite (e), and nitrate (f). Values are means $\pm$ SEM ($n = 3$)

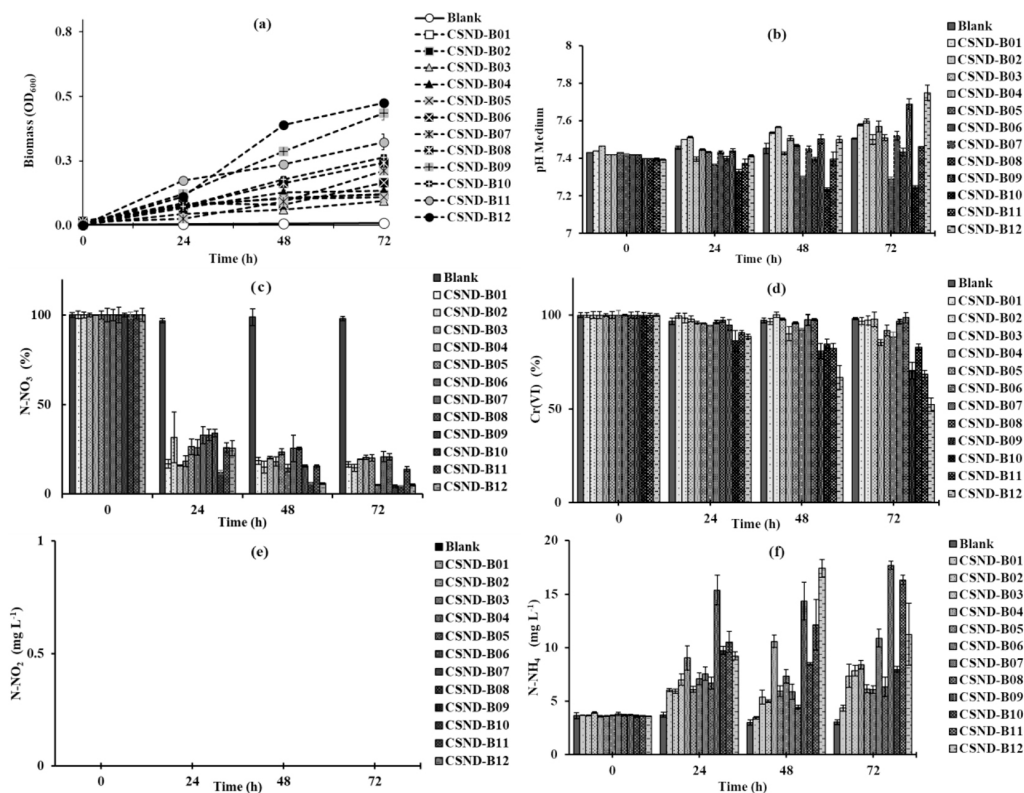


Figure 5. Growth, pH, and dissolved nitrogen and Cr(VI) profiles of the twelve CSND isolates in MM-NCr containing 100 mg L⁻¹ initial nitrate and Cr(VI) over 72 h: OD₆₀₀ (a), pH (b), nitrate (c), Cr(VI) (d), nitrite (e), and ammonium (f). Values are means ± SEM (n = 3)

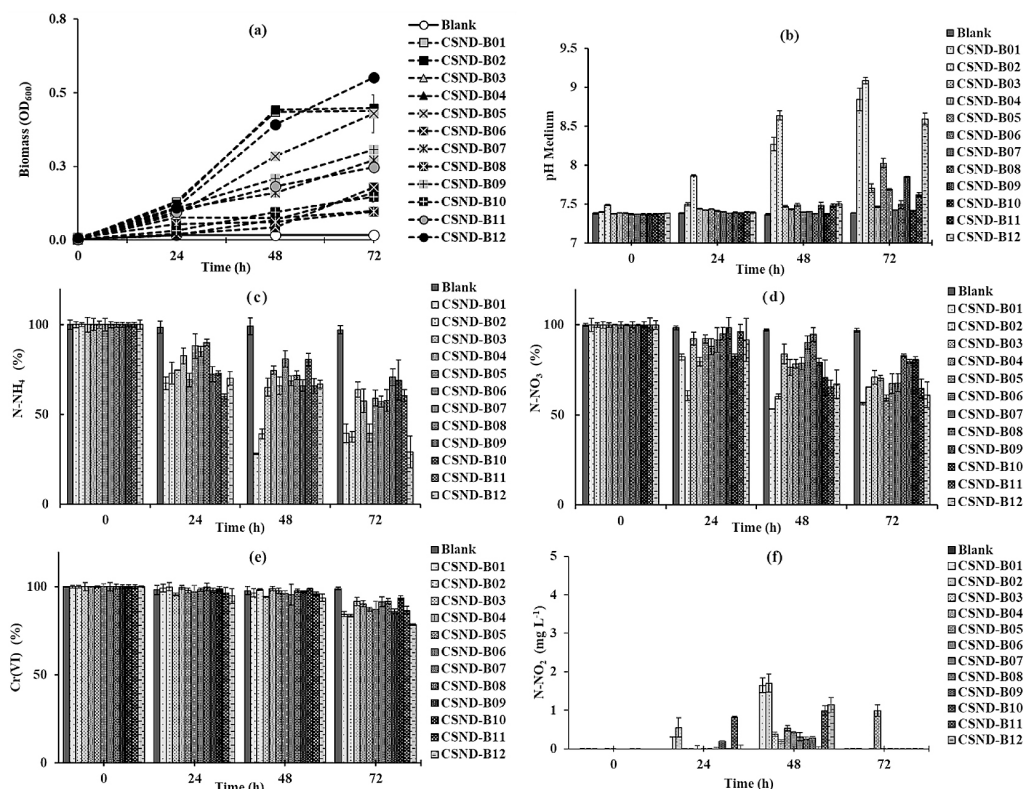


Figure 6. Growth, pH, and dissolved nitrogen and Cr(VI) profiles of the twelve CSND isolates in MM-ANCr containing 100 mg L⁻¹ initial ammonium, 100 mg L⁻¹ nitrate, and 100 mg L⁻¹ Cr(VI) over 72 h: OD₆₀₀ (a), pH (b), ammonium (c), nitrate (d), Cr(VI) (e), and nitrite (f). Values are means ± SEM (n = 3)

(Supplementary Table S2–S4). In MM-ACr, CSND-B01 and CSND-B12 achieved the highest apparent ammonium removal (63.06% and 66.80%, respectively), and CSND-B12 achieved the greatest Cr(VI) removal (50.42%), followed by CSND-B11, CSND-B04, and CSND-B09. In MM-NCr, CSND-B06, CSND-B10, and CSND-B12 showed statistically comparable apparent nitrate removal efficiencies (94.93–96.10%), while CSND-B12 again achieved the greatest Cr(VI) removal (47.76%), followed by CSND-B09 and CSND-B11. In MM-ANCr, CSND-B12 showed 54.69% apparent removal of total nitrogen from the combination of ammonium and nitrate pools, together with 21.52% Cr(VI) removal. The uninoculated abiotic controls showed only minor apparent removal of the measured contaminants. Cr(VI) removal in the Blank-B0 controls was 3.72%, 1.81%, and 1.01% in MM-ACr, MM-NCr, and MM-ANCr, respectively, while nitrogen-species removal remained low. These values were substantially lower than those observed in the inoculated cultures, indicating that abiotic processes contributed only minimally to the observed decreases under the tested conditions. Taken together, these results identify CSND-B12 as the most consistent multifunctional performer, combining high apparent removal of dissolved nitrogen species with substantial Cr(VI) removal across the three nitrogen regimes tested. Figure 7 summarizes the growth and functional performance of the twelve isolates.

The concurrent decreases in dissolved nitrogen species and Cr(VI) concentration indicate that several CSND isolates remained functionally active under combined nutrient limitation and Cr(VI) stress. The observed decreases in ammonium and nitrate may reflect a combination of assimilation and transformation processes, as supported by changes in other inorganic nitrogen species during incubation. However, because we did not quantify nitrogen mass balance, biomass-associated nitrogen, or gaseous nitrogen products, this study cannot determine the fraction of the observed decrease in dissolved nitrogen attributable to cellular assimilation, conversion to gaseous products such as N_2 , or other transformation processes. Therefore, the reported values represent apparent removal of dissolved nitrogen species rather than confirmed net nitrogen removal from the system. Similarly, the decrease in Cr(VI) concentration demonstrates removal of dissolved Cr(VI) from the liquid phase but does not establish the underlying removal mechanism. One possible explanation is that nitrogen metabolism contributed to the cellular reducing capacity associated with Cr(VI) transformation. However, this remains a mechanistic hypothesis because this study did not determine either intracellular reducing equivalents or the enzymatic or genetic basis of Cr(VI) transformation. Nitrogen metabolism may generate reducing power that contributes to Cr(VI) removal through enzymatic reduction, biosorption, intracellular sequestration, or

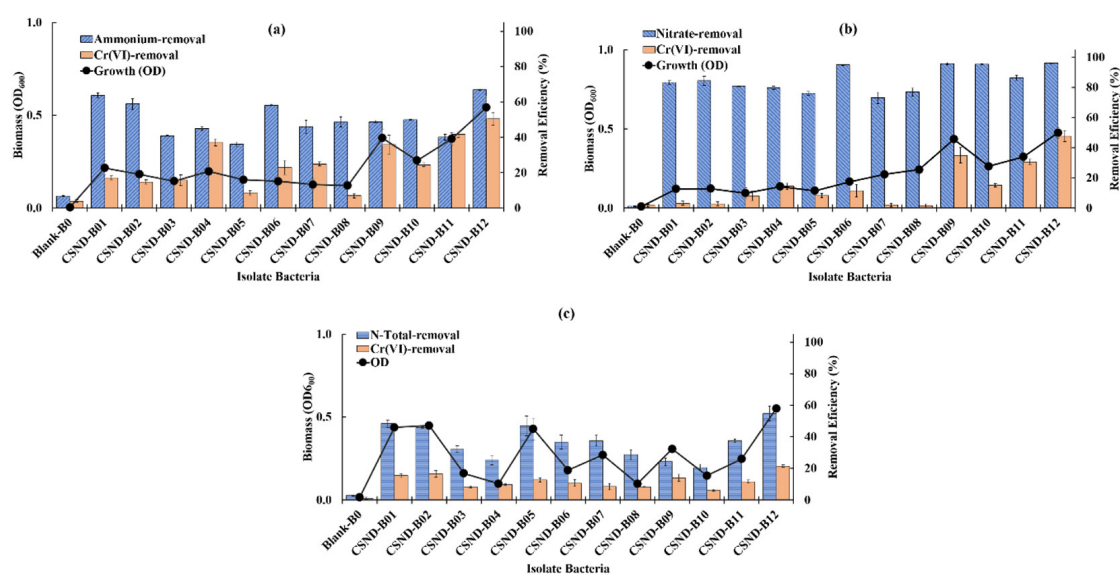


Figure 7. Comparison of growth and functional performance of CSND isolates for simultaneous removal of dissolved nitrogen species and Cr(VI) in MM-ACr (a), MM-NCr (b), and MM-ANCr (c) after 72 h. Values are means $\pm$ SEM ($n = 3$)

other physicochemical processes (Rahman and Thomas, 2021; Shi et al., 2023). The coupling of nitrogen transformation and Cr(VI) removal has been reported in heterotrophic nitrification–aerobic denitrification (HN-AD) bacteria capable of dual functionality, including *Pseudomonas aeruginosa* LX (Zhang et al., 2020), *Acinetobacter baumannii* AL-6 (An et al., 2020), and *Pseudomonas aeruginosa* G12 (An, Deng, et al., 2020).

The comparison among isolates further demonstrated that biomass production, apparent dissolved nitrogen removal, and Cr(VI) removal were not always directly coupled. For example, CSND-B06 and CSND-B10 achieved nitrate removal comparable to CSND-B12 in MM-NCr but accumulated considerably less biomass (OD_{600} 0.166–0.264) and reduced only 11–15% of Cr(VI). These observations indicate that Cr(VI) removal was not directly proportional to biomass production or the apparent removal of dissolved nitrogen species, suggesting that isolate-specific physiological characteristics may have contributed to the observed Cr(VI) removal. Similar uncoupling between bacterial growth and Cr(VI) removal has been reported previously, suggesting that Cr(VI) removal may depend on isolate-specific physiological processes beyond general cell proliferation (Niu et al., 2025; Wei et al., 2026).

Comparative evaluation of bacterial performance under mixed-nitrogen regimes in the presence of 100 mg L⁻¹ Cr(VI) remains limited. Several isolates achieved higher removal efficiencies when supplied with either ammonium or nitrate

alone, suggesting that the coexistence of multiple nitrogen forms may have altered substrate utilization and associated metabolic demands. Similar substrate-dependent responses have been reported in bacteria that simultaneously remove nitrogen and Cr(VI), where nitrogen availability influences metabolic activity and contaminant removal performance (An, Deng, et al., 2020). Across nearly all isolates, both nitrogen and Cr(VI) removal were consistently lower in MM-ANCr than in the single-nitrogen media. This trend was particularly evident in CSND-B12, whose Cr(VI) removal declined from approximately 50% in MM-ACr and MM-NCr to approximately 21% in MM-ANCr. This lower functional performance in MM-ANCr may reflect the combined effect of nitrogen-source complexity and the lower C/N ratio used in this medium. In MM-ANCr, both ammonium and nitrate were supplied simultaneously, while the C/N ratio was adjusted to 5 compared with 10 in MM-ACr and MM-NCr. The simultaneous availability of two inorganic nitrogen sources may have altered the relative utilization of nitrogen substrates and associated metabolic demands.

In contrast, the lower C/N ratio may have limited the carbon available relative to the total nitrogen supplied. These factors could have contributed to the lower apparent nitrogen and Cr(VI) removal observed in MM-ANCr. Previous studies have reported that simultaneous nitrogen and Cr(VI) removal improved under C/N ratios between approximately 10 and 15 (An, Deng, et al., 2020; An, Zhou, et al., 2020). However,

Table 4. Comparison of reported bacterial performance for simultaneous nitrogen and Cr(VI) removal under aerobic conditions

