



## Bioremediation of Alkaline Textile Effluent by *Corynebacterium casei*: Evaluation of Physicochemical Parameter Reduction

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### KEYWORDS

Bioremediation

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### ABSTRACT:

**Introduction:** Textile wastewater is a significant environmental issue due to its high organic loads, dissolved salts, and nutrient pollutants, which can threaten water quality and aquatic ecosystems.

**Objectives:** The present study evaluated the bioremediation potential of *Corynebacterium casei* for the treatment of raw textile effluent and 50 and 25% diluted effluent.

**Methods:** The present study evaluated the bioremediation potential of *Corynebacterium casei* for treating raw textile effluent and 50% and 25% diluted effluent. The efficacy of bacterial treatment was evaluated by comparing relevant physicochemical parameters before and after effluent treatment, including electrical conductivity (EC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), sulphate, phosphate, and nitrate content.

**Results:** bacterial treatment led to distinct reductions in pollutant load across effluent concentrations. In raw effluent, mean BOD value was reduced from 393.1 to 54.4 mg/L, COD value from 3932.7 to 1720.2 mg/L, EC reduced from 15.3 to 8.3, TDS reduced 10840.3 to 6276.7mg/L, sulphate reduced 411.0 to 108.8 mg/L, phosphate reduced from 12.7 to 2.5 mg/L and nitrate reduced from 54.4 to In 50% diluted effluent mean BOD was reduced from 284.5 to 32.4 mg/L, COD was reduced from 2277.6 to 1240.4 mg/L and TDS was reduced from 7656.3 to 4646.2 mg/L. At the same time, nutrient ions were also significantly reduced following treatment. The 25% diluted effluent exhibited the best overall treatment performance, with the mean BOD, COD, EC, TDS, sulphate, phosphate, and nitrate being reduced to 17.6 mg/L, 299.2 mg/L, 2.8, 1827.0 mg/L, 31.0 mg/L, 0.7 mg/L, and 1.1 mg/L, respectively.

**Conclusions:** The research supports the concept of bacterial treatment as an eco-friendly and sustainable way of reducing the physicochemical pollution load of textile wastewater before discharging it into the environment.

### 1. Introduction

The textile industry is globally recognized as one of the largest consumers of freshwater resources and a major contributor to severe environmental pollution. [1]. During the various stages of fabric production, including dyeing and finishing, large amounts of structurally complex synthetic dyes, heavy metals, and toxic chemical additives are inevitably released into aquatic ecosystems [2]. The direct discharge of this untreated or

inadequately treated effluent causes severe ecological degradation. The dark coloration of the dyes prevents sunlight penetration, drastically reducing the photosynthetic activity of aquatic flora. At the same time, bioaccumulation of heavy metals has serious long-term toxicological impacts on marine life and human health [3, 4]. Consequently, the sustainable development of the global environment is a pressing priority and has become an urgent global concern for reducing the environmental impact of textile wastewater.



Conventionally, treatment of such industrial effluents has relied heavily on physical and chemical methods, such as chemical coagulation, flocculation, membrane filtration, and advanced oxidation processes. However, these traditional methodologies are often economically unviable at large-scale industrial scale, are very energy-consuming, and notoriously produce copious amounts of hazardous secondary sludge that require complex further disposal [5]. To overcome these inherent limitations, biological treatment, namely microbial bioremediation, has emerged as a highly promising, eco-friendly, and cost-effective alternative [6, 7]. Microorganisms, especially highly adapted bacteria, have shown an exceptional ability to mineralize and detoxify a wide range of synthetic dyes and persistent industrial pollutants through specific enzymatic pathways, effectively converting them into benign, non-toxic metabolites [8].

Among microbial candidates, bacteria of the genus *Corynebacterium* have received considerable attention from scientists due to their high metabolic flexibility, unique cell wall resistance, and exceptional tolerance to extreme conditions, such as heavy metal poisoning and variable pH [9, 10]. Previous studies have widely described the strong azoreductase and oxidative enzyme activities of *Corynebacterium* species, enabling them to decolorize and degrade complex dye structures quickly [11, 12]. Furthermore, closely related strains, such as *Corynebacterium casei*, have shown a high propensity to perform concomitant bioremediation of toxic metals and complex organic effluents and are therefore ideal candidates for treating challenging industrial wastewater matrices [12, 13].

Despite the huge potential of microbial remediation, the extreme toxicity of raw textile effluent, represented by very high chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), and varying alkaline pH, can have a devastating effect on bacterial growth and enzymatic activity [14]. Therefore, assessment of the bioremediation potential using a gradient of effluent concentrations (i.e., 25% diluted, 50% diluted, and 100% raw effluent) is an experimental step. This step-wise approach avoids an immediate shock to the microbes, permits acclimation, and creates optimal treatment conditions. Furthermore, continuously monitoring effluent physicochemical parameters before and after biological treatment is the most appropriate and universally accepted way to quantify the efficiency of the remediation process accurately [15].

The present study comprehensively focuses on the bioremediation effectiveness of *Corynebacterium casei* on textile industry effluent. The main goals are to

accurately isolate and identify the bacterium using 16S rRNA sequencing, followed by a detailed evaluation of its degradation ability at different textile effluent concentrations (raw, 50%, and 25%). Finally, the success of the bioremediation process as a whole is systematically confirmed by analyzing key physicochemical parameters before and after bacterial treatment.

## 2. Methods and materials

### 2.1 Study Site and Sampling Collection

The study area was in an industrial area, Y N Hosakote, Tumakuru district (14°29'55.2"N 77°1286'E), Karnataka, India. A 20-litre grab sample of raw textile effluent was taken from the raw textile effluent directly at the primary discharge point using a sterile high-density polyethylene (HDPE) container. Physical parameters such as color, odor, temperature, pH, and electrical conductivity (EC) were measured and recorded on-site.

Soil samples for microbial isolation were collected using random sampling, with a uniform spacing of 2 meters between sample collections. Surface debris was removed before sampling, and soil was aseptically collected at a depth of approximately 15 cm (6 inches) using a sterilized spatula and placed in sterile low-density polyethylene (LDPE) bags to keep the sample intact. All collected samples were transported to the Environmental Science Research Laboratory at Kuvempu University and stored at 4 °C until further processing.

### 2.2 Isolation and Molecular Characterization of Bacterial Isolate

Bacterial strains were isolated from the contaminated soil samples using the serial dilution plating method. Molecular identification was performed by PCR amplification of the 16S rRNA gene using universal primers 16SF (5'-AGAGTTTGATCCTGGCTCAG-3') and 16SR (5'-CGGTTACCTGTTACGACTT-3'), as previously described. The amplified products were purified and sent for Sanger sequencing. The resulting sequences were then subjected to a BLAST similarity search of the nucleotide database of the National Centre for Biotechnology Information, and the best-scoring hits were retrieved and aligned. A neighbour-joining phylogenetic tree was constructed using MEGA X, and the topology was visualized using FigTree version 1.4.4.

### 2.3 Physicochemical Analysis and Experimental Setup for Biodegradation

The effluent collected was divided into three experimental groups. Sterilization was performed by



autoclaving at 121 °C for 20 min in order to remove indigenous microbiota. The effluents were prepared at 100%, 50% and 25% concentration using sterile distilled water.

All biodegradation experiments were performed in triplicate using 250 mL Erlenmeyer flasks with 100 mL of effluent supplemented with Minimal Salt Medium. (MSM; composition:  $\text{KH}_2\text{PO}_4$  1.5 g/L,  $\text{K}_2\text{HPO}_4$  3.5 g/L,  $(\text{NH}_4)_2\text{SO}_4$  1.0 g/L,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g/L,  $\text{CaCl}_2$  0.01 g/L,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.001 g/L). The bacterial inoculum, standardized to an optical density of 0.8 at 600 nm, was added to the treated groups at a 10% v/v concentration. All flasks were incubated at  $35 \pm 2$  °C for 7 days under static conditions. Physicochemical parameters, such as COD, BOD, TDS, Sulphate, Nitrate, and Phosphate, were determined before and after treatment by the APHA (2017) standard methods.

### 3. Results and discussion

The physicochemical parameters of the effluent were analyzed across three concentration gradients: 100% raw effluent, 50% diluted effluent, and 25% diluted effluent. The parameters, Temperature (Temp), Electrical Conductivity (EC), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Sulphates (SO), Phosphates (PO), and Nitrates were determined before and after the treatment of the bacteria in three independent trials to ensure statistical reliability. The results show a marked, concentration-dependent improvement in water quality following the introduction of *C. casei* (Table 1). The visual color was reduced after bacterial treatment (Fig. 1).

**Table 1.** Physicochemical parameters of textile effluent before and after treatment with *Corynebacterium casei* at three concentration gradients. Values are mean  $\pm$  SD (n = 3). RTE: raw textile effluent; T-RTE: treated raw effluent; RTE50/RTE25: 50% and 25% diluted effluent; T-RTE50/T-RTE25: respective treated dilutions. EC (mS/cm); BOD, COD, TDS, sulphate, phosphate, nitrate (mg/L).

Parameter	RTE	T-RTE	RTE50	T-RTE50	RTE25	T-RTE25
pH	11 $\pm$ 0.2	8.30 $\pm$ 0.10	10.9 $\pm$ 0.2	7.90 $\pm$ 0.10	10 $\pm$ 0.21	7.75 $\pm$ 0.07
EC	15.33 $\pm$ 0.32	8.10 $\pm$ 0.10	10.27 $\pm$ 0.15	5.23 $\pm$ 0.15	6.4 $\pm$ 0.26	2.8 $\pm$ 0.07
BOD	393 $\pm$ 15	54.4 $\pm$ 1.68	284.4 $\pm$ 4.31	32.4 $\pm$ 0.90	148.6 $\pm$ 3.42	17.4 $\pm$ 0.57
COD	3932 $\pm$ 76	1720 $\pm$ 71	2277 $\pm$ 130	1240 $\pm$ 36.36	1458 $\pm$ 50	298 $\pm$ 7.14
TDS	10840 $\pm$ 182	6276 $\pm$ 301	7656 $\pm$ 551	4646 $\pm$ 36	3583 $\pm$ 7	1799 $\pm$ 24
Sulphate	411 $\pm$ 12.37	108 $\pm$ 1.26	196 $\pm$ 3.35	70.7 $\pm$ 1.78	85 $\pm$ 0.8	32 $\pm$ 1.4

Phosphate	12.73 $\pm$ 0.15	2.47 $\pm$ 0.06	8.73 $\pm$ 0.15	1.70 $\pm$ 0.10	3.67 $\pm$ 0.06	0.65 $\pm$ 0.07
Nitrates	54.37 $\pm$ 0.65	4.43 $\pm$ 0.21	34.00 $\pm$ 3.00	2.03 $\pm$ 0.21	24.00 $\pm$ 2.65	1.1 $\pm$ 0.14



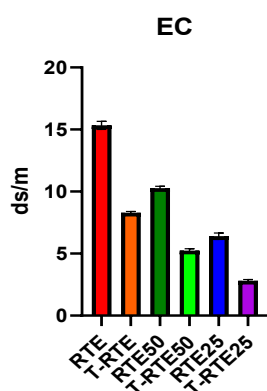
**Fig. 1.** Visual appearance of textile effluent samples before and after bioremediation with *Corynebacterium casei*.

#### 3.1 Physical Parameters: pH, Electrical Conductivity

pH is one of the most important parameters in wastewater treatment because it directly affects microbial enzyme activity and the metabolic rates of the remediation microorganisms [16]. Most bacteria thrive within a pH range of 6.5 to 8.5, and even short-term exposure to extreme pH values can cause significant inhibition or destruction of microbes. In the present study, the average initial raw effluent pH was 11.1. Following biological treatment with *C. casei*, the raw effluent pH decreased by 25.2%, averaging 8.3. Similar pH-reduction effects were observed in the diluted samples, with the 50% effluent showing a 28.1% decrease (from an adjusted average of 10.9 to 7.9) and the 25% effluent showing a 22.3% decrease (from 10.0 to 7.8). This pH normalization is ecologically significant because highly alkaline industrial discharges reduce the solubility of dissolved oxygen and endanger aquatic fauna in receiving water bodies [17]. The post-treatment pH stability indicates that the strongly alkaline chemical conditions present in the raw toxic matrix were predominantly neutralized by bacterial metabolic processes, including  $\text{CO}_2$  production as an acidic metabolic by-product, making the effluent more ecologically compatible [18]. Electric Conductivity (EC), taken as an indicator of the total concentration of ionized species and dissolved salts in the wastewater, also showed significant improvement. The raw effluent's initial EC was extremely high, averaging 15.3 mS/cm (Fig 2), indicating the extensive use of salts to promote dye fixation in the textile manufacturing process [18]. Treatment with *C. casei* achieved a significant reduction



