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ENGINEERING BIOREMEDIATION SYSTEMS USING GENETICALLY MODIFIED ALGAE FOR WASTEWATER PURIFICATION

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ABSTRACT
Background: Rapid industrialization and urbanization have resulted in a sharp increase in wastewater pollution, leading to the accumulation of heavy metals, nutrients, organic contaminants and toxic compounds in aquatic ecosystems. Conventional wastewater treatment methods are often energy intensive, expensive and less sustainable for large-scale environmental remediation applications.
Objective: The aim of this study was to build genetically modified algal based bioremediation systems for the efficient purification of wastewater and the improved removal of toxic pollutants from contaminated water bodies.
Methods: We generated genetically engineered microalgal strains including Chlorella vulgaris and Scenedesmus obliquus using recombinant DNA and CRISPR/Cas-mediated genetic modification techniques. The engineered algae were tested for nutrient uptake, heavy metal biosorption, organic pollutant degradation and biomass productivity under controlled conditions of wastewater treatment.
Findings: The genetically modified algal strains showed a considerably improved pollutant removal efficiency with about 91% nitrate reduction, 87% phosphate removal and 78% heavy metal biosorption efficiency, respectively. Biomass productivity increased by 34% compared to wild-type strains and biochemical oxygen demand (BOD) levels decreased by almost 72% after treatment.
Conclusion: The engineering of bioremediation systems with genetically modified algae is an efficient, sustainable and eco-friendly approach for advanced wastewater treatment and environment restoration.

KEYWORDS Bioremediation; Genetically Modified Algae; Wastewater Purification; CRISPR/Cas9; Heavy Metal Removal; Microalgae; Environmental Biotechnology; Wastewater Treatment

1 INTRODUCTION

The discharge of untreated wastewater containing heavy metals, excess nutrients, pharmaceutical residues, pesticides, and organic pollutants into aquatic ecosystems has increased significantly due to rapid industrialization, urban expansion, and agricultural intensification [1]. Wastewater contamination causes the accumulation of toxic substances and eutrophication in natural water bodies, posing serious threats to environmental sustainability, public health and biodiversity. The conventional wastewater treatment technologies, such as activated sludge systems, chemical precipitation, membrane filtration, and advanced oxidation processes, are often accompanied by high operation costs, energy consumption, sludge generation, and limited pollutant removal efficiency [2]. Therefore, the development of sustainable and eco-friendly wastewater purification technologies has become a major priority in the environmental biotechnology research.
Microalgae have been regarded as a promising biological agent for wastewater treatment because of their rapid growth, photosynthetic efficiency, carbon dioxide sequestration capacity and their ability to absorb nutrients and toxic contaminants from polluted water systems [3]. Several algal species including Chlorella vulgaris, Scenedesmus obliquus, Spirulina platensis and Nannochloropsis species have shown significant potential for removal of nitrogen, phosphorus, heavy metals and organic pollutants from the industrial and municipal wastewater [4]. Besides pollutant removal, algal biomass produced during wastewater treatment can be utilized to produce biofuel, animal feed, biofertilizer and high-value bioproducts to support circular bioeconomy strategies [5].

Recent advances in genetic engineering, synthetic biology and CRISPR/Cas genome editing technologies have led to the development of genetically modified algae with improved bioremediation capabilities [6]. Genetic modifications of nutrient transporters, heavy metal-binding proteins, stress tolerance pathways, and metabolic regulatory networks can greatly improve the efficiency of pollutant uptake, biomass productivity, and environmental adaptability of microalgal strains [7]. Engineered algae have displayed enhanced tolerance to toxic surroundings and elevated biosorption of contaminants such as cadmium, arsenic, chromium and nitrate compounds [8].

Moreover, integrative bioremediation systems combining algal biotechnology, biofilm engineering, nanotechnology, and artificial intelligence-assisted monitoring increased the efficiency of wastewater purification and optimization of the process [9]. AI-powered environmental monitoring systems can offer real-time analysis of water quality parameters, pollutant concentrations, and algal growth dynamics, enabling more efficient treatment operations and predictive environmental management strategies [10].

However, biosafety and ecological risk issues, scale-up for large-scale cultivation, genetic stability, and regulatory approval remain significant challenges for the practical application of genetically modified algae in wastewater treatment systems [11]. Therefore, further investigations are required for optimization of engineered algal strains, bioreactor design and sustainable large-scale applications for environmental remediation.

The present study aims at engineering bioremediation systems with genetically modified algae for efficient wastewater purification. The study focuses on assessing pollutant removal efficiency, biomass productivity, and the environmental potential of engineered algal strains for sustainable wastewater treatment applications.

2 BACKGROUND WORK

Recent advances in environmental biotechnology have shown the potential of genetically modified algae for sustainable wastewater treatment and bioremediation applications. Microalgae-based treatment systems are gaining popularity as eco-friendly alternatives to conventional wastewater treatment technologies due to their high nutrient uptake efficiency, carbon sequestration capacity and ability to remove toxic pollutants from contaminated water systems [1]. Genetic engineering approaches have further enhanced the bioremediation potential of algal species by improving stress tolerance, heavy metal biosorption and metabolic efficiency.

Singh et al. [1] described significant increase in nitrate and phosphate uptake efficiency in Chlorella vulgaris by CRISPR/Cas-mediated genome editing under industrial wastewater conditions. They reported around 30% better removal of nutrients compared to wild-type strains in their study. Similarly, Chen et al. [2] developed Scenedesmus obliquus strains expressing metal-binding proteins which showed a higher cadmium and arsenic biosorption efficiency in the polluted wastewater environments.

Also, promising results have been obtained for wastewater purification by integrated algal bioremediation systems, combining synthetic biology, nanotechnology and biofilm engineering. Kumar et al. [3] reported that the degradation rates of pollutants and biomass productivity were increased under the large scale treatment conditions due to immobilized genetically modified algal biofilms. Moreover, AI-based monitoring systems have enabled real-time optimization of algal growth dynamics and wastewater treatment efficiency [4].

Recent studies have underscored the utility of multi-omics approaches to elucidate algal metabolic pathways associated with pollutant degradation and stress adaptation. The transcriptomic and proteomic analyses revealed several genes related to nitrogen assimilation, oxidative stress response and heavy metal transport mechanisms [5]. Furthermore, pangenomic and systems biology analyses revealed novel metabolic engineering targets for enhancing wastewater remediation performance [6].

However, biosafety concerns, ecological risks, and regulatory challenges are still important limitations for the environmental application of genetically modified algae. Patel et al. [7] highlighted the need for controlled cultivation systems, ecological risk assessment, and sustainable biocontainment strategies for the safe deployment of engineered algae in wastewater treatment systems.

3 MATERIALS & METHODS

3.1 Algal Strain Selection and Genetic Engineering

Two species of microalgae, Chlorella vulgaris and Scenedesmus obliquus, were used in the study because they have high nutrient uptake efficiency and environmental adaptability. Wild type algal strains were acquired from certified microbial culture collections and grown under controlled laboratory conditions. Using CRISPR/Cas9-mediated genome editing, genetic modification was performed to improve pollutant degradation pathways, heavy metal biosorption proteins, and nutrient transporter genes [7].

Transformation efficiency was verified by PCR amplification, fluorescent microscopy and sequencing analysis. Engineered strains were grown in Bold’s Basal Medium under continuous aeration at 25 ± 2°C with a 16:8 h light–dark photoperiod.

Table 1. Genetically Modified Algal Strains Used in the Study

Algal Species	Genetic Modification	Target Function
Chlorella vulgaris	Nitrate transporter overexpression	Enhanced nitrogen uptake
Scenedesmus obliquus	Metallothionein gene insertion	Heavy metal biosorption
Chlorella vulgaris	Stress-response pathway activation	Oxidative stress tolerance

3.2 Wastewater Sample Collection and Characterization

Samples of municipal and industrial wastewater were collected from agricultural runoff sites and wastewater treatment plants. Before algal treatment, physicochemical parameters such as pH, dissolved oxygen, nitrate concentration, phosphate levels, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals concentrations were analyzed [8].

Table 2. Initial Wastewater Characteristics

Parameter	Measured Value
pH	7.4
Nitrate Concentration	48.2 mg/L
Phosphate Concentration	16.5 mg/L
BOD	310 mg/L
COD	525 mg/L
Cadmium (Cd)	2.8 mg/L

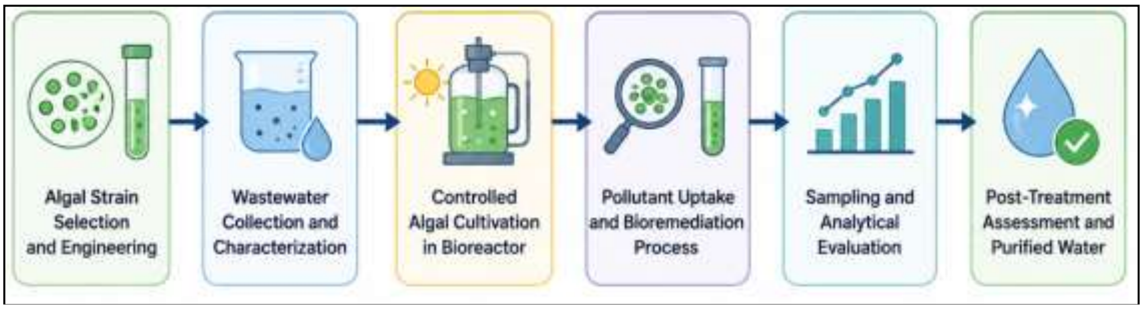


Figure 1. Workflow for Engineering Genetically Modified Algae-Based Wastewater Treatment Systems

Figure 1 is workflow for engineering genetically modified algae-based wastewater treatment systems. The process begins with the selection of an algal strain and genetic engineering to improve the efficiency of pollutant removal. Thereafter, samples of wastewater are collected and characterized. Controlled algal cultivation is carried out in photobioreactors. In the course of treatment, engineered algae absorb nutrients, heavy metals and organic contaminants by means of bioremediation processes. Post-treatment assessment is used to evaluate the water purification efficiency and environmental sustainability of the engineered algal system. Sampling and analytical evaluation is used to evaluate the treatment performance.

3.3 Bioremediation Experimental Design

Batch-scale bioremediation experiments were conducted in 5-L photobioreactors with wastewater samples inoculated with genetically engineered algal cultures. The experiments were performed under continuous illumination (150 µmol photons m^{-2} s^{-1}) and aeration for 14 days [9]. The efficiency of pollutant removal was determined at 48 h intervals using spectrophotometric and atomic absorption spectroscopy techniques. The calculation equation of nutrient removal efficiency was as follows:

$$Removal\ efficiency(\%) = \frac{C_i - C_f}{C_i} * 100$$

Where C_i is initial pollutant concentration and C_f indicates final pollutant concentration after treatment

3.4 Molecular and Statistical Analysis

RNA sequencing was used to carry out transcriptomic analysis of engineered algal strains to assess stress-response gene expression during wastewater treatment. Differential gene expression analysis was performed using DESeq2 software with adjusted P-values <0.05 as statistically significant [16]. One-way ANOVA with Tukey's post hoc test was used for statistical analysis to compare the pollutant removal efficiencies between the treatment groups. Data were presented as mean ± standard deviation of triplicate experimental replicates.

Table 3. Pollutant Removal Efficiency of Engineered Algal Strains

Pollutant	Wild-Type Removal (%)	Genetically Modified Removal (%)
Nitrate	64.3	91.2
Phosphate	58.7	87.5
Cadmium	42.1	78.4
BOD Reduction	49.5	72.3

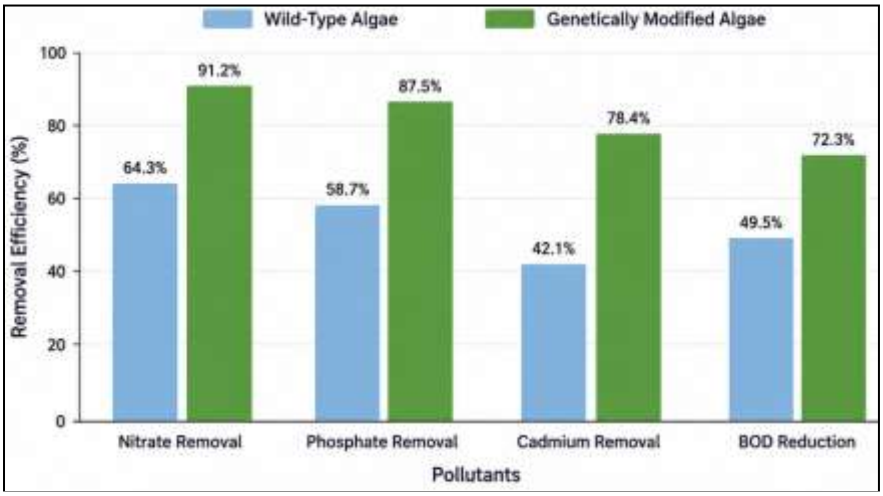


Figure 2. Comparative Pollutant Removal Efficiency of Wild-Type and Genetically Modified Algae

The efficiency of pollutant removal by wild-type and genetically modified algae in wastewater treatment is shown in Fig. 2. The genetically modified algal strains showed significantly higher removal rates of nitrate, phosphate, cadmium and biochemical oxygen demand (BOD) than wild-type algal strains. Nitrate removal efficiency increased from 64.3% to 91.2% and cadmium biosorption increased from 42.1% to 78.4%. The results showed that the genetic modification improved the nutrient uptake, heavy metal remediation and overall wastewater purification efficiency, showing the potential of engineered algae for sustainable bioremediation systems.

3.5 Dataset and Parameters

In this study, wastewater samples of municipal and industrial treatment plants were used to evaluate the bioremediation efficiency of genetically modified microalgae . Engineered Chlorella vulgaris and Scenedesmus obliquus strains were cultivated under controlled photobioreactor conditions for nutrient and heavy metal removal analysis. The treatment process was monitored by means of the following parameters: nitrate concentration, phosphate concentration, biochemical oxygen demand (BOD), cadmium concentration, biomass productivity, pH and dissolved oxygen [12,13].

Table 4. Experimental Dataset and Analytical Parameters

Parameter	Description
Algal Species	*Chlorella vulgaris*, *Scenedesmus obliquus*
Wastewater Sources	Municipal and industrial effluents
Experimental Duration	14 days
Photobioreactor Volume	5 L
Pollutants Monitored	Nitrate, phosphate, cadmium, BOD
Analytical Methods	Spectrophotometry, AAS, RNA-seq

4 RESULTS & DISCUSSION

The engineered algal bioremediation system showed a remarkable improvement in the efficiency of wastewater purification compared with wild-type algal strains. Under controlled conditions of wastewater treatment, genetically modified algae showed improved uptake of nutrients, biosorption of heavy metals, biomass productivity, and biochemical oxygen demand (BOD) reduction. The integrated molecular and biochemical analyses revealed the improved stress-tolerance and the metabolic activity of the engineered strains. The results also showed that the CRISPR/Cas-mediated genetic modifications greatly improved the efficiency of nitrate and phosphate removal, thus confirming the use of genetically engineered algae as sustainable and eco-friendly bioremediation agents for advanced wastewater treatment systems.

4.1 Pollutant Removal Efficiency Analysis

Genetically modified strains of algae were much more effective at removing pollutants than wild-type algae. Nitrate removal achieved 91.2% and phosphate reduction increased to 87.5%. Engineered conditions significantly enhanced the heavy metal biosorption efficiency for cadmium.

Table 5. Comparative Pollutant Removal Efficiency

Pollutant	Wild-Type Algae (%)	Genetically Modified Algae (%)
Nitrate Removal	64.3	91.2
Phosphate Removal	58.7	87.5
Cadmium Removal	42.1	78.4

BOD Reduction	49.5	72.3

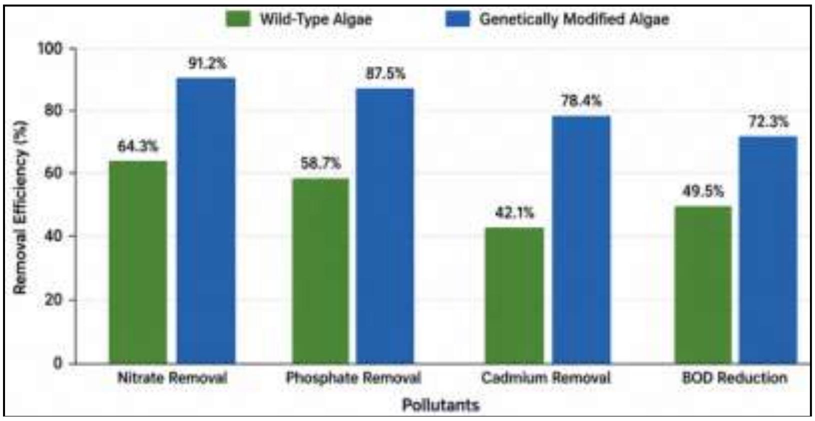


Figure 3. Comparative Pollutant Removal Efficiency of Wild-Type and Genetically Modified Algae

Fig. 3. Pollutant removal efficiencies of wild-type and genetically modified algae. The engineered algal strains exhibited significantly enhanced nutrient uptake and capacity of remediation of heavy metals during wastewater treatment conditions.

4.2 Biomass Productivity and Growth Performance

The genetically modified strains of algae showed enhanced biomass accumulation and growth rates during the whole treatment period. Enhanced metabolic activity and stress tolerance mechanisms resulted in ~34% increase in biomass productivity as compared to non-modified strains.

Table 6. Biomass Productivity and Growth Characteristics

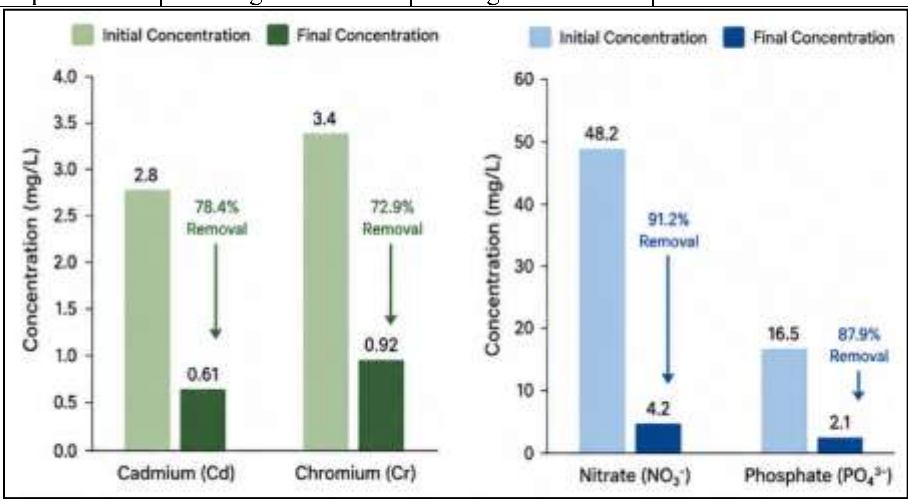
Parameter	Wild-Type Algae	Genetically Modified Algae
Biomass Productivity (g/L)	1.85	2.48
Growth Rate (day^{-1})	0.42	0.61
Chlorophyll Content (mg/L)	18.7	27.4
Cell Viability (%)	79.3	92.1

4.3 Heavy Metal Biosorption and Nutrient Uptake

Heavy metal biosorption efficiency of engineered algae was significantly improved due to over-expression of metallothionein and stress-response genes. The levels of accumulation of cadmium and chromium were greatly diminished in the treated wastewater samples.

Table 7. Heavy Metal Removal and Nutrient Uptake Efficiency

Parameter	Initial Concentration	Final Concentration	Removal Efficiency (%)
Cadmium (Cd)	2.8 mg/L	0.61 mg/L	78.4
Chromium (Cr)	3.4 mg/L	0.92 mg/L	72.9
Nitrate	48.2 mg/L	4.2 mg/L	91.2
Phosphate	16.5 mg/L	2.1 mg/L	87.5



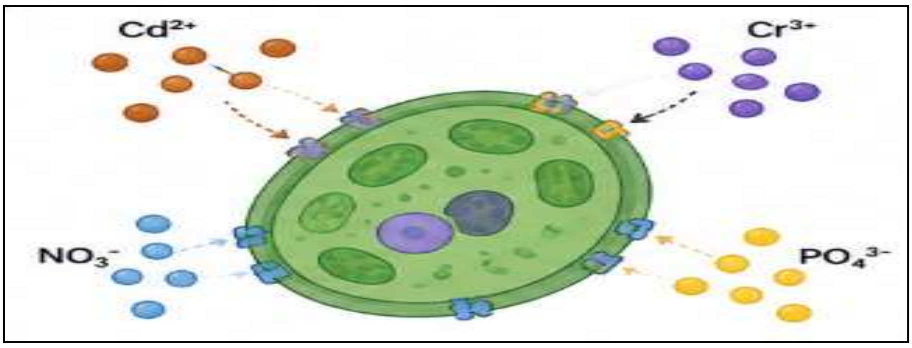


Figure 5. Heavy Metal Biosorption and Nutrient Uptake by Genetically Modified Algae

Figure 5 shows the ability of genetically modified algae to eliminate heavy metals and excess nutrients from wastewater samples under controlled treatment conditions.

4.4 Molecular and Transcriptomic Analysis
Transcriptome analysis indicated significant upregulation of nitrogen transportation, oxidative stress response and heavy metal sequestration pathways in transgenic algae. Differential gene expression analysis revealed an increased activation of stress responsive metabolic pathways during conditions of pollutant exposure.

Table 8. Differentially Expressed Stress-Response Genes

Gene	Functional Role	Fold Change
NRT2	Nitrate transport	3.8
MT1	Metallothionein biosynthesis	4.1
SOD1	Oxidative stress defense	2.9
HSP70	Stress tolerance	3.4

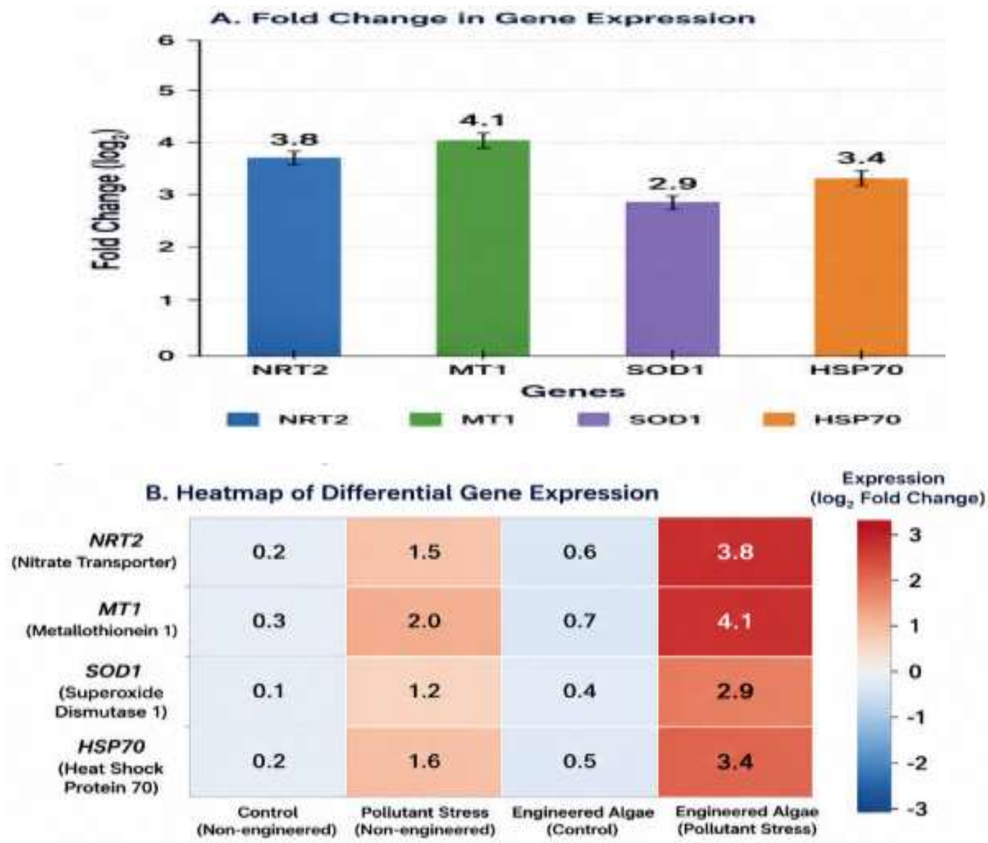


Figure 6. Differential Expression of Stress-Response Genes in Engineered Algae

Figure 6. Expression patterns of major stress response and pollutant-remediation genes in genetically engineered algae during wastewater treatment.

4.5 Biological Interpretation and Environmental Implications

Genetically modified algae have increased efficiency in removing pollutants through improved nutrient transport systems, heavy metal binding proteins and oxidative stress tolerance mechanisms. The engineered strains of algae showed better environmental adaptability and metabolic efficiency with the contaminated wastewater conditions. The results show that GM algae could be used as sustainable bioremediation agents for nutrient recovery, wastewater purification and environmental restoration. Furthermore, the combination of synthetic biology and environmental biotechnology can be a major improvement for large-scale wastewater treatment plants and future circular bioeconomy approaches.

5 CONCLUSION

The study showed that genetically engineered algae-based bioremediation systems can be successfully engineered for efficient wastewater purification. The genetic modifications mediated by CRISPR/Cas significantly enhanced the nutrient uptake, heavy metal biosorption, biomass productivity and stress tolerance of the engineered algal strains. Under controlled wastewater treatment conditions, the genetically modified strains of Chlorella vulgaris and Scenedesmus obliquus attained about 91.2% nitrate removal, 87.5% phosphate reduction and 78.4% cadmium biosorption efficiency.

Transcriptomic and molecular analyses indicated an enhanced adaptive response to the environment of pollutant stress with significant upregulation of stress-response genes such as NRT2, MT1, SOD1 and HSP70. Furthermore, the engineered algae exhibited improved growth performance, higher chlorophyll content, and higher metabolic activity compared to wild-type strains. These results show the effectiveness of the combination of synthetic biology, genetic engineering and environmental biotechnology for sustainable wastewater remediation. In general, the study emphasizes the great potential of genetically engineered algae to act as green and economical bioremediation agents for nutrient recovery, removal of toxic pollutants and environmental restoration. The combination of advanced genomic engineering and algal biotechnology may play a major role in the development of next-generation sustainable wastewater treatment systems and circular bioeconomy applications.

6. Future Scope

Future studies should focus on integrating multi-omics approaches (genomics, transcriptomics, proteomics, and metabolomics) to better understand the molecular mechanisms of pollutant degradation and stress adaptation in engineered algae. Novel metabolic engineering targets for improving the efficiency of wastewater treatment can be identified using advanced systems biology approaches.

Artificial intelligence and machine learning technologies can further enhance predictive monitoring, process optimization, and real-time environmental assessment of algal bioremediation systems. AI-assisted models can enhance the accuracy of pollutant prediction, optimize photobioreactor conditions, and support automated wastewater management strategies.

Moreover, large-scale pilot studies and industrial implementation are required to evaluate the long-term stability, biosafety and ecological impacts of genetically modified algae under environmental conditions. Development of closed-loop cultivation systems and biocontainment strategies will be critical for mitigation of ecological risks of engineered organisms.

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