Strains	Nitrogen regime	Stressor (mg L ⁻¹)			Efficiency (%)		Incubation time	References
		N-NH ₄	N-NO ₃	Cr(VI)	N-removal	Cr(VI)-removal		
<i>Pseudomonas aeruginosa</i> LX	NH ₄	200	0	10	81.69	94.44	48 h	(Zhang et al., 2020)
<i>Acinetobacter baumannii</i> AL-6	NH ₄	100	0	10	93.9	45.3	48 h	(An, Zhou, et al., 2020)
<i>Pseudomonas aeruginosa</i> G12	NH ₄	500	0	10	77.4	92.5	72 h	(An, Deng, et al., 2020)
<i>Pseudomonas aeruginosa</i> G12	NO ₃	0	500	10	80.3	54.6	72 h	(An, Deng, et al., 2020)
<i>Pseudomonas brassicacearum</i> LZ-4	NO ₃	0	500	10	80.2	100	84 h	(Yu et al., 2016)
CSND-B12	NH ₄	100	0	100	66.80	50.42	72 h	This study
	NO ₃	0	100	100	96.10	47.76	72 h	This study
	NH ₄ + NO ₃	100	100	100	54.69	21.52	72 h	This study

because nitrogen-source composition and C/N ratio changed simultaneously in the present experimental design, we cannot distinguish their individual contributions. The potential involvement of reduced carbon availability and competition for reducing equivalents therefore remains tentative. Based on the present data, we cannot directly attribute this to a specific electron-transport or enzymatic pathway. Independent evaluation of nitrogen regime and C/N effects would be required to determine their respective contributions to nitrogen and Cr(VI) removal.

Table 4 compares CSND-B12 performance with previously reported bacteria capable of simultaneous nitrogen transformation and Cr(VI) removal under aerobic conditions. Several previously reported strains achieved higher nitrogen or Cr(VI) removal efficiencies than CSND-B12 when evaluated at approximately 10 mg L⁻¹ Cr(VI), whereas the present study evaluated CSND-B12 at 100 mg L⁻¹. The ability of CSND-B12 to maintain measurable removal of dissolved nitrogen species and Cr(VI) at 100 mg L⁻¹ Cr(VI) indicates that the isolate remained functionally active under relatively high chromium exposure across different nitrogen regimes. However, interpret direct comparisons of removal efficiencies cautiously because nitrogen composition, carbon availability, inoculum conditions, pH, incubation time, and analytical procedures differed among studies.

Identification of selected isolates by 16S rRNA

The physiological screening revealed substantial variation in growth and functional

performance among the twelve CSND isolates. In this study, CSND-B12 was selected because it showed the most consistent growth and functional performance across the three nitrogen regimes, including the highest biomass accumulation and consistently high apparent removal of the measured dissolved nitrogen species and Cr(VI). We selected CSND-B09 as a second representative isolate because it showed comparatively strong growth and Cr(VI) removal in the single-nitrogen media and exhibited distinct phenotypic characteristics from CSND-B12. Therefore, these two isolates were selected as representatives of the functionally diverse CSND population for initial taxonomic identification using 16S rRNA gene sequencing. The remaining isolates were retained as phenotypically and functionally characterized strains but were not subjected to molecular identification in the present study. The 16S rRNA gene sequences were deposited in GenBank under accession numbers PX205042.1 for CSND-B09 and PV789641.1 for CSND-B12. Based on 16S rRNA gene sequence analysis, CSND-B09 was placed within the *Brevibacterium* lineage, with its closest database match corresponding to *Brevibacterium sediminis*. At the same time, CSND-B12 showed very high sequence similarity to *Achromobacter xylosoxidans* reference sequences (Table 5 and Figure 8). Because 16S rRNA gene sequences may provide limited resolution among closely related taxa, the taxonomic assignment of CSND-B09 and CSND-B12 should be interpreted as 16S-based identification rather than definitive species-level confirmation.

CSND-B09 showed high 16S rRNA gene sequence similarity to *Brevibacterium sediminis*,

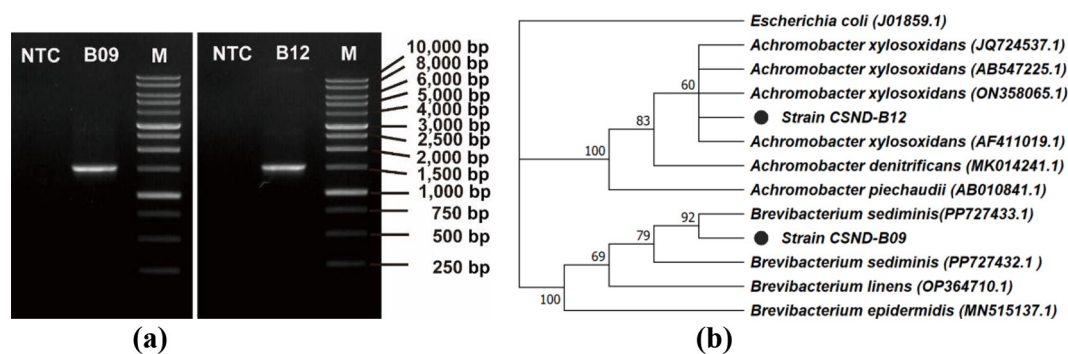


Figure 8. Agarose gel electrophoresis (0.8%) of PCR-amplified 16S rRNA gene fragments (~1500 bp) from selected CSND isolates (a) and maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences using the Tamura–Nei model with 1000 bootstrap replicates (b). Bootstrap values >50% are shown at the corresponding internal nodes

Table 5. Molecular identification of selected CSND isolates based on 16S rRNA gene sequences

Parameter	CSND-B09	CSND-B12
GenBank accession	PX205042.1	PV789641.1
16S sequence length	1.380 bp	1.389 bp
Query coverage (%)	100	100
Highest Identity (%)	99.78	99.93
Closest reference taxon	<i>Brevibacterium sediminis</i> (PP727432.1)	<i>Achromobacter xylosoxidans</i> (ON358065.1;JQ724537.1;AB547225.1;AF411019.1)
Phylogenetic placement	<i>Brevibacterium</i> clade	<i>Achromobacter xylosoxidans</i> clade
Bootstrap*	92%	60%

Note: Bootstrap value refers to the corresponding internal phylogenetic node.

suggesting that this isolate belongs to the *Brevibacterium* lineage. Its occurrence in chromium-impacted tannery activated sludge may therefore warrant further investigation. CSND-B12 showed very high 16S rRNA gene sequence similarity to *Achromobacter xylosoxidans* reference sequences, consistent with its placement within the *Achromobacter* lineage in the phylogenetic analysis. Closely related *Achromobacter* strains have previously been reported to exhibit heterotrophic nitrification, aerobic denitrification, and chromium tolerance (Istiaq et al., 2019; Liu et al., 2023). Chromate reductase activity has also been reported in *Achromobacter xylosoxidans* under aerobic conditions, providing a possible context for the Cr(VI) removal observed in the present study. However, the present study did not directly assess the enzymatic, genetic, or chemical basis of Cr(VI) removal; therefore, the underlying mechanism remains unresolved.

Integrating the molecular and physiological results identified CSND-B12 as a candidate strain for further investigation of simultaneous dissolved nitrogen and Cr(VI) removal. However, the present study did not resolve the genetic or biochemical basis of the observed activities. Further characterization using approaches such as whole-genome sequencing, functional-gene analysis, nitrogen mass balance, gaseous nitrogen measurements, and chromium speciation would therefore be required to clarify the mechanisms underlying the observed contaminant removal.

Environmental safety and future practical perspective

As an isolation and functional screening study, this work characterizes the physiological

potential of indigenous bacteria under defined laboratory conditions and does not constitute an environmental deployment or risk assessment. Although CSND-B12 demonstrated the capacity for simultaneous apparent removal of dissolved nitrogen species and Cr(VI) under the laboratory conditions tested, its suitability for direct environmental application therefore remains undetermined. This study did not evaluate the environmental safety and ecological effects of CSND-B12. Accordingly, future studies should evaluate the strain under contained, controlled conditions, with appropriate laboratory bio-safety and wastewater management practices, before considering environmental application.

From a practical perspective, further studies should first evaluate CSND-B12 in actual tannery wastewater to determine whether its observed performance can be maintained under more complex wastewater conditions. Subsequent studies could investigate immobilized-cell systems to improve biomass retention and recovery and to assess simultaneous removal performance compared with freely suspended cells. If stable performance is demonstrated under these contained conditions, continuous-flow reactor experiments could then be conducted under controlled operating conditions. Parameters such as hydraulic retention time, influent nitrogen and Cr(VI) concentrations, carbon availability, pH, dissolved oxygen, biomass retention, and long-term treatment stability should be evaluated. Appropriate handling and decontamination of chromium-containing laboratory waste should also be maintained throughout these studies. These studies would represent subsequent development stages beyond the preliminary isolation and screening conducted in the present study.

CONCLUSION

This study isolated and screened indigenous bacteria from tannery activated sludge that remained physiologically active under simultaneous nitrogen and Cr(VI) exposure in defined aerobic media containing 100 mg L⁻¹ Cr(VI). Among the twelve isolates screened, CSND-B12 consistently showed the highest biomass accumulation and overall apparent removal of the measured dissolved nitrogen species and Cr(VI) across ammonium, nitrate, and mixed-nitrogen regimes. 16S rRNA gene sequence analysis placed CSND-B12 within the *Achromobacter xylosoxidans* lineage. Overall, the findings demonstrate the capacity of selected indigenous isolates to remove dissolved nitrogen species and Cr(VI) under the tested laboratory conditions, with *Achromobacter* sp. CSND-B12 showing the most consistent overall performance among the isolates evaluated.

Acknowledgments

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REFERENCES

1. Acharya, C., Sarangi, A., Rath, S., Mohapatra, R. K., Panda, C. R., Thatoi, H. (2023). Screening and evaluation of Cr(VI) biosorption potential of metal-resistant bacteria isolated from Sukinda chromite mine soil of Odisha. *Systems Microbiology and Biomanufacturing*, 4, 203–214. <https://doi.org/10.1007/s43393-023-00170-7>
2. An, Q., Deng, S., Xu, J., Nan, H., Li, Z., Song, J.-L. (2020). Simultaneous reduction of nitrate and Cr(VI) by *Pseudomonas aeruginosa* strain G12 in wastewater. *Ecotoxicology and Environmental Safety*, 191(December 2019), 110001. <https://doi.org/10.1016/j.ecoenv.2019.110001>
3. An, Q., Zhou, Y., Zhao, B., Huang, X. L. (2020). Efficient ammonium removal through heterotrophic nitrification-aerobic denitrification by *Acinetobacter baumannii* strain AL-6 in the presence

of Cr(VI). *Journal of Bioscience and Bioengineering*, 130(6), 622–629. <https://doi.org/10.1016/j.jbiosc.2020.07.010>

4. APHA. (2017). Standard methods for the examination of water and wastewater. In E. W. Rice, R. B. Baird, A. D. Eaton (Eds.), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association (23rd Edition). American Public Health Association, American Water Works Association, Water Environment Federation.
5. Ashfaq, H., Amin, A., Cheema, M. T., Majeed, M. N. (2026). Harnessing chromium-resistant bacteria from tannery wastewater: Innovative in vitro and in silico strategies for bioremediation. *Water, Air, and Soil Pollution*, 237(11), 1–17. <https://doi.org/10.1007/s11270-026-09156-2>
6. Bhardwaj, A., Kumar, S., Singh, D. (2023). Tannery effluent treatment and its environmental impact: a review of current practices and emerging technologies. *Water Quality Research Journal*, 58(2), 128–152. <https://doi.org/10.2166/wqrj.2023.002>
7. Boros-Lajszner, E., Wyszowska, J., Baćmaga, M., Kucharski, J. (2025). Analysis of changes in the microbial biodiversity of soil contaminated with Cr(III) and Cr(VI). *Applied Sciences (Switzerland)*, 15(20). <https://doi.org/10.3390/app152010951>
8. Carneiro Fidélis Silva, L., Santiago Lima, H., Antônio de Oliveira Mendes, T., Sartoratto, A., de Paula Sousa, M., Suhett de Souza, R., Oliveira de Paula, S., Maia de Oliveira, V., Canêdo da Silva, C. (2019). Heterotrophic nitrifying/aerobic denitrifying bacteria: Ammonium removal under different physical-chemical conditions and molecular characterization. *Journal of Environmental Management*, 248(June), 1–9. <https://doi.org/10.1016/j.jenvman.2019.109294>
9. Devi, A., Verma, M., Saratale, G. D., Saratale, R. G., Ferreira, L. F. R., Mulla, S. I., Bharagava, R. N. (2023). Microalgae: A green eco-friendly agents for bioremediation of tannery wastewater with simultaneous production of value-added products. *Chemosphere*, 336(March), 139192. <https://doi.org/10.1016/j.chemosphere.2023.139192>
10. Dixit, S., Yadav, A., Dwivedi, P. D., Das, M. (2015). Toxic hazards of leather industry and technologies to combat threat: A review. *Journal of Cleaner Production*, 87, 39–49. <https://doi.org/10.1016/j.jclepro.2014.10.017>
11. Dubey, P., Farooqui, A., Patel, A., Srivastava, P. K. (2024). Microbial innovations in chromium remediation: mechanistic insights and diverse applications. *World Journal of Microbiology and Biotechnology*, 40(5), 1–20. <https://doi.org/10.1007/s11274-024-03936-w>
12. Essien, E. E., Said Abasse, K., Côté, A., Mohamed, K. S., Baig, M. M. F. A., Habib, M., Naveed, M., Yu,

- X., Xie, W., Jinfang, S., Abbas, M. (2022). Drinking-water nitrate and cancer risk: A systematic review and meta-analysis. *Archives of Environmental & Occupational Health*, 77(1), 51–67. <https://doi.org/10.1080/19338244.2020.1842313>
13. Faye, G., Sibali, L. L. (2025). A review on characterization, treatment, and impact of tannery wastewater in Ethiopia. *Environmental Quality Management*, 34(3), 1–15. <https://doi.org/10.1002/tqem.70015>
14. Fito, J., Abewaa, M., Nkambule, T. (2023). Magnetite-impregnated biochar of parthenium hysterophorus for adsorption of Cr(VI) from tannery industrial wastewater. *Applied Water Science*, 13(3), 1–23. <https://doi.org/10.1007/s13201-023-01880-y>
15. Fu, W., Song, G., Wang, Y., Wang, Q., Duan, P., Liu, C., Zhang, X., Rao, Z. (2022). Advances in research into and applications of heterotrophic nitrifying and aerobic denitrifying microorganisms. In *Frontiers in Environmental Science* (Vol. 10). <https://doi.org/10.3389/fenvs.2022.887093>
16. Ghasemian-Dastjerdi, M., Mohammadi-Sichani, M., Ahadi, A. M., Ghiasian, M., Rastegari, A. (2026). Isolation and molecular-proteomic characterization of metallothionein-producing heavy metal-resistant bacteria for industrial wastewater bioremediation. *Antonie van Leeuwenhoek*, 119(6), 1–13. <https://doi.org/10.1007/s10482-026-02279-w>
17. Harshan Gandhi, M., Krishnaveni, A., Maheswari, M., Prabu, P. C., Aneesha Rani, M. S., Dhevagi, P., Pazhanivelan, S. (2025). Understanding the ecological footprint of chromium from tannery operations. *Environmental Geochemistry and Health*, 47(8), 1–19. <https://doi.org/10.1007/s10653-025-02618-2>
18. Henze, M., van Loosdrecht, M. C. M., George, A. E., Damir, B. (2008). Biological wastewater treatment. In *Chemical Engineering* (Vol. 112, Number 10). IWA Publishing Alliance.
19. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., Williams, S. T. (1994). *Bergey's Manual of determinate bacteriology*.
20. Hu, B., Lu, J., Qin, Y., Zhou, M., Tan, Y., Wu, P., Zhao, J. (2023). Journal of water process engineering a critical review of heterotrophic nitrification and aerobic denitrification process : Influencing factors and mechanisms. *Journal of Water Process Engineering*, 54(March), 103995 Contents. <https://doi.org/10.1016/j.jwpe.2023.103995>
21. Istiaq, A., Shuvo, M. S. R., Jaminur Rahman, K. M., Siddique, M. A., Hossain, M. A., Sultana, M. (2019). Adaptation of metal and antibiotic resistant traits in novel β -Proteobacterium *Achromobacter xylosoxidans* BHW-15. *PeerJ*, 7, e6537. <https://doi.org/10.7717/peerj.6537>
22. Kalola, V., Desai, C. (2020). Biosorption of Cr(VI) by *Halomonas* sp. DK4, a halotolerant bacterium isolated from chrome electroplating sludge. *Environmental Science and Pollution Research*, 27(22), 27330–27344. <https://doi.org/10.1007/s11356-019-05942-0>
23. Karim, M. E., Sharmin, S. A., Moniruzzaman, M., Fardous, Z., Das, K. C., Banik, S., Salimullah, M. (2020). Biotransformation of chromium (VI) by *Bacillus* sp. isolated from chromate contaminated landfill site. *Chemistry and Ecology*, 36(10), 922–937. <https://doi.org/10.1080/02757540.2020.1799992>
24. Lejri, R., Younes, B. S., Ellafi, A., Bouallegue, A., Moussaoui, Y., Chaieb, M., Mekki, A. (2022). Physico-chemical, microbial and toxicity assessment of industrial effluents from the southern Tunisian tannery. *Journal of Water Process Engineering*, 47(February), 102686. <https://doi.org/10.1016/j.jwpe.2022.102686>
25. Liu, X., Zhang, Q., Yang, X., Wu, D., Li, Y., Di, H. (2023). Isolation and characteristics of two heterotrophic nitrifying and aerobic denitrifying bacteria, *Achromobacter* sp. strain HNDS-1 and *Enterobacter* sp. strain HNDS-6. *Environmental Research*, 220(January), 1–10. <https://doi.org/10.1016/j.envres.2023.115240>
26. Mpongwana, N., Rathilal, S., Tetteh, E. K. (2022). Recovery strategies for heavy metal-inhibited biological nitrogen removal from wastewater treatment plants: A review. *Microorganisms*, 10(9). <https://doi.org/10.3390/microorganisms10091834>
27. Niu, J., Song, J., Wang, Q., Cao, G., Yuan, X., Chao, C., Zhang, J., Nuramkhaan, M., Zhao, Y. (2025). Effects of Cr(VI) shock on microbial metabolic pathways: From simultaneous nitrification and denitrification to complete chromium reduction. *Journal of Environmental Chemical Engineering*, 13(6). <https://doi.org/10.1016/j.jece.2025.120458>
28. Pal, R. R., Khardenavis, A. A., Purohit, H. J. (2015). Identification and monitoring of nitrification and denitrification genes in *Klebsiella pneumoniae* EGD-HP19-C for its ability to perform heterotrophic nitrification and aerobic denitrification. *Functional and Integrative Genomics*, 15(1), 63–76. <https://doi.org/10.1007/s10142-014-0406-z>
29. Rahman, Z., Thomas, L. (2021). Chemical-assisted microbially mediated chromium (Cr) (VI) reduction under the influence of various electron donors, redox mediators, and other additives: An outlook on enhanced Cr(VI) removal. *Frontiers in Microbiology*, 11(January), 1–19. <https://doi.org/10.3389/fmicb.2020.619766>
30. Shi, Y., Wang, Z., Li, H., Yan, Z., Meng, Z., Liu, C., Chen, J., Duan, C. (2023). Resistance mechanisms and remediation potential of hexavalent chromium in *Pseudomonas* sp. strain AN-B15. *Ecotoxicology and Environmental Safety*, 250(December 2022), 1–9. <https://doi.org/10.1016/j.ecoenv.2023.114498>
31. Singh, D., Singh, D., Mishra, V., Kushwaha, J., Sengar, M., Sinha, S., Singh, S., Giri, B. S. (2024).