in EC of the raw effluent, from 13.2 mS/cm to 8.3 mS/cm (45.9%) (Fig 2). The efficiency of salt removal increased as effluent concentration decreased, with EC reductions of 49.0 and 56.2% for the 50% and 25% effluent groups, respectively. The final EC of the 25% treated effluent was significantly lowered to 2.8 mS/cm (Fig 2). High electrical conductivity in irrigation water severely limits water uptake by plants due to changes in osmotic pressure in the root zone, which is a major cause of physiological drought and reduced crop yield [19, 20]. The ability of *C. casei* to promote the precipitation or bioaccumulation of these dissolved ions is what makes them useful in counteracting the osmotic stress implicated in the discharge of raw textiles [21].



**Fig. 2.** Effect of *C. casei* treatment on pH and electrical conductivity (EC) of textile effluent at 100%, 50%, and 25% concentrations. Values are mean  $\pm$  SD (n = 3).

### 3.2 Organic Load Reduction: BOD and COD

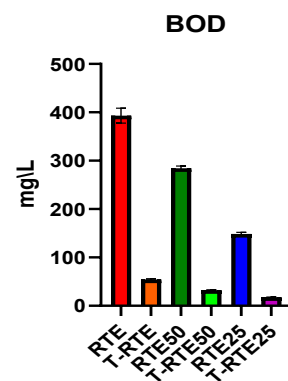
Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are perhaps the most important indices for assessing the organic pollution load and the overall toxicity of industrial wastewater. Textile effluents are well known for their high BOD and COD values due to the presence of unfixed synthetic dyes, sizing agents, thickeners, and various spinning lubricants [22].

The pre-treatment BOD of the raw effluent was significantly high at 393.1 mg/L. Incredibly, in the presence of *C. casei*, the BOD reduction is outstanding, with an 86.2 percent reduction in wastewater BOD, and concentrations dropped to an average of 54.4 mg/L (Fig 3). This deep reduction trend was replicated in the diluted treatments, where the 50% effluent showed an 88.6% reduction (from 284.4 mg/L to 32.4 mg/L), and the 25% effluent showed an 88.2% reduction (from 148.6 mg/L to 17.6 mg/L) (Fig 3). The final BOD values of the diluted treatments lie well within permissible limits of industrial

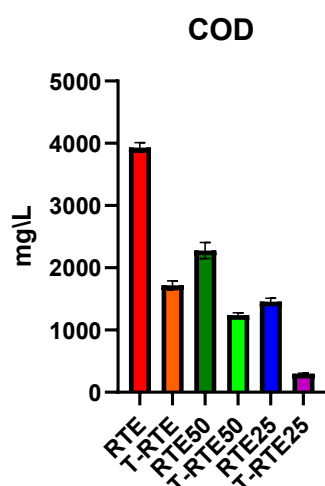
discharge by global environmental protection agencies, which usually dictate BOD values below 30-50 mg/L depending upon the recipient water body [23].

Correspondingly, the COD levels decreased substantially, but presumably to varying degrees depending on the initial load. The raw effluent had an initial average COD value of 3932.7 mg/L (Fig 4). Post-treatment, the COD value decreased by 56.3%, from 1720.1 mg/L to 755.8 mg/L (Fig 4). Although this is a significant decrease from a mathematical point of view, the final COD concentration of the raw treated effluent still exceeds typical safe discharge levels, highlighting the extreme chemical recalcitrance of the undiluted textile dyes [24]. However, when the effluent was diluted, the biological treatment became vastly more efficient. The 50% effluent demonstrated a 45.5% reduction (from 2277.5 to 1240.4 mg/L), while the 25% effluent demonstrated the highest efficiency, with a 79.5% reduction (from 1458.1 to 299.2 mg/L).

