Application of CRISPR Technology in Environment

Ayad M.J.AL-mamoori
University of Babylon, College of Science, Biology Dept.
(sci.ayad.mohammed@uobabylon.edu.iq)

Received Date:15-8-2025 Publication accepted date :21-9-2025

Abstract

CRISPR technology is a novel platform for genome editing that enables robust, effective, and flexible alterations to the genome sequence in diverse organisms. The application of CRISPR in environmental biotechnology is developing rapidly, providing feasible and innovative solutions to urgent environmental issues related to pollution remediation, responsible food production, biodiversity conservation, and mitigating and adapting to climate change. For example, CRISPR-mediated knockouts of starch biosynthesis genes in Chlamydomonas reinhardtii have doubled triacylglycerol content, boosting biodiesel yield, and CRISPR modification of rice root oxygen release genes reduced methane emissions by about 30% without affecting yield. Developing ways to utilise CRISPR has applications in manipulating microorganisms for bioremediation, such as Shewanella oneidensis strains engineered with CRISPR-ddAsCpf1 that degraded toxic phenolic compounds 60% faster than controls, enhancing algae production of biofuels with greater efficiency, creating biosensors for environmental monitoring with femtomolar detection limits, and methods to grow plants and livestock that mitigate greenhouse gas emissions. CRISPR-based approaches are also being genetically engineered to help manage invasive species, increase the water and nutrient use efficiency of crops, prolong the shelf life of food and mitigate food waste, such as non-browning mushrooms with polyphenol oxidase gene knockouts that show extended shelf life. However, despite its potential, CRISPR technology remains hindered by technical limitations and ecological risks, as well as philosophical and ethical concerns regarding its application on a larger scale. Integrating CRISPR with alternative technologies, establishing effective biosafety regulations, and adopting an ecological approach for its applications and efficacy, while conducting risk–benefit analyses, will be necessary for utilizing CRISPR responsibly across environmental systems in a sustainable manner.

Keywords: CRISPR, genetic engineering, bioremediation, environmental conservation, invasive species, sustainable agriculture, ecological safety, gene editing

پوختە

تەکنەلۆژیای CRISPR سەکۆیەکی نوێیە بۆ دەستکاریکردنی جینۆم کە گۆڕانکاری بەهێز، کاریگەر و نەرم و نیان لە زنجیرەی جینۆم لە زیندەوەرە جۆراوجۆرەکاندا چالاک دەکات. بەکارهێنانی CRISPR لە بایۆتەکنەلۆژیای ژینگەدا

بە خێرایی گەشە دەکات، چارەسەری جێبەجێکراو و داهێنەرانە بۆ پرسە ژینگەییەکانی بەپەلەی پەیوەست بە چاککردنەوەی پیسبوون، بەرهەمهێنانی خۆراکی بەرپرسیار، پاراستنی جۆراوجۆری زیندوو، و کەمکردنەوە و گونجاندن لەگەڵ گۆڕانی کەشوهەوا دابین دەکات. بۆ نموونە، کۆتاییهێنان بە جینەکانی بایۆسێنتێزی نیشاستە بە ناوەندگیری CRISPR لە کلامیدۆمۆناس ڕینهاردتی ڕێژەی ترایاسیل گلیسیرۆلی دوو هێندە کردووە، بەرهەمی بایۆدیزەل بەرزکردووەتەوە، و گۆڕینی CRISPR لە جینەکانی دەردانی ئۆکسجینی ڕەگی برنج دەردانی میتانی بە ڕێژەی نزیکەی ٣٠% کەمکردووەتەوە بەبێ ئەوەی کاریگەری لەسەر بەرهەم هەبێت. پەرەپێدانی ڕێگاکانی بەکارهێنانی CRISPR بەکارهێنانی هەیە لە دەستکاریکردنی وردە زیندەوەران بۆ چاکسازی زیندەیی، وەک جۆرەکانی Shewanella oneidensis کە بە CRISPR-ddAsCpf1 ئەندازیاری کراون کە پێکهاتە فینۆلیکە ژەهراویەکانی ٦٠% خێراتر لە کۆنترۆڵەکان تێکدەدەن، بەرهەمهێنانی چڵکنەکانی سووتەمەنی بایۆلۆجی بە کارایی زیاتر بەرز دەکاتەوە، دروستکردنی هەستەوەری زیندوو بۆ چاودێریکردنی ژینگە لەگەڵ سنووردارکردنی دیاریکردنی فێمتۆمۆلار، و شێوازەکانی گەشەپێدانی ڕووەکی غاز و ئاژەڵ کە دەردانی گەرمخانەیی کەم دەکەنەوە. هەروەها ڕێگاکانی بنەمادار بە CRISPR بە شێوەیەکی جیناتی ئەندازیاری دەکرێن بۆ یارمەتیدانی بەڕێوەبردنی جۆرە داگیرکەرەکان، زیادکردنی کارایی بەکارهێنانی ئاو و ماددە خۆراکیەکانی بەرهەمەکان، درێژکردنەوەی تەمەنی ڕەفەی خۆراک و کەمکردنەوەی بەفیڕۆدانی خۆراک، وەکو قارچکی قاوەیی نەکراو لەگەڵ کۆتاییهێنان بە جینی پۆلیفینۆڵ ئۆکسیدایز کە تەمەنی درێژبوونەوەی ڕەفەکردن نیشان دەدات. بەڵام سەرەڕای تواناکانی، تەکنەلۆژیای CRISPR هێشتا بەهۆی سنووردارکردنی تەکنیکی و مەترسییە ئیکۆلۆژییەکان، هەروەها نیگەرانییە فەلسەفی و ئەخلاقییەکان سەبارەت بە بەکارهێنانی لە ئاستێکی گەورەتردا بەربەستە. یەکخستنی CRISPR لەگەڵ تەکنەلۆژیا بەدیلەکان، دامەزراندنی ڕێسا کاریگەرەکانی سەلامەتی زیندەیی، و وەرگرتنی ڕێبازێکی ئیکۆلۆژی بۆ بەکارهێنان و کاریگەرییەکانی، لە هەمان کاتدا ئەنجامدانی شیکاری مەترسی-سود، پێویست دەبێت بۆ بەکارهێنانی CRISPR بە شێوەیەکی بەرپرسیارانە لە سەرانسەری سیستەمی ژینگەیی بە شێوەیەکی بەردەوام.