- Strategies for biological treatment of wastewater: A critical review. *Journal of Cleaner Production*, 454(April), 142266. <https://doi.org/10.1016/j.jclepro.2024.142266>
32. Singhal, P., Sharma, V., Kumar, R., Alkhanjaf, A. A. M., Umar, A., Baskoutas, S. (2026). Mitigating chromium (VI) toxicity through microbial and biotechnological innovations: a critical review. *International Journal of Environmental Science and Technology*, 23(5), 1–18. <https://doi.org/10.1007/s13762-026-07141-0>
33. Sodhi, V., Singh, C., Pal Singh Cheema, P., Sharma, R., Bansal, A., Kumar Jha, M. (2021). Simultaneous sludge minimization, pollutant and nitrogen removal using integrated MBBR configuration for tannery wastewater treatment. *Bioresource Technology*, 341(August), 125748. <https://doi.org/10.1016/j.biortech.2021.125748>
34. Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027.
35. Tejada-Meza, K., Arenazas-Rodríguez, A., Garcia-Chevesich, P. A., Flores-Farfan, C., Morales-Paredes, L., Romero-Mariscal, G., Ticona-Quea, J., Vanzin, G., Sharp, J. O. (2023). Acute ecotoxicity potential of untreated tannery wastewater release in Arequipa, Southern Peru. *Sustainability (Switzerland)*, 15(21). <https://doi.org/10.3390/su152115240>
36. Tripathy, S., Prakash, O. (2025). Challenges and innovations in industrial wastewater treatment: Safeguarding water resources and promoting sustainable practices. *Water, Air, & Soil Pollution*, 236(92), 1–4. <https://doi.org/10.1007/s11270-025-07742-4>
37. Wang, Q., Zhao, Y., Chen, Z., Zhang, C., Jia, X., Zhao, M., Tong, Y., Liu, Y. (2023). Nitrate bioreduction under Cr(VI) stress: Crossroads of denitrification and dissimilatory nitrate reduction to ammonium. *Environmental Science & Technology*, 57(29), 10662–10672. <https://doi.org/10.1021/acs.est.2c09624>
38. Wang, Z., Liu, H., Cui, T. (2023). Identification of a strain degrading ammonia nitrogen, optimization of ammonia nitrogen degradation conditions, and gene expression of key degrading enzyme nitrite reductase. *Fermentation*, 9(4), 1–18. <https://doi.org/10.3390/fermentation9040397>
39. Wei, Y., Zheng, N., An, Q., Chen, C., Pan, J., Wang, S., Peng, L., Qin, Y. (2026). Cr(VI) stress-driven enrichment of rhizosphere microbial functions and metabolic regulation synergistically reduce chromium bioavailability. *Journal of Hazardous Materials*, 501(December 2025), 140786. <https://doi.org/10.1016/j.jhazmat.2025.140786>
40. Xi, H., Zhou, X., Arslan, M., Luo, Z., Wei, J., Wu, Z., Gamal El-Din, M. (2022). Heterotrophic nitrification and aerobic denitrification process: Promising but a long way to go in the wastewater treatment. *Science of the Total Environment*, 805. <https://doi.org/10.1016/j.scitotenv.2021.150212>
41. Xia, Y., Wen, X., Zhang, B., Yang, Y. (2018). Diversity and assembly patterns of activated sludge microbial communities: A review. *Biotechnology Advances*, 36(4), 1038–1047. <https://doi.org/10.1016/j.biotechadv.2018.03.005>
42. Yang, Q., Yang, T., Shi, Y., Xin, Y., Zhang, L., Gu, Z., Li, Y., Ding, Z., Shi, G. (2021). The nitrogen removal characterization of a cold-adapted bacterium: *Bacillus simplex* H-b. *Bioresource Technology*, 323(November 2020), 124554. <https://doi.org/10.1016/j.biortech.2020.124554>
43. Yu, X., Jiang, Y., Huang, H., Shi, J., Wu, K., Zhang, P., Lv, J., Li, H., He, H., Liu, P., Li, X. (2016). Simultaneous aerobic denitrification and Cr(VI) reduction by *Pseudomonas brassicacearum* LZ-4 in wastewater. *Bioresource Technology*, 221, 121–129. <https://doi.org/10.1016/j.biortech.2016.09.037>
44. Zhang, X., Yan, J., Luo, X., Zhu, Y., Xia, L., Luo, L. (2020). Simultaneous ammonia and Cr (VI) removal by *Pseudomonas aeruginosa* LX in wastewater. *Biochemical Engineering Journal*, 157. <https://doi.org/10.1016/j.bej.2020.107551>
45. Zhao, J., Wu, Q., Tang, Y., Zhou, J., Guo, H. (2022). Tannery wastewater treatment: conventional and promising processes, an updated 20-year review. *Journal of Leather Science and Engineering*, 4(10). <https://doi.org/10.1186/s42825-022-00082-7>