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Original Article

Simultaneous Denitrification and Sodium Dodecyl Sulfate (SDS) Biodegradation by *Pseudomonas citronellolis* L1

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Abstract: Sodium dodecyl sulfate (SDS) and nitrate (NO_3^-) are prevalent contaminants in industrial wastewater that pose significant environmental threats to aquatic ecosystems. Strain L1 was isolated from anaerobic SDS-degrading denitrifying sludge and identified as *Pseudomonas citronellolis* based on the 16S rRNA gene sequence. This study investigated the capacity of strain L1 to biodegrade both contaminants simultaneously under three distinct oxygen conditions (anaerobic, anoxic, and aerobic environments). Strain L1 was a facultative bacterium capable of degrading NO_3^- and SDS simultaneously. At initial concentration of 100 mg/L NO_3^- and 200 mg/L SDS, NO_3^- removal efficiencies after 24 h were highest at 96.9% under anaerobic, followed by 56.6% under anoxic, and 10.8% under aerobic conditions. Additionally, SDS removal efficiencies were 76.4%, 99.8%, and 99.9% under anaerobic, anoxic, and aerobic conditions, respectively. Strain L1 also showed adaptation to SDS concentrations of 200-1000 mg/L and NO_3^- concentrations of 100-500 mg/L. After 24 h, the highest SDS degradation rate reached 29.37 mg/L/h at SDS of 1000 mg/L, while the maximum NO_3^- removal rate was 4.28 mgN/L/h at NO_3^- of 150 mg/L. These findings indicate the potential of strain L1 for biological treatment of wastewater contaminated with nitrogen compounds and anionic surfactants.

Keywords: Denitrification, nitrogen removal, sodium dodecyl sulfate (SDS), SDS removal, *Pseudomonas citronellolis.*

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1. Introduction

The accumulation of sodium dodecyl sulfate (SDS) and nitrate (NO_3^-) in wastewater has emerged as a significant environmental concern. SDS, a widely used anionic surfactant in domestic and industrial cleaning products [1-3], is frequently detected in municipal and industrial effluents. Due to its amphiphilic structure, SDS can disrupt biological membranes, posing toxicity risks to aquatic organisms, including the tissue damage in fish [4]. Moreover, SDS has been reported to inhibit algal and microbial growth by interfering with microbial cell membranes [5, 6], which may affect the performance of biological treatment processes in wastewater systems. To mitigate these impacts of SDS, biological treatment primarily relies on microbial degradation pathways to drive the complete mineralization of this surfactant [4].

In parallel, nitrate contamination remains a persistent issue in industrial and municipal effluents, contributing to eutrophication and deterioration of receiving water bodies. Biological denitrification, the sequential reduction of nitrate and nitrite to dinitrogen gas, is widely recognized as an efficient and cost-effective strategy for nitrogen removal in wastewater treatment plants (WWTPs) [7, 8]. Facultative denitrifying bacteria exhibit metabolic flexibility, shifting between aerobic respiration and denitrification in response to oxygen availability. While oxygen serves as the preferred terminal electron acceptor under aerobic conditions, nitrate is utilized as an alternative electron acceptor under oxygen-limited conditions. During this transition, the electron transport chain diverts from oxygen toward nitrate reductases [9, 10].

In many wastewaters, surfactants such as SDS coexist with nitrogen compounds, potentially affecting microbial activity and nitrogen removal efficiency. Previous studies have predominantly focused on isolating bacterial strains capable of either SDS degradation or nitrate reduction individually. Several SDS-degrading bacteria have been reported, including *Pseudomonas aeruginosa* PA01, *Pseudomonas plecoglossicida* S5 [4, 11], as well as *Staphylococcus saprophyticus* ASOI-01 and *Bacillus pumilus* ASOI-02 [12]. Similarly, various nitrate-reducing strains have been characterized and efficiently reduced nitrate at an initial concentration of 100 mg/L-N under both aerobic and anaerobic conditions, such as *P. citronellolis* WXP-4 [7] and *B. subtilis* JD-014 [13].

However, studies investigating bacterial strains that simultaneously degrade SDS and reduce nitrate, particularly across multiple environmental conditions, remain limited. Only a few heterotrophic strains, such as *P. stutzeri* SN1 and *P. nitroreducens* SN2, isolated from activated sludge, have been reported to perform both functions under anoxic conditions [14]. The development and characterization of bacteria capable of integrated SDS degradation and nitrate removal would therefore represent a promising approach for improving the efficiency of wastewater treatment systems.

In this study, a bacterial strain with dual SDS-degrading and denitrifying capabilities was isolated and characterized. Strain L1 was obtained from anaerobic sludge of a denitrification system in which SDS served as the carbon source. The capacity of strain L1 for nitrate removal and SDS degradation was evaluated under aerobic, anoxic, and anaerobic conditions. Additionally, its tolerance to different concentrations of SDS and nitrate was assessed under anoxic conditions.

2. Materials and Methods

2.1. Sample Collection

Sludge was collected from a laboratory-scale anaerobic reactor operated for 108 days, which achieved removal efficiencies of 99.8% SDS (influent SDS concentration of 200 mg/L) and 94.1% nitrate (influent nitrate concentration of 100 mg-N/L). Microbial community analysis showed that the initial sludge was dominated by the phylum *Pseudomonadota*, accounting for 40.5% of the total abundance (data not shown).

The collected sludge was enriched three times, with each enrichment lasting for 3 days. The enrichment was performed in 120-mL vials containing 100 mL of SDS-degrading denitrifying medium (SDM) under anoxic conditions (closed vials without gas purging) at 30 °C with shaking at 150 rpm.

2.2 Media and Chemicals

The SDM contained the following components (g/L): 0.7214 KNO_3, 0.2 SDS, 0.2 KH_2PO_4, 0.4 $MgSO_4.7H_2O$, and a trace element solution, 2 mL. The trace element solution [15], contained (g/L): 50 EDTA; 4.4 $ZnSO_4$; 11 $CaCl_2$; 10.2 $MnCl_2 \cdot 4H_2O$; 10 $FeSO_4.7H_2O$; 2.2 $(NH_4)_6Mo_7O_{24}.4H_2O$; 3.2 $CuSO_4.5H_2O$; 3.2 $CoCl_2$ medium was adjusted to pH 7.0 - 7.5 and sterilized at 121 °C for 20 min.

SDS chemical used in the medium was purchased from Japan, and others from China.

2.3. Screening isolate

After three enrichments in SDM under anoxic conditions, five strains were isolated. Among these, strain L1 had the highest SDS degradation and nitrate removal efficiencies of 99.8% and 55.7%, respectively, after 24 h (data not shown). Strain L1 was streaked onto SDM agar plates at 30 °C for 3 days to observe colony morphology. Representative colony was then transferred to liquid SDM and cultured at 30 °C with shaking at 150 rpm for 24 h. Colonial morphology description and Gram stain were conducted to identify isolated bacteria. Then L1 was stored in 30% glycerol solution at - 80 ºC.

2.4. DNA Isolated Sequencing of 16S rRNA and Phylogenetic Analysis

The 16S rRNA gene of the isolate was amplified by PCR using the bacterial primers 27F (5'-GAGGAACCTGCGTTCGATTAG-3') and 1492R (5'-TACACTGGGAATTCCACAACC-3'). PCR amplification was performed under the following thermal cycling conditions: an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 68 °C for 1 min 30 s, with a final extension at 68 °C for 5 min. The 16S rRNA amplicons were Sanger-sequenced using the BigDye™ Terminator v3.1 Kit [16]. Forward and reverse reads were trimmed using MEGA12 [17] and assembled into a consensus sequence in Geneious Prime (version 2025.1.3). The resulting sequence was identified by BLASTn against the NCBI GenBank database. Twelve closely related strains exhibiting denitrification and/or SDS-degradation capabilities were selected for subsequent phylogenetic analysis. A phylogenetic tree was constructed in MEGA12 using the Maximum Likelihood method based on the Tamura–Nei model, with bootstrap analysis performed using 1,000 replicates [18].

2.5. Assessment of SDS and Nitrate Removal Capability

The capacities for SDS degradation and nitrate reduction of strain L1 were further determined under anaerobic, anoxic, and aerobic conditions. The strain was inoculated into closed 120-mL vials containing 80 mL of SDM for anaerobic and anoxic conditions, and into 250-mL flasks containing 80 mL of SDM for aerobic conditions. For anaerobic cultures, argon was used to purge the vials to remove oxygen; anoxic cultures were not purged, and aerobic cultures were continuously shaken. The initial optical density at 600 nm was 0.5 for each sample. All samples were incubated at 30 °C, shaken at 150 rpm for 24 h. Each condition was performed in duplicate, and samples were collected at 0, 12, and 24 hours to measure concentrations of SDS, NH_4^+-N, NO_3^--N, and NO_2^--N. The removal efficiency (RE) for nitrate and SDS was calculated using the following equation:

$$RE\ (\%) = \frac{m_0 - m_1}{m_0} \times 100$$

Where m_0, m_1 (mg/L) represent the initial concentration and concentration at sampling time, respectively.

2.6. Effect of SDS and Nitrate Tolerance Levels

The study was designed to determine tolerance to SDS and nitrate across a range of

concentrations: NO_3^--N (100 - 500 mg/L) and SDS (200 - 1000 mg/L), with an unchanging SDS/N ratio of 2/1 under anoxic conditions. The concentration tolerance levels were illustrated in Table 1.

Table 1. SDS and nitrate concentrations for tolerance assessment of the strain

Sample	2S	3S	4S	5S	6S	8S	10S
SDS (mg/L)	200	300	400	500	600	800	1000
NO_3^--N (mg/L)	100	150	200	250	300	400	500

All tests were carried out in 120-mL vials containing 80 mL of SDM, with an initial inoculum at an optical density at 600 nm of 0.5. The culture was incubated at 30 °C and shaked at 150 rpm. Each concentration was performed in duplicate, and the samples were collected at 0 h and 24 h, and the concentrations of SDS, NO_3^--N, and OD_{600} were determined.

2.7. Analytical Method

SDS concentration was determined by the Methylene Blue Active Substances (MBAS) method as described by Hayashi et al., (1975) [19]. The concentrations of nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), and ammonium nitrogen (NH_4^+-N) were determined by the NitraVer 5 kit (Hach) [20]; N-(1-naphthyl)-ethylenediamine spectrophotometry, indophenol blue spectrophotometry [21], respectively. The pH values were measured with an AS800 pH meter (As one, Japan), and the optical density at 600 nm was determined using a UV-1800 spectrophotometer (Shimadzu, Japan).

3. Results and Discussion

3.1. Identification of Stain L1

On SDM agar, strain L1 formed circular, opaque, white colonies with wrinkled edges, approximately 1-2 mm in diameter (Figure 1A). Microscopic observation revealed that the cells were rod-shaped and Gram-negative (Figure 1B).

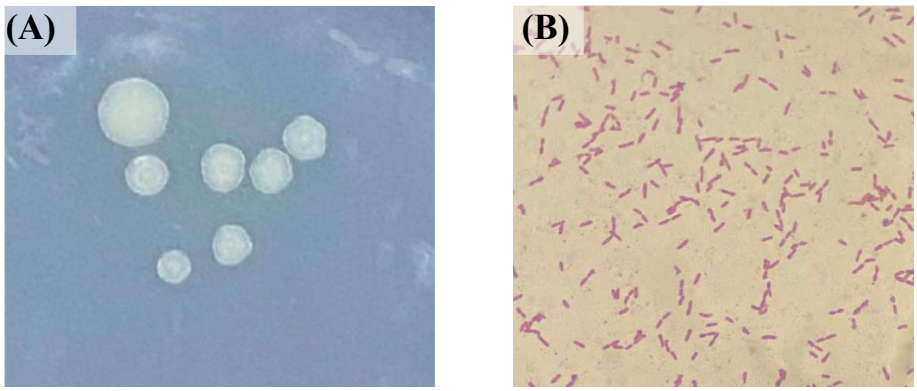

Figure 1. Morphology of strain L1: (A) colony morphology on SDM plates; (B) Gram-stained cells under light microscopy (100X).

As shown in Figure 2, the neighbor-joining phylogenetic tree clustered strain L1 within the Gram-negative *Pseudomonas* genus. Phylogenetic analysis was conducted using reference *Pseudomonas* strains reported to possess SDS-degrading and/or nitrate-reducing capabilities. Based on 16S rRNA gene sequence analysis, strain L1 showed the highest sequence

similarity to *P. citronellolis* WXP-4 (99.86%), a known denitrifying bacterium. Notably, WXP-4 has been documented to reduce nitrate under both anaerobic and aerobic conditions; however, its potential for SDS degradation has not yet been reported [7]. Several other *Pseudomonas* species have been reported to possess nitrate-reducing activity, such as *P. hunanensis* PAD-1, *P. denitrificans* G1, *P. citronellolis* YN-21, *P. mendocina* X49, and *P. hunanensis* DC-2 [8, 9, 22-24]. In contrast, *Pseudomonas* strains also demonstrated the ability to degrade SDS, like *P. jessenii* AP3-22 and *P. plecoglossicida* S5 [1, 4]. However, only two denitrifying strains, *P. stutzeri* SN1 and *P. nitroreducens* SN2, have been reported to simultaneously degrade SDS while performing denitrification [14]. The 16S rRNA gene sequence of strain L1 (1,363 bp) has been deposited in the GenBank database under accession number PV840605.

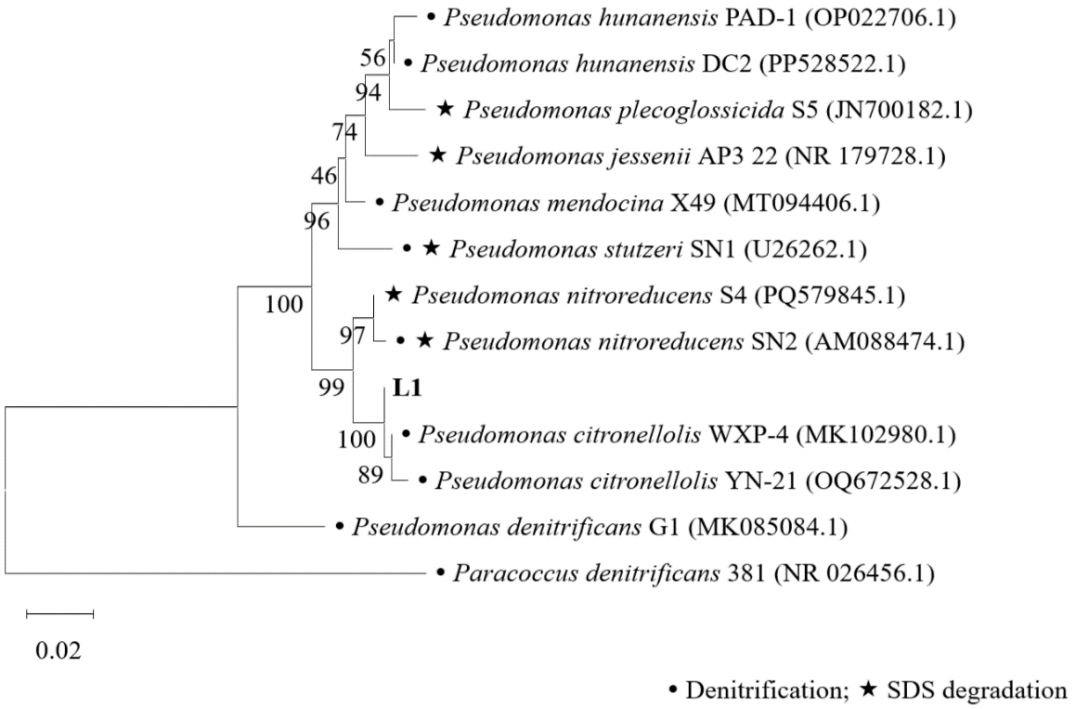

Figure 2. Phylogenetic tree constructed using the partial 16S rRNA gene sequence of strain L1 and other related strains.

3.2. Evaluation of Different Conditions for SDS and Nitrate Removal

The ability of strain L1 to simultaneously degrade SDS and reduce nitrate was investigated under anaerobic, anoxic, and aerobic conditions.

3.2.1. Determination of Denitrification Capacity

Figure 3 illustrates the nitrate removal performance of strain L1 under three distinct oxygen conditions, with an initial nitrate concentration of approximately 100 mg/L. Under anaerobic conditions (Figure 3A), nitrate removal was highest, reaching 94.7% after 12 h and 96.9% after 24 h, corresponding to NO_3^-–N concentrations of 5.42 mg/L and 3.19 mg/L, respectively. In addition, L1 exhibited incomplete denitrification with nitrite accumulation (39.48 mg/L) and nitrogen gas production accounting for 18.8% of the total gas volume at 24 h (data not shown). The nitrate removal efficiency of strain L1 was lower than that of previously reported denitrifying strains, such as *P. citronellolis* WXP-4 (100% removal

within 9 h at 100 mg/L) [7]. This may be due to differences in carbon sources. These strains were cultivated on biodegradable and non-toxic carbon sources, such as sodium acetate or sodium succinate [7, 23, 25], whereas strain L1 utilized SDS as its sole carbon source. Yin et al. (2020) reported that SDS inhibited nitrite reductase more strongly than nitrate reductase [15]. As a result, nitrate was converted to nitrite more rapidly than nitrite was further processed, leading to NO_2^- accumulation [15].

Under anoxic conditions (Figure 3B), nitrate reduction proceeded at a lower rate, with 57.7 mg/L removed after 24 h (56.6% efficiency), accompanied by a 24.56 mg/L accumulation of nitrite. The removal efficiency was intermediate, as the strain began to couple substrate oxidation with nitrate reduction under limited oxygen [10]. Paulo et al. (2013) reported that *P. stutzeri* SN1 and *P. nitroreducens* SN2 also reduced nitrate and utilized SDS as a sole carbon source [14]. However, strain L1's nitrate removal efficiency under anoxic conditions was higher than SN1 and SN2, which required 50 hours of incubation to remove 96.6% and 83.1% of nitrate, respectively, at an initial concentration of 60.2 mg-N/L [14]. Moreover, denitrification was markedly inhibited under aerobic conditions (Figure 3C), where only 10.5 mg/L nitrate was removed after 24 h (10.8% efficiency), and nitrite accumulation remained minimal (0.4 mg/L). The L1's nitrate removal was lower than that reported for *Pseudomonas* sp. G16 (92.6% removal at 120 mg/L) [25], and *P. mendocina* X49 (72.6 mg/L removed within 16 h) [23]. The low nitrate reduction in L1 can be explained by the inactivation of nitrate reductases under aerobic conditions, where electrons are prioritized for oxygen, thereby terminating denitrification [26, 27].

Trace concentrations of ammonium (NH_4^+–N) were detected under both anaerobic (0.23 mg/L) and anoxic (0.30 mg/L) conditions, suggesting the minor occurrence of dissimilatory nitrate reduction to ammonium (DNRA) [22]. The negligible accumulation of NH_4^+ suggests that DNRA contributed insignificantly to nitrogen transformation, with denitrification being the dominant pathway in strain L1. These results showed that strain L1 reduced nitrate under all three oxygen conditions. Oxygen strongly inhibited denitrification efficiency, with removal highest under anaerobic conditions, lower under anoxic conditions, and minimal under aerobic conditions.

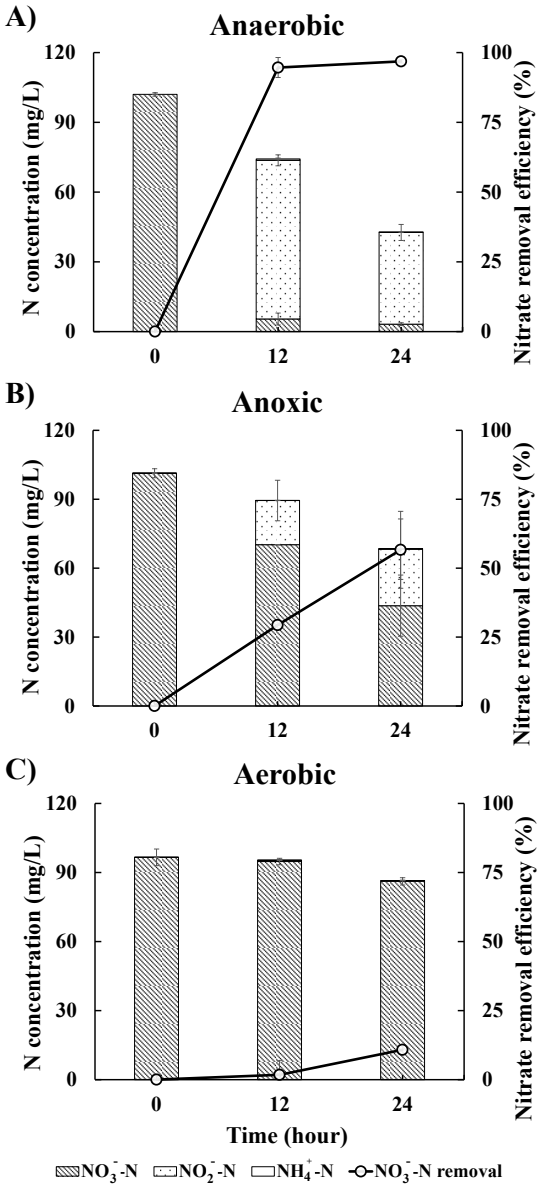

Figure 3. Nitrogen removal efficiency of strain L1 under different oxygen conditions: (A) anaerobic, (B) anoxic, (C) aerobic conditions.

3.2.2. Determination of SDS Removal Capacity

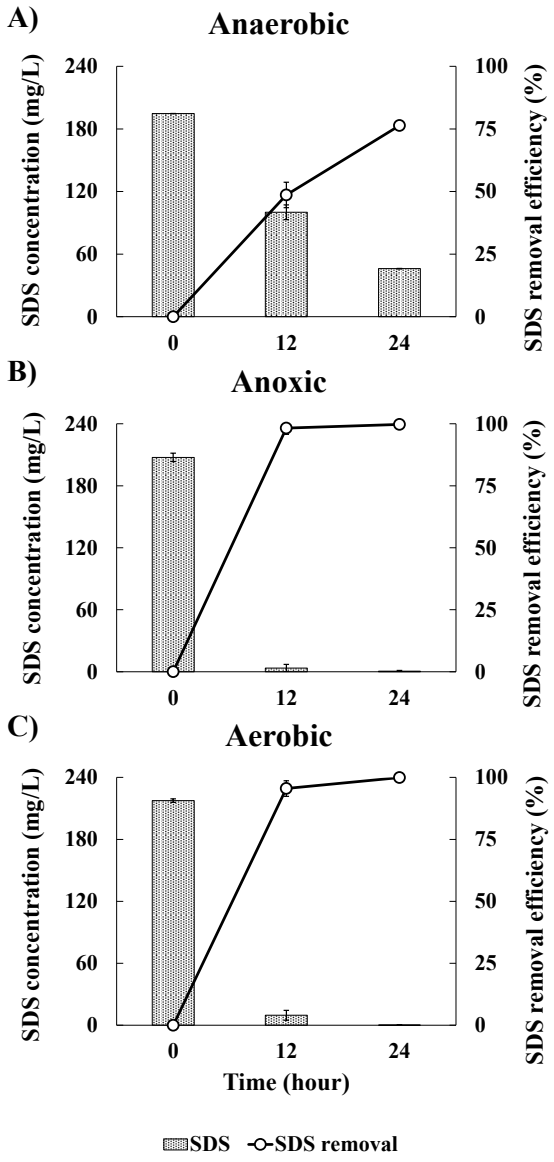

Figure 4. SDS removal efficiency of strain L1 under different oxygen conditions: (A) anaerobic, (B) anoxic, (C) aerobic conditions.

As shown in Figure 4, after 12 h, SDS was almost removed under oxygen available conditions, including 98.3% under anoxic and 95.5% under aerobic, while only 48.6% SDS was removed under anaerobic conditions. The performance under anoxic conditions may be attributed to the prior enrichment of strain L1 under anoxic conditions. After 24 h, residual SDS concentrations under anoxic and aerobic conditions decreased to 0.5 mg/L and 0.1 mg/L, corresponding to removal efficiencies of 99.8% and 99.9%, respectively. In contrast, degradation under anaerobic conditions proceeded more slowly, reaching 76.4% removal at 24 h.

The observed differences in SDS and nitrate removal can be explained by the availability of terminal electron acceptors and their role in microbial metabolism. Under aerobic and anoxic conditions, oxygen drives rapid SDS mineralization (via alkyl sulfatases and β-oxidation) by providing a high cellular energy yield [3, 9, 11, 14]. However, oxygen simultaneously outcompetes nitrate and suppresses denitrification enzymes, resulting in minimal nitrate reduction [27]. In contrast, under anaerobic conditions, nitrate becomes the only electron acceptor, leading to high nitrate removal efficiency. Nevertheless, the energy yield of anaerobic respiration is lower than that of aerobic pathways [10], which reduces overall metabolic activity, thereby slowing SDS removal. Under anoxic conditions, the strain can effectively couple SDS oxidation with nitrate reduction [14]. In this case, nitrate serves as an alternative electron sink for electrons from nicotinamide adenine dinucleotide (NADH), effectively maintaining cellular redox homeostasis and ensuring the sustained, efficient mineralization of substrates [28]. Therefore, this relationship between carbon oxidation and electron acceptor availability explains the superior simultaneous removal performance observed under anoxic conditions.

The SDS-degrading capability of strain L1 was compared with that of other strains. For example, under anoxic conditions, strains SN1 and SN2 achieved degradation efficiencies of 94.9% and 92.4% at an initial concentration of 100 mg/L SDS, respectively [14]. Moreover, under aerobic conditions, some strains can degrade SDS without coupling to denitrification, such as *P. plecoglossicida* S5 [4], which was able to be degraded 89.3% of SDS at 10 g/L

within 20 h, and *P. jessenii* AP3-22, which completely removed 1 g/L SDS within 24 h [1].

3.3. SDS and Nitrate Tolerance Levels of Strain L1

Strain L1 exhibited strong SDS degradation and nitrate removal capacities under anoxic conditions. Therefore, the effects of varying initial SDS and nitrate concentrations on removal efficiencies were further evaluated under anoxic conditions over a 24 h incubation period.

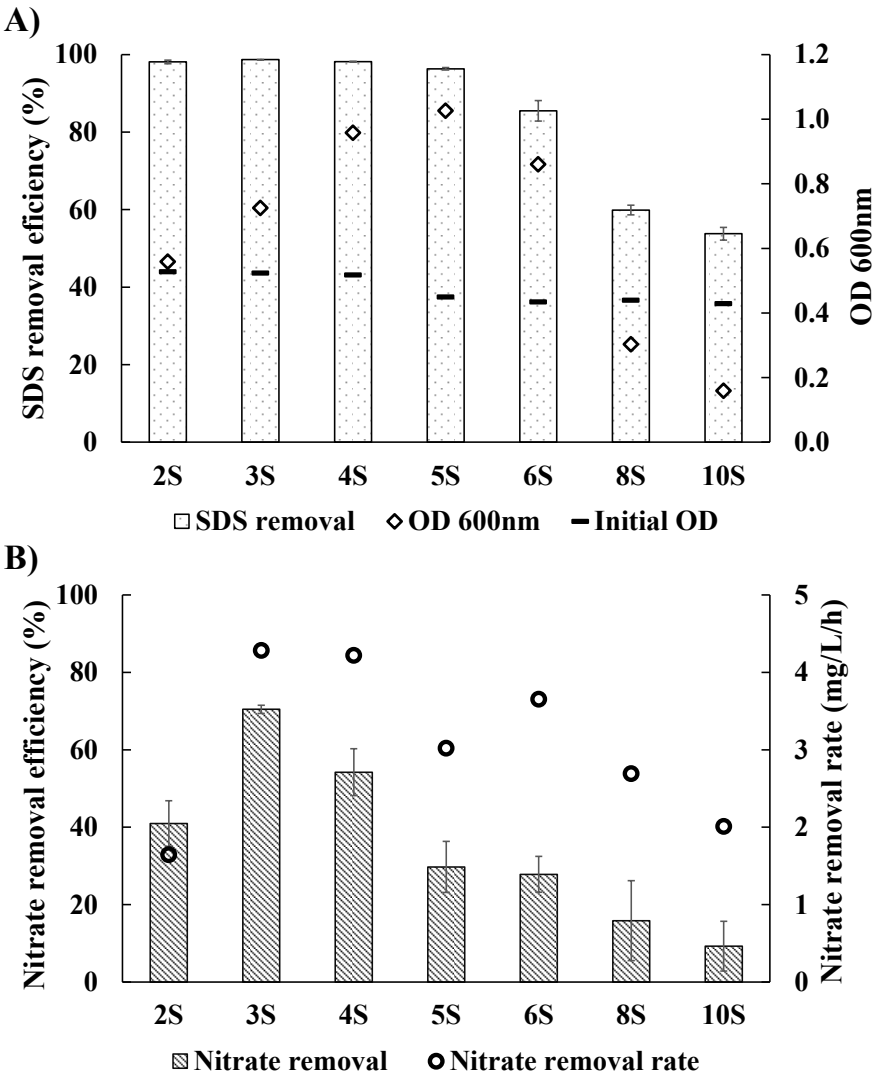

Figure 5. Effects of different stress levels on SDS and nitrate removal efficiencies and OD_{600} under anoxic conditions (24 h): (A) SDS removal and OD_{600}; (B) Nitrate removal.

At SDS concentrations ranging from 200 mg/L (2S) to 600 mg/L (6S), strain L1 achieved more than 80% SDS removal and maintained stable growth, as reflected by OD_{600} values of 0.6 (2S), 0.7 (3S), 1.0 (4S), 1.0 (5S), and 0.9 (6S) (Figure 5A). At higher concentrations (8S and 10S), initial removal efficiencies decreased to 59.9% and 53.8% for SDS, and 15.9% and 9.3%

for nitrate, respectively (Figure 5A, 5B). Biomass at these levels was lower than the initial OD_{600} at 0 h, indicating acute surfactant-induced inhibition. Nevertheless, the highest SDS degradation rate was observed at 10S (29.4 mg/L/h). Extended incubation showed that biomass stabilized without further decline at 48 and 72 h, while SDS degradation increased to approximately 80% in both 8S and 10S treatments (data not shown). Nitrate removal was most efficient at 3S, reaching 70.5% with a removal rate of 4.3 mg N/L/h, whereas lower efficiencies were recorded at other concentrations (Figure 5B). The maximum SDS and nitrate removal rates were observed in this study, markedly higher than those reported by Paulo et al. (2013), where strain SN1 exhibited removal rates of 2.8 mg SDS/L/h and 4.4 mg N/L/h, and strain SN2 showed 3.8 mg SDS/L/h and 4.4 mg N/L/h at initial concentrations of 100 mg/L SDS and 266.6 mg/L nitrate [14].

The tolerance profile of strain L1 is comparable to that of *P. nitritireducens* I2S26, which completely degrades SDS at low concentrations (50 - 400 mg/L) but exhibits reduced degradation efficiencies and extended lag phases at elevated concentrations (800 - 1000 mg/L) [3]. According to Furmanczyk et al. 2018, when bacteria are in direct contact with the surfactant SDS, which initially disrupts cell membrane integrity and triggers a global stress response, they adjust their membrane lipid composition to maintain structural stability and activate repair mechanisms [29].

4. Conclusions

In this study, a facultative bacterium, *P. citronellolis* strain L1, was isolated from an anaerobic reactor operated under SDS-denitrifying conditions. The strain demonstrated the capacity to simultaneously remove SDS and nitrate under anaerobic, anoxic, and aerobic conditions. Under anoxic conditions, strain L1 degraded SDS and reduced nitrate at elevated concentrations of 200-1000 mg/L and 100-500 mg/L, respectively. Further studies are needed to investigate the nitrate and SDS removal at higher pollutant concentrations under anoxic conditions for application L1 in wastewater treatment systems.

Acknowledgments

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