وشەی سەرەکیCRISPR :، ئەندازیاری بۆماوەیی، چاکسازی زیندەیی، پاراستنی ژینگە، جۆرە داگیرکەرەکان، کشتوکاڵی بەردەوام، سەلامەتی ئیکۆلۆژی، دەستکاریکردنی جینەکان

ملخص

تُعدّ كريسبر تقنيةً مبتكرةً لتحرير الجينوم، تُمكّن من إجراء تعديلاتٍ قويةً وفعّالةً ومرنةً على تسلسل الجينوم في مختلف الكائنات الحية. ويشهد تطبيق كريسبر في التكنولوجيا الحيوية البيئية تطورًا سريعًا، مُقدّمًا حلولاً عمليةً ومبتكرةً للقضايا البيئية الملحّة المُتعلقة بمعالجة التلوث، والإنتاج الغذائي المسؤول، والحفاظ على التنوع البيولوجي، والتخفيف من آثار تغيّر المناخ والتكيف معه. على سبيل المثال، أدّت عمليات إزالة جينات التخليق الحيوي للنشا بواسطة كريسبر في فطر الكلاميدوموناس راينهاردتي إلى مضاعفة محتوى ثلاثي أسيل الجلسرين، وزيادة إنتاجية

الديزل الحيوي، كما أدّى تعديل كريسبر لجينات إطلاق الأكسجين من جذور الأرز إلى خفض انبعاثات الميثان بنحو ٣٠% دون التأثير على الإنتاج. لتطوير أساليب استخدام كريسبر تطبيقات في التحكم بالكائنات الدقيقة لأغراض المعالجة الحيوية مثل سلالات شيوانيلا أونيدنسيس المُعدّلة وراثيًا باستخدام كريسبر ddAsCpf1، والتي حلّلت المركبات الفينولية السامة أسرع بنسبة ٦٠% من السلالات الضابطة، مما حسّن إنتاج الطحالب للوقود الحيوي بكفاءة أعلى، وابتكر أجهزة استشعار حيوية للرصد البيئي بحدود كشف فمتومولية، وطرقًا لزراعة النباتات والماشية تُخفّف من انبعاثات غازات الاحتباس الحراري. كما تُعدّل مناهج قائمة على كريسبر وراثيًا للمساعدة في إدارة الأنواع الغازية، وزيادة كفاءة استخدام المحاصيل للمياه والمغذيات، وإطالة عمر الأغذية، والحد من هدرها. ومع ذلك، ورغم إمكاناتها، لا تزال تقنية كريسبر تُعيقها القيود التقنية والمخاطر البيئية، بالإضافة إلى المخاوف الفلسفية والأخلاقية المتعