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Bioremediation of Chloride and Nitrate in Industrial Wastewater Using Native *Enterococcus faecalis* FF2021 and Two Algal Strains

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Abstract

Industrial plants and microalgae require clean water for proper growth and have been increasingly explored for their roles in wastewater treatment. Wastewater commonly contains anions such as chloride, sulfate, and nitrate, originating from aquaculture, hatcheries, and industrial processes. These anions can contaminate surface and groundwater, posing serious threats to aquatic life and environmental health. This study investigated the bioremediation of chloride and nitrate from aqueous environments using *Enterococcus faecalis* FF2021, a native bacterial strain isolated from industrial wastewater, in combination with two algal species under laboratory conditions. Identification of *Enterococcus faecalis* FF2021 was confirmed through biochemical tests, and *16s rRN*A gene sequencing. Experimental data on chloride and nitrate removal were analyzed using the ion chromatography (IC) method. To evaluate the ion-removal efficiency, factors such as culture medium, bacterial concentration, and microorganism type were assessed. Findings indicated that *Chlorella vulgaris* regularly achieved higher efficiency than *Chlorococcum* sp. in removing nitrate and chloride ions. *E. faecalis* FF2021 possesses strong ionic tolerance but limited remediation capacity alone, which can be significantly enhanced through synergistic interaction with *Chlorella vulgaris* and *Chlorococcum sp.* in a consortium system. Moreover, integrating tolerant bacteria with high-performing microalgae might form synergistic consortia capable of improving overall bioremediation efficiency in saline or nutrient-rich industrial effluents.

1. Introduction

Urban development, rapid industrialization, and unsustainable agricultural practices have significantly contributed to environmental pollution, creating serious risks for humans, animals, and plants (Ontañon *et al.*, 2015; Sandrin & Maier, 2003). Industrial facilities consume large volumes of water and chemicals, which, if not remediated, are discharged as waste (Ali *et al.*, 2009). Among the major pollutants are anion compounds, such as chloride, sulfate, and nitrate, which are common in wastewater (Li *et al.*, 2019; Mook *et al.*, 2012). These ions are released from activities such as aquaculture, hatcheries, and industrial activities, among others (Pal & Chakraborty, 2017). Once they reach surface or groundwater, their toxicity poses a danger to aquatic ecosystems and other living organisms (Bakshi *et al.*, 2021; Ghasemi *et al.*, 2023). Numerous reports have documented the direct and indirect toxic effects of anionic contaminants. Exposure to these contaminants has been linked to various health problems, including increased cancer risks and allergic reactions (Barbin *et al.*, 1997; Jaakkola & Knight, 2008; Marcusson, 1996). Chloride and nitrate are considered the most widespread anionic pollutants in industrial and aquaculture wastewater. High chloride concentrations increase salinity, disrupt the osmotic balance of aquatic species, and reduce freshwater biodiversity (Alcalde & Gawlik, 2014). Similarly, nitrate contamination contributes to eutrophication, groundwater pollution, and health risks such as methemoglobinemia (Camargo *et al.*, 2005; Ward *et al.*, 2018). Industrial effluents, aquaculture operations, and hatchery are recognized as main contributors of these contaminants, often releasing both ions simultaneously (Sonone *et al.*, 2020).

Conventional physicochemical methods for chloride and nitrate removal, such as ion exchange and reverse osmosis, are often energy-intensive and costly (Alkhadra *et al.*, 2022; Singh & Saxena, 2022). Other techniques, such as membrane filtration, ion exchange, and precipitation, can also be applied; however, they generate secondary waste that must be managed (Peng & Guo, 2020). As a result, biological approaches such as bioremediation have gained attention as effective and sustainable alternatives (Huët & Puchooa, 2017). Bioremediation refers to the use of microorganisms to

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transform or degrade harmful pollutants into less toxic forms (Fulekar, 2009; Ghasemi *et al.*, 2022; Singh, 2014). Many microorganisms are capable of remediating various contaminants, although their effectiveness depends on environmental compatibility and metabolic activity (Popli & Patel, 2015; Zarei *et al.*, 2022). Some species can convert contaminants into relatively harmless end products such as carbon dioxide, methane, water, or microbial biomass (Singh, 2014).

Given the frequent co-occurrence of chloride and nitrate in reclaimed industrial water, it is necessary to evaluate the bioremediation potential of both bacteria and algae. Such studies not only clarify their pollutant removal efficiency but also address possible risks related to microbial persistence and growth under high ion concentrations. Biological remediation approaches have therefore received growing attention as eco-friendly and cost-effective alternatives. Certain bacterial genera, including *Enterococcus* and *Pseudomonas*, have demonstrated tolerance and ion-removal capacity under saline or nitrate-rich conditions (Bhatnagar & Sillanpää, 2017). Among microalgae, Chlorella vulgaris has been repeatedly reported as particularly effective, especially for nitrate removal and tolerance to saline wastewater. Microalgae, including *Chlorella vulgaris* and *Chlorococcum sp.*, can assimilate nitrogen compounds and contribute to chloride stress mitigation through ion regulation and biomass accumulation (Abinandan *et al.*, 2018; Sial *et al.*, 2021). Microalgae have emerged as promising biological agents for wastewater bioremediation due to their ability to assimilate nutrients, tolerate high ionic stress, and produce valuable biomass. Species such as *Chlorella vulgaris, Scenedesmus obliquus*, and *Chlorococcum sp.* efficiently remove nitrogenous compounds (nitrate, ammonium) and phosphates through uptake for cellular metabolism and protein synthesis (Abinandan *et al.*, 2018; Sial *et al.*, 2021). In addition, some microalgae maintain ionic balance and mitigate chloride toxicity by accumulating compatible solutes and excreting extracellular polymers that enhance pollutant adsorption (Gupta *et al.*, 2019).

Integrating microalgae into wastewater treatment systems is increasingly recognized as a sustainable strategy for the removal of both organic and inorganic pollutants. Compared with conventional methods, algal-based systems have two major advantages: (i) reduction of nutrient and ion loads, and (ii) generation of biomass suitable for biofuels, feed, or biofertilizers (Rawat *et al.*, 2013). Moreover, the synergistic interaction between microalgae and bacteria further enhances removal efficiency. Combined algal–bacterial systems are particularly effective, as algae provide oxygen that supports bacterial metabolism, while bacteria supply CO_2 and micronutrients that stimulate algal growth (Subashchandrabose *et al.*, 2011).

The use of native microbial communities also plays a critical role in wastewater bioremediation. Local bacteria are naturally adapted to the physicochemical characteristics of effluents, making them both cost-effective and efficient for chloride and nitrate removal (Ontañon *et al.*, 2015). These native bacteria can convert pollutants into simpler, water-soluble end products such as organic acids, gases, or biomass (Sharma, 2012; Shukla *et al.*, 2010; Zarei *et al.*, 2023).

Considering the specific conditions of the effluent from the Sooreh industrial plant (Iran), the present study focused on elevating the physicochemical properties of sewage and the bioremediation processes for chloride and nitrate. The self-purification ability of the wastewater was assessed by analyzing the potential of isolated native bacteria under laboratory conditions. To our knowledge, this is the first report examining the viability of *Enterococcus faecalis* and its contribution to chloride and nitrate removal in industrial wastewater.

2. Materials and Methods

2.1. Site description and sample collection

Samples were collected from the drum filter of the Sooreh industrial plant (Iran). The samples were transported to the laboratory under sterile conditions for further processing. The main physicochemical properties of the effluent were measured as follows: pH~7.97, COD 793.6 ppm, TDS 74.1 ppm, dissolved chloride 6115 mg/L, nitrate 46700 mg/L (Association, 1926).

2.2. Isolation and Characterization Processes

Approximately 40 mg/L of the effluent (containing 6115 mg/L chloride and 46700 mg/L nitrate) was added to 160 mL of Mueller medium. A 1 µL aliquot of this mixture was plated on Mueller Hinton agar and incubated for 48 h at 30 °C on a shaker incubator at 180 rpm until microbial colonies developed. Distinct colonies were selected based on morphology after 48 h at 30 °C incubation. The selected colonies were cultured separately using the lawn culture method on Mueller Hinton agar plates. An isolated strain was obtained by repeating the above plating method, and its phenotypic and genotypic characteristics were investigated (Sanjay *et al.*, 2020).

2.2.1. Phenotypic identification procedures

Colony and cellular characteristics were examined along with standard biochemical tests, including Gram staining, motility, oxidase, catalase, urease, hippurate hydrolysis, hyaluronidase, lecithinase, lipase, and gelatin hydrolysis (Vandamme *et al.*, 1994).

2.2.2. Genotypic identification procedures

To sequence the bacterial partial genome, a colony of the bacteria was dissolved in sterile distilled water using a sterile microtube. The sample was incubated at 90°C for 30 min to extract the genome (Adeniji *et al.*, 2019). Genomic DNA was extracted using a DNA extraction kit (Sinaclon, Iran). Then, *16s rRNA* was amplified using universal primers (27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′)) (Edwards *et al.*, 1989). PCR amplifications were carried out using the HotStar HiFidelity PCR kit (Qiagen, Germany). The PCR program consisted of one cycle of DNA denaturation for 5 min at 95°C, and then 35 cycles were performed using the following parameters: 1 min at 94°C to denature, 1 min at 51.3°C to anneal, and 1 min at 72°C to extend, followed by a final extension of 15 min at 72°C. Then, the genome was quality-assessed using 1.5% agarose gel electrophoresis, and bands were observed by illuminating Safe stain-stained gels on a UV transilluminator. After that, *16s rRNA* fragments were extracted from the agarose gel using a gel purification kit (Bioneer, Korea), and PCR products were sequenced (Jahani *et al.*, 2015). Finally, sequence alignment was performed on the NCBI website (https://www.ncbi.nlm.nih.gov/BLAST/), and phylogenetic trees were constructed with MEGA 5 software using the neighbor-joining method.

2.3. Bioremediation Experiments

2.3.1. Bacterial and algal strains

Bioremediation of polluted sites needs inoculation with algae and bacterial strains that have exhibited the capacity for pollution biodegradation in treatability tests (Paisio *et al.*, 2014). The bacterial sample was isolated from the wastewater, and the pure stocks of the algae *Chlorella vulgaris* and *Chlorococcum* sp. were provided by the microbial collection of Dr. Porfakhrai (Microbial Collection, Beheshti University, Iran).

2.3.2. Culture media

The growth medium utilized for algae samples was N8 medium (Zhu *et al.*, 2020). The bacterial isolate was grown in Mueller medium (Merck, Germany) and in a modified mineral salt medium (Table 1). All operations were performed under sterile conditions, and all chemicals were of analytical grade. The algal growth media were prepared according to the manufacturer's instructions. Growth media were sterilized by autoclaving, and the final composition of the algal medium is shown in Table 2.

2.3.3. Inoculum preparation

Bacterial inoculum was prepared in the medium by growing the bacterial strain and incubating it at 34°C for 24-48 h in an orbital shaker at 150 rpm (Awais *et al.*, 2007) using buffer phosphate (Optical density/ 0.5 McFarland standard) and a pH of 8 (Christofilogiannis, 2001). A microalgae inoculum was prepared by adding 20% (v/v) of actively growing cultures to the growth medium in the culture medium, followed by incubation under fluorescent light (Metsoviti *et al.*, 2019).

2.3.4. Chloride and Nitrate Tolerance Capacity

Isolated Bacteria

Real wastewater treatment

Experiments were conducted in shaking flasks to assess chloride removal from real wastewater. Removal studies were also performed on two groups (Table 3).

The control and treatment groups were as follows:

- Treatment 1: IB 30mL + WW 170 mL
- Treatment 2: IB 60mL + WW 140 mL
- Control 1: Buffer 30mL + WW 170 mL
- Control 2: Buffer 60mL + WW 140 mL

(IB = Isolated Bacteria, WW = sterile Waste Water)

The samples were analyzed for chloride and nitrate removal using the ion chromatographic method (Buldini *et al.*, 2002).

Table 1. Components of the Modified Mineral Salt Base Medium

Modified Mineral Salt Base Medium	100 ml Stok (100X)
$NaNO_3$	5 g
NH_4Cl	10 g
$MgSO_4.7H_2O$	2 g
$CaCl_2.2H_2O$	1.8 g
$Na_2HPO_4.2H_2O$	21.4 g
KH_2PO_4	10.9 g
Na_2Mg EDTA (triplex III)	0.01 g
Ferric ammonium citrate	0.3 g
Citric Acid	0.33g
Trace Salts Solution:	1 mL

Table 2. Optimization Experiments Groups

Synthetic wastewater	Composition	Amounts of	
		Chloride	Nitrate
Artificial Medium (AM)	AM1: Mueller medium+ Distilled water AM2:Modified Mineral salt base medium+ Distilled water	6115 mg/L	46700 mg/L

Table 3. Amounts of Chloride, Nitrate, and Synthetic Wastewater Composition

Synthetic wastewater	Composition	Amounts of	
		Chloride	Nitrate
Artificial Medium (AM)	AM1: Mueller medium+ Distilled water AM2:Modified Mineral salt base medium+ Distilled water	6115 mg/L	46700 mg/L

Synthetic wastewater and Artificial culture medium treatment

For the startup, the reactor was fed synthetic wastewater (Table 4) composed of Mueller and modified mineral salt base medium containing NH_4Cl (chloride source), KNO_3 (nitrate source), and trace elements.

The concentration of the ions of interest was 6115 (mg/L) for chloride and 46700 (mg/L) for nitrate. The ability to remove nitrate and chloride was tested during 24 and 72 hours of exposure in an isolated strain. Chloride removal was emphasized because preliminary trials showed higher variability in removal efficiency and stronger microbial tolerance effects compared to nitrate. This justified a deeper experimental focus on chloride while still reporting nitrate data in parallel. Samples of 34mL were taken from the sterile wastewater before adding the isolate bacteria (0 h); then, 6 mL of isolate bacteria was added. The samples were incubated at 30 °C on a shaker incubator at 160rpm. The control and treatment groups were as follows:

- Treatment 1: IB 20mL + AM1180 mL
- Treatment 2: IB 20mL + AM2180 mL
- Treatment 3: IB 20mL + EWW1180 mL
- Treatment 4: IB 20mL + EWW2180 mL
- Control 1: Buffer 20mL + AM1180 mL
- Control 2: Buffer 20mL + AM2180 mL
- Control 3: Buffer 20mL + EWW1180 mL
- Control 4: Buffer 20mL + EWW2180 mL

(IB = Isolated Bacteria, AM = Artificial Medium, EWW = Enriched Waste Water)

Three replications were performed for each sample and centrifuged at 7000 rpm for 15 min to obtain a separation between the supernatant at the top and the remnant at the bottom. 1 mL of the supernatant was removed and transferred to a sterile container. Ions were analyzed by ion chromatography (Fritz, 1987).

Algae

Algal bioremediation experiments were conducted at 25 ºC, pH=8, with a final volume of 200 mL, under sterile conditions, on a shaker at 180 rpm. The control and treatment groups were as follows:

- Treatment: CH.v / CH.l 20ml + WW 180 ml
- Control: Buffer 20mL + WW 180 mL

(CH.v = *Chlorella vulgaris*, CH.l = *Chlorococcum* sp., AM = Artificial Medium, WW = Waste Water). The cultured medium was then centrifuged at 7000 rpm for 10 min, and 1 mL of the supernatant was transferred to a sterile microtube for analysis.

2.4. Statistical Analysis

Data were analyzed using SPSS 20 software. One-way ANOVA followed by a Tukey's post-hoc test was performed to assess significant differences ($p < 0.05$). The Excel 2010 software was used to do the graphical analysis. All experiments were carried out in triplicate.

3. Results and Discussion

3.1. Isolation and Characterization Processes

After 96 h of incubation at 30 °C, single colonies developed on the Mueller–Hinton agar. Distinct colonies were selected for further phenotypic and genotypic characterization.

3.1.1. Phenotypic identification

The isolated bacteria were subjected to different morphological and biochemical characterization. Isolated strain colonies on Mueller Hinton solid medium were circular, convex, smooth, and white to creamy in color. Microscopic observations of the isolated bacteria revealed that they are cocci-shaped and non-motile. Gram staining confirmed the isolate as Gram-positive.

3.1.2. Genotypic identification procedures

The isolated strain was further identified by the partial nucleotide sequence of 16s rRNA. Amplification conditions were optimized using genomic DNA from pure cultures of FJ1-3 as templates. The determined partial nucleotide sequences of 16s rRNA were used to find the most likeness with the bacterial strains in the GenBank database. A comparative phylogenetic analysis of the 16s rRNA genes of the isolated bacteria was performed with some strains of Enterococcus sp., and their phylogenetic tree is shown in Figure 1.

The figure shows that the isolated strain is most closely related to Enterococcus faecalis with 99.8% similarity. Nucleotide sequences of gene fragments of Enterococcus faecalis isolated were recorded in the NCBI database (Enterococcus faecalis strain: *Enterococcus faecalis* FF2021; Accession numbers OL905957.1). Enterococcus species are Gram-positive bacteria (Elahi *et al.*, 2021) and core constituents of the intestinal flora of many animal species (Pereira *et al.*, 2023).

While some studies have shown Enterococcus concentrations were not associated with waterborne illnesses (Arnone & Perdek Walling, 2007), other studies have shown that Enterococcus concentrations correlate with the occurrence of waterborne illness (Harwood *et al.*, 2004; Shailemo *et al.*, 2016; Zeki *et al.*, 2021), making identifying and controlling its extent in aquatic environments important.

3.2. Bioremediation process

3.2.1. Isolated Bacteria

Real wastewater treatment

Rapid population growth increases water consumption and wastewater generation (Rodríguez-Chueca *et al.*, 2013). Disinfection-resistant bacteria in municipal wastewater may pose risks to public health and the environment (Nagulapally *et al.*, 2009). Therefore, it is important to estimate the tolerance of disinfectants by pathogens (Owoseni & Okoh, 2017).

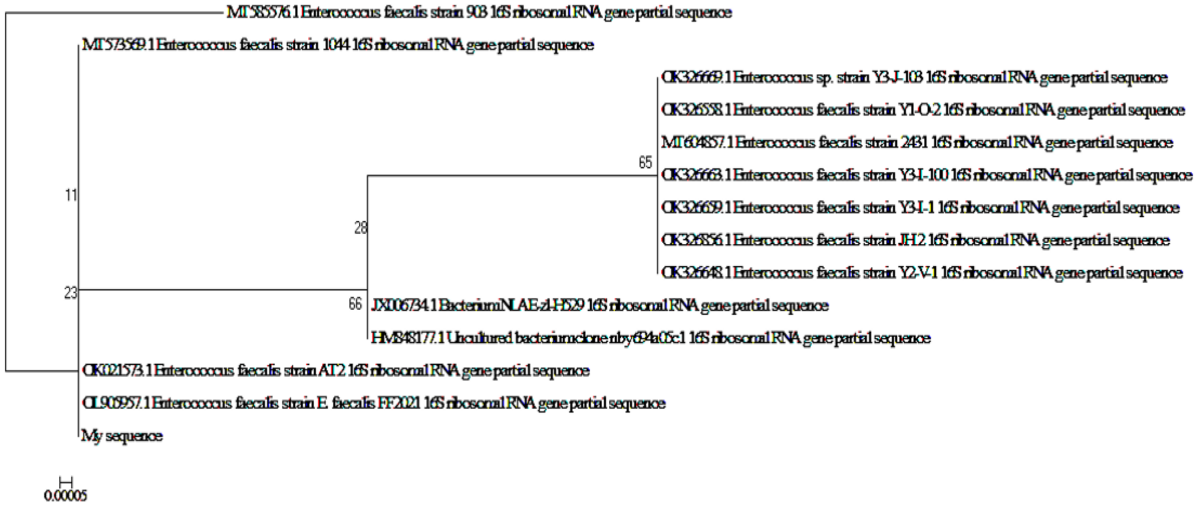

Figure1. Phylogenetic Tree of Enterococcus faecalis Strain Based on 16s rRNA

Enterococci are present in the gastrointestinal tract (Blanch *et al.*, 2003) and are important nosocomial pathogens (Doud *et al.*, 2014). These bacteria are released into the environment via wastewater (Blanch *et al.*, 2003; Manero *et al.*, 2002; Nagulapally *et al.*, 2009).

Bioremediation of anionic pollutants such as chloride and nitrate involves both tolerance mechanisms and active removal pathways. Tolerance refers to the microbial strategies that allow survival under high ion concentrations without necessarily reducing pollutant levels. *Enterococcus faecalis* regulates osmotic balance by accumulating compatible solutes (e.g., proline, glycine betaine) and by adjusting membrane transporters to prevent ionic toxicity (Empadinhas & da Costa, 2008). Similarly, microalgae respond to chloride stress by activating antioxidant defense systems, synthesizing osmolytes, and adjusting ion transport channels to maintain cellular homeostasis (Gupta *et al.*, 2019).

Active removal involves direct uptake or transformation of ions. Nitrate can be assimilated into amino acids and proteins by both bacteria and algae through nitrate reductase pathways (Kaviraj *et al.*, 2024), while some bacteria can also denitrify under oxygen-limiting conditions, reducing nitrate to gaseous nitrogen (NO, N_2O, N_2). Chloride removal is less metabolically versatile but may occur through biosorption onto cell walls, precipitation, and entrapment in extracellular polymeric substances (EPS), particularly in algal-bacterial consortia (Qi *et al.*, 2024).

The chloride remediation ability of *Enterococcus faecalis* was evaluated in real wastewater and is shown in Figure 2. After 72 h of incubation, a significant decrease ($p<0.05$) in the amount of chlorine ion was observed across all groups (control and treatment), which suggests that other factors played a role in the bioremediation process. The decline in the chloride remediation amount by bacterial biomass was negligible for all samples; in other words, *Enterococcus faecalis* biomass has not shown the ability to remediate chloride ($p>0.05$) and only exhibits tolerance. On the other hand, given the significant difference between the treatments, it can be concluded that the 30% bacterial inoculum treatment's activity in the wastewater led to greater ion reduction compared to the 15% treatment ($p<0.05$).

Nitrate is one of the adverse water quality parameters (Abdelsalam *et al.*, 2021). These compounds, accompanied by elevated bacterial counts in the water, render aquatic organisms susceptible to bacterial diseases; thus, it is important to remove them from the water treatment system (Abdelsalam *et al.*, 2021; Mahmoud *et al.*, 2016). However, the apparent ion reduction observed in laboratory conditions may also be influenced by confounding factors such as changes in medium pH, volatilization, adsorption onto experimental surfaces, or competition between microbial populations (Camargo *et al.*, 2005; Gupta *et al.*, 2019). Therefore, it is essential to distinguish between genuine bioremediation effects and abiotic or indirect processes when interpreting ion concentration data.

The results (Figure 3) show there is a significant reduction in the number of ions in all groups ($p<0.05$). However, since there is no significant difference between the treatment and control groups ($p>0.05$), it can be concluded that the bacteria biomass is not related, and other factors involved in the experiment have caused the reduction of the effective ions. Considering the significant difference between the 15% and 30% treatment groups, it can be stated that the increase in bacterial concentration caused a significant increase in nitrate modulation ($p<0.05$). In other words, *Enterococcus faecalis* biomass has not shown the ability to remidiate nitrate ($p>0.05$) but only exhibits a tolerance to high nitrate concentrations.

Some studies have reported that the durability of *Enterococcus* species in chlorine-treated effluents is indicative of chlorine tolerance, and the presence of chlorine-tolerant bacteria reduces water modality, posing a risk to public health and the environment (Owoseni *et al.*, 2017). Comparative studies indicate that *Chlorella vulgaris* consistently exhibits higher nitrate uptake rates compared to *Chlorococcum* sp., likely due to its efficient nitrate reductase system and faster growth kinetics (Perumalsamy, 2024). Meanwhile, bacterial isolates, such as *Enterococcus faecalis*, show strong tolerance to high chloride and nitrate loads but contribute less to active ion removal compared to microalgae. These differences highlight the importance of integrating both microorganisms for optimal bioremediation performance in complex wastewater matrices.

Synthetic wastewater and Artificial culture medium treatment

The experimental data obtained for chloride removal using the IC method were used to identify the bioremediation process. The results of chlorine bioremediation for the artificial culture medium and enriched wastewater are shown in Figure 4.

According to the significant difference in the treatment groups ($p<0.05$), it can be concluded that the highest effect in reducing chlorine ions by bacteria ($p<0.05$) was the Mueller medium among the artificial culture mediums, and the Modified Mineral salt base with wastewater among the enriched wastewater group.

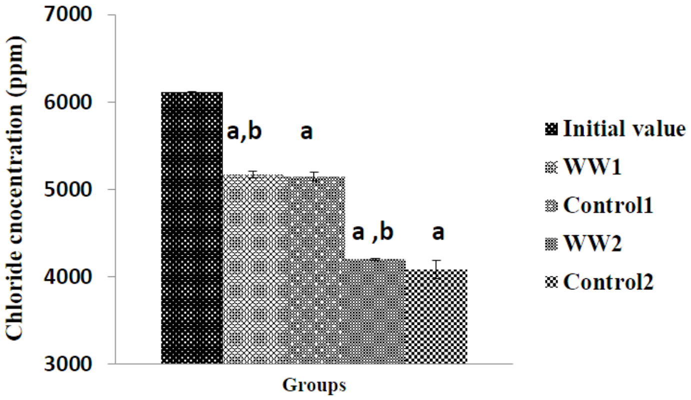

Figure 2. Bioremediation of Chloride in in Real Wastewater (**Note**. a: Significant difference with the initial value, b: Significant difference with the control group.)

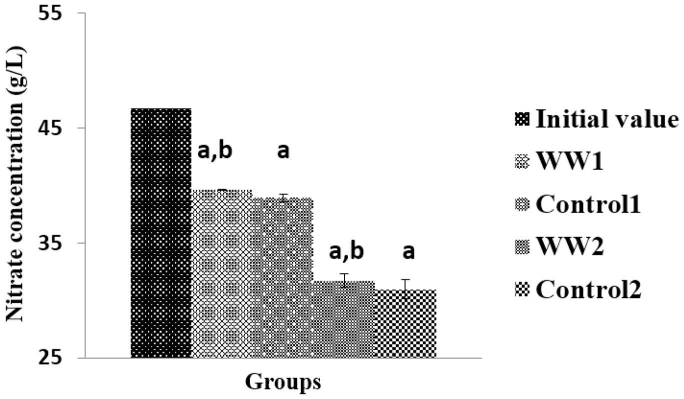

Figure 3. Bioremediation of Nitrate in Real Wastewater. (**Note**. a: Significant difference with the initial value, b: Significant difference with the control group.)

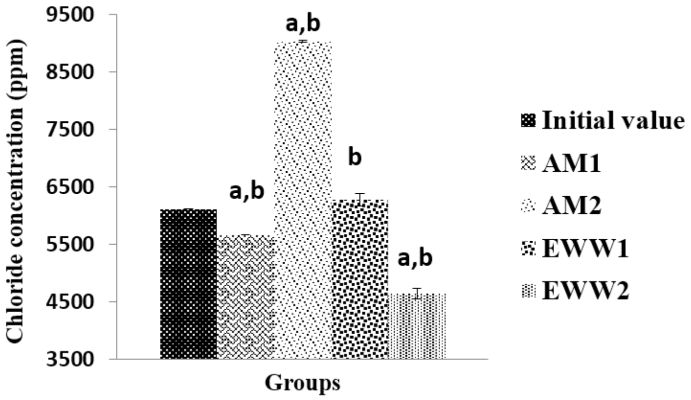

Figure 4. Bioremediation of Chloride in artificial culture medium (AM) and enriched wastewater (EWW) (**Note**. a: Significant difference with the initial value, b: Significant difference within groups.)

The results of nitrate bioremediation for enriched wastewater are shown in Figure 5. Accordingly, it can be concluded that both enriched wastewaters caused a significant reduction of ions by bacteria ($p<0.05$), but the treatment of enriched wastewater with modified mineral salt resulted in a better performance than the treatment of enriched wastewater with Mueller ($p<0.05$).

Generally, the bacterial isolates' biomass showed no significant reduction when acclimatized against high concentrations of chloride and nitrate ions after culturing on AM, EWW, and real wastewater. The results show that *Enterococcus faecalis* exhibited strong tolerance to elevated chloride and nitrate concentrations, but its contribution to active ion removal was limited. This distinction is critical, as tolerance ensures bacterial survival but does not guarantee pollutant reduction. The persistence of *E. faecalis* in reclaimed industrial wastewater may therefore pose public health risks, as this species is opportunistic and may spread resistance traits during water reuse. These findings emphasize the importance of monitoring bacterial populations in conjunction with measuring ion removal efficiency (Kim *et al.*, 2020). However, the resistance effect mechanism is not clear, and it has been linked to microorganisms' density or population dissimilarity (Ramseier *et al.*, 2011; Simonet & Gantzer, 2006).

3.2.2. Algal Bioremediation

Microalgae have been shown to be effective in removing pollutants from wastewater (Bahar *et al.*, 2013; Drewniak & Sklodowska, 2013; Pandey, 2012; Rai *et al.*, 2013). Upadhyay *et al.* (2020), Rath *et al.* (2012), and Inthorn *et al.* (2001) reported that *Chlorella vulgaris* and *Chlorococcum* sp. have the potential for wastewater bioremediation, improving water quality (Inthorn *et al.*, 2002; Rath, 2012; Upadhyay *et al.*, 2022). Chlorine bioremediation by *Chlorella vulgaris* and *Chlorococcum* sp. after 72 h is shown in Figure 6. Both algal species have caused an ion reduction. However, considering the significant difference between the control group and the initial value ($p < 0.05$) and the absence of a significant difference between the treatment and control groups ($p > 0.05$), it can be concluded that other factors contributed to the reduction. Among the algae, *Chlorella vulgaris* consistently outperformed *Chlorococcum sp.* ($p<0.05$). Previous studies have confirmed that *Chlorella vulgaris can* tolerate high chloride levels and can remove pollutants from industrial wastewater (Lim *et al.*, 2010; Phang & Chu, 2004).

The results of nitrate bioremediation by *Chlorella vulgaris* and *Chlorococcum* sp. after 72 h are shown in Figure 7. According to the results, there is a significant difference between the initial value and the *Chlorella vulgaris* treatment ($p<0.05$), which means *Chlorella vulgaris* has shown a better performance ($p<0.05$). Algal bioremediation, particularly by *Chlorella vulgaris*, showed substantial capacity for active removal of nitrate and partial mitigation of chloride stress. The superior performance of *Chlorella* compared to *Chlorococcum* sp. highlights its potential for practical application in wastewater treatment systems. The integration of microalgae into photobioreactors or pond-based treatment schemes could not only reduce nutrient loads but also generate biomass for biofuels, fertilizers, or feed, thereby offering a sustainable and cost-effective alternative to conventional technologies. This inherent tolerance positions *E. faec*alis as a robust biological chassis for genetic engineering. By introducing specific metabolic pathways, such as denitrification or chloride transport systems, this strain could be engineered to enhance active ion removal capabilities, thereby improving bioremediation efficiency in wastewater treatment systems. Such an approach aligns with emerging strategies that focus on engineered microbial consortia, which offer advantages like metabolic flexibility and ecological resilience in pollutant degradation (Renganathan *et al.*, 2025). Many studies have considered bioremediation in algae; Das *et al.* (2017) reported that *Chlorella vulgaris* can complete the removal of NO_3-N from tannery wastewater (Phang & Chu, 2004). Asaad *et al.* demonstrated that *Chlorella vulgaris* effectively removed phosphate and nitrate ions from wastewater. The process highlighted the potential of this alga as an eco-friendly and cost-effective option for wastewater treatment (Asaad & Amer, 2024). Aldaby *et al.* showed that *Chlorococcum humicola* can produce significant biomass and up to 30% oil content under optimal C:N ratios, with fatty acid profiles strongly influenced by nutrient balance. Additionally, the defatted biomass fermented by *Bacillus coagulans* yielded notable hydrogen production, with the highest efficiency (Aldaby *et al.*, 2024).

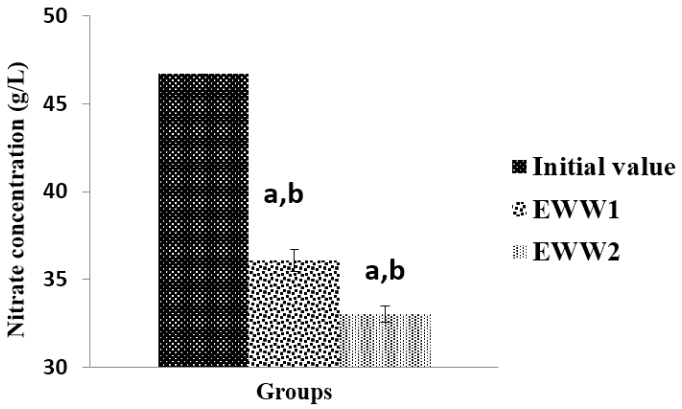

Figure 5. Bioremediation of Nitrate by Chlorella vulgaris and Chlorococcum sp. at real Waste Water. **(Note.** a: Significant difference with the initial value, b: Significant difference within groups.)

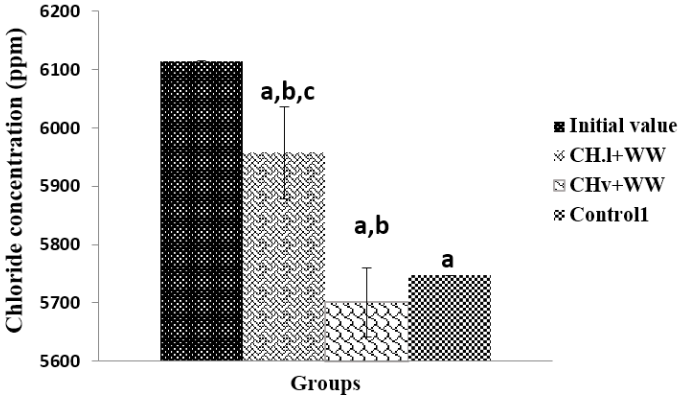

Figure 6. Bioremediation of Chloride by Chlorella vulgaris and Chlorococcum sp. at real Waste Water. (**Note**. a: Significant difference with the initial value, b: Significant difference within groups, c:Significant difference with control.)

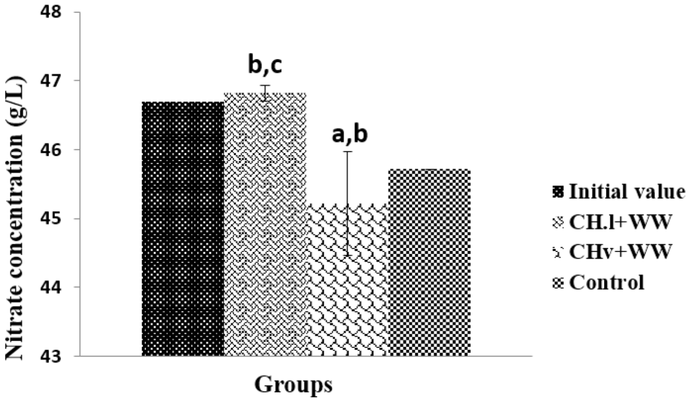

Figure 7. Bioreduction of Nitrate by Chlorella vulgaris and Chlorococcum sp. at real Waste Water. (**Note**: a: Significant difference with the initial value, b: Significant difference within groups, c Significant difference with control)

Given the remarkable tolerance of *Enterococcus faecalis* to elevated chloride and nitrate levels, this strain may serve as a robust biological chassis for future genetic engineering approaches aimed at enhancing active ion removal pathways. Moreover, its potential integration with high-performing microalgae such as *Chlorella vulgaris* could lead to resilient algal–bacterial consortia for sustainable wastewater treatment in high-salinity environments. However, the persistence of *E. faecalis* in reclaimed water also highlights the need for public health risk assessment and continuous monitoring of antimicrobial resistance genes.

In conclusion, the natural tolerance of *E. faecalis* is not merely a passive trait but a valuable asset that can be harnessed through genetic engineering to develop advanced bioremediation strategies. This approach not only enhances the efficiency of pollutant removal but also contributes to the sustainable management of wastewater resources (Carey *et al.*, 2016).

4. Conclusion

In this study, native bacteria isolated from a drum filter at the Sooreh plant (Iran) were characterized using staining procedures, biochemical tests, and *16s rRNA* sequencing. The isolated bacteria belonged to the *Enterococcus* family and were very similar to *Enterococcus faecalis* (99.8%). Thus, *Enterococcus faecalis* and two groups of algae (*Chlorella vulgaris* and *Chlorococcum* sp.) were utilized to examine the removal of chloride and nitrate from real and synthetic wastewater.

The native Enterococcus faecalis FF2021, isolated from industrial wastewater, exhibited strong tolerance to high concentrations of chloride and nitrate but limited capacity for their active removal. The bacterium's survival under ion-rich conditions suggested robust osmotic and physiological adaptation mechanisms rather than efficient pollutant degradation. In contrast, microalgal strains, particularly *Chlorella vulgaris*, showed a significant potential for nitrate and chloride removal, emphasizing the higher metabolic flexibility of algae in nutrient assimilation.

Analysis revealed that in real wastewater, WW1 (sterile Wastewater + 30% isolated bacteria) demonstrated high effectiveness toward nitrate and chloride in the bioremediation process. Also, AM1 (Mueller medium + Distilled water) and EWW2 (Modified Mineral salt base medium + Wastewater) showed the highest ion remediation in synthetic wastewater. Although a decrease was observed in the test samples, it was found that this decrease was not related to the bacterial biomass.

Overall, when comparing bacterial and algal bioremediation performance, findings indicate that while native *E. faecalis* FF2021 exhibits strong tolerance mechanisms enabling survival in ion-rich wastewater, its bioremediation potential can be greatly enhanced when combined with efficient microalgae (*Chlorella vulgaris* and *Chlorococcum sp.*) in a consortium system. *Enterococcus faecalis* exhibited remarkable tolerance to elevated chloride and nitrate levels, a trait consistent with its well-documented environmental resilience.

While the strain showed limited capacity for active ion removal, its ability to survive under high-salinity and nitrate-rich conditions is of particular importance. From a practical standpoint, the persistence of *E. faecalis* in reclaimed wastewater highlights both opportunities and risks.

On one hand, its resilience makes it a valuable candidate for designing engineered microbial consortia, where its stability could complement the active removal efficiency of microalgae like *Chlorella vulgaris*. On the other hand, its opportunistic pathogenicity necessitates careful monitoring during wastewater reuse to minimize potential public health risks. From a public health perspective, the persistence of *E. faecalis* FF2021 in treated wastewater underscores the importance of continuous microbial monitoring, particularly regarding antimicrobial resistance dissemination during water reuse. Overall, this study highlights both the limitations and opportunities of native microbial isolates in sustainable wastewater management and paves the way for advanced biotechnological strategies that combine natural tolerance with bioremediation capacity.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Open access

Data Availability Statement

The datasets generated during and/or analysed during the current study are available in the [NCBI] repository, [https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=1169554].

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Authors' Contributions

Sabere Naij wrote the manuscript. Javad Rafiei analyzed the data. Faezeh Fatemi designed the experiment and analyzed the data. Somayeh Farahmand managed the experiment and collected the data. Razieh Ghasemi edited the text and completed the manuscript submission process. All authors have read and approved the final version of the manuscript.

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