The differential efficiency in COD reduction across concentrations suggests the presence of a toxicity threshold for *C. casei*. The high concentrations of toxic heavy metals and complex azo dyes in the 100% raw effluent likely hindered bacterial enzymatic activity, including azoreductases and laccases, which are necessary to break the resistant nitrogen-nitrogen double bonds of synthetic dyes [25]. In comparison, the 25% dilution provided a less hostile environment, allowing the bacteria to metabolise the organic pollutants at an optimal rate without being subject to substrate inhibition [26]. The large simultaneous reduction in both BOD and COD confirms that *C. casei* does not simply adsorb the dyes passively to its cell surface, but actually catabolizes complex organic molecules into simpler, less toxic metabolic byproducts.



**Fig. 3.** Reduction in BOD (mg/L) of textile effluent after treatment with *C. casei* at 100%, 50%, and 25% concentrations. Values are mean  $\pm$  SD (n = 3).

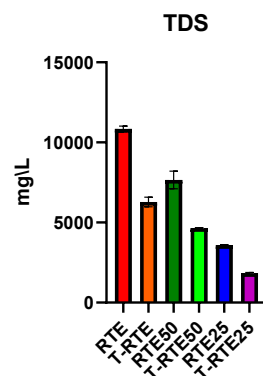


**Fig. 4.** Reduction in COD (mg/L) of textile effluent after treatment with *C. casei* at 100%, 50%, and 25% concentrations. Values are mean  $\pm$  SD (n = 3).

### 3.3 Mitigation Efforts for Total Dissolved Solids (TDS)

Total Dissolved Solids (TDS) is the sum of the contents of all inorganic and organic compounds contained in a liquid in molecular, ionized, or micro-granular suspended form. In the textile industry, mainly through carbonates, bicarbonates, chlorides, sulphates, and heavy metals used as mordants, high TDS is contributed [27]. High TDS in water systems increases salinity, alters water density, and severely affects the respiratory systems of aquatic organisms [28].

The initial TDS of the raw effluent was very high, averaging 10,840.3 mg/L (**Fig 5**). Treatment with *C. casei* resulted in a 42.1 percent reduction in TDS levels, from 8,000 mg/L to 6,276.7 mg/L. The 50% effluent group had a 39.3% reduction (from 7,656.3 mg/L to 4,646.2 mg/L), and the 25% effluent group had the most effective removal rate of 49.0% (from 3,583.0 mg/L to 1,827.0 mg/L) (**Fig 5**). The process of reducing TDS by bacterial action is complex [29]. These negatively charged biopolymers function as natural bio-flocculants that actively bind soluble cationic salts, heavy metals, and particulate matter in the water, thereby pulling them from the aqueous phase and precipitating them into biological sludge [30]. While biological treatment alone is rarely sufficient to remove TDS, the nearly 50% reduction at the 25% dilution is a highly significant accomplishment for a single-strain remediation system.



**Fig. 5.** Effect of *C. casei* treatment on TDS (mg/L) of textile effluent at 100%, 50%, and 25% concentrations. Values are mean  $\pm$  SD (n = 3).

### 3.4 Nutrient Depletion: Sulphates, Phosphates, and Nitrates

The presence of excessive nutrients, in this case sulphates, phosphates, and nitrates, is the main cause of anthropogenic eutrophication in freshwater ecosystems [31]. Textile effluents involve a large use of sulphuric acid in dyeing baths, the use of detergents with phosphate structures for fabric scouring, and the use of nitrogenous compounds inherent to certain dye structures. [32].

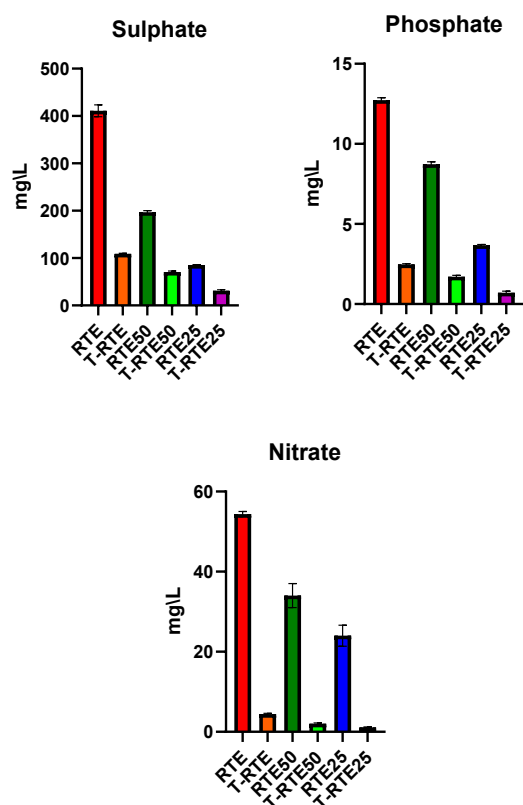
The bioremediation assay proved extraordinarily efficient at reducing these particular anions. Initial levels of sulphate (SO) in the raw effluent averaged 411.0 mg/L. *C. casei* was able to reduce sulphates by an impressive 73.5%, leaving only 108.8 mg/L. In diluted groups, the sulphate reductions were consistently high: 64.0% for the 50% effluent and 63.6% for the 25% effluent (**fig 6a**). The marked reduction in sulphates suggests that *C. casei* efficiently utilises sulphur as a macronutrient for the production of sulphur-containing amino acids (cysteine and methionine) necessary for bacterial biomass production, or is involved in assimilatory sulphate reduction pathways to neutralise oxidative stress [33].

Phosphate (PO) reduction was highly efficient across all experimental groups. The initial raw effluent contained 12.7 mg/L of phosphates, which was reduced by 80.6% to a mere 2.4 mg/L after treatment. The 50% and 25% effluent groups had nearly identical efficiencies, at 80.5% and 80.9%, respectively (**fig 6b**). The final phosphate concentration in the 25% treated effluent was an extraordinarily low 0.7 mg/L. In the natural environment, organisms sequester phosphorus for the synthesis of nucleic acids (DNA and RNA), phospholipids for cell membrane integrity, and adenosine triphosphate (ATP) for intracellular energy



transfer [33]. The rapid removal of phosphates is a strong indication of vigorous bacterial growth and high metabolic activity of the effluent matrix during treatment.

Similarly, the remediation of nitrates was also the most successful parameter overall. The initial nitrate concentration of the raw effluent was, on average, 54.3 mg/L. After using the culture of *C. casei*, the nitrate concentration dropped by an incredible 91.8%, to 4.4 mg/L. This efficiency was in perfect scale with the dilution, providing a 94.0% reduction in the 50% effluent and an almost complete 95.6% reduction in the 25% effluent (from 24.0 mg/L to only 1.0 mg/L) (Fig 6c). Nitrates are often used by bacteria as alternative terminal electron acceptors in the absence of dissolved oxygen during rapid organic degradation (denitrification), thereby reducing nitrates to harmless nitrogen gas (N<sub>2</sub>) that escapes to the atmosphere [34]. The almost complete removal of nitrates in the diluted samples ensures that the discharged water will not contribute to the eutrophication of downstream aquatic environments.



**Fig. 6.** Reduction in sulphate, phosphate and nitrate (mg/L) of textile effluent after treatment with *C. casei* at 100%, 50%, and 25% concentrations. Values are mean  $\pm$  SD (n = 3).

#### 4. Conclusion

The present study demonstrated the strong bioremediation potential of *Corynebacterium casei*, as identified by 16S rRNA sequencing, for treating highly toxic textile industry effluent. The biological treatment showed significant improvements in all measured physicochemical parameters, including biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and critical nutrient ions such as nitrates, phosphates, and sulphates. Of note, the degradation efficiency was highly dependent on concentration. While the raw effluent exhibited high levels of absolute reductions, the 25% diluted effluent showed the best and ecologically safe final parameter values. In the 25% effluent matrix, *C. casei* achieved near-complete elimination of nitrates (over 95% reduction) and a substantial reduction in organic load, driving BOD and COD down to environmentally compliant levels.

These results indicate that an initial dilution, combined with targeted treatment of the bacteria, is a highly effective, eco-friendly approach to addressing recalcitrant textile wastewater. Future research should investigate the integration of *C. casei* into mixed microbial consortia to further improve the degradation of complex synthetic dyes in totally undiluted raw effluents. Ultimately, the use of such specialized biological agents could provide a long-term, cost-effective solution to alleviate the effects of industrial water pollution and safeguard sensitive aquatic ecosystems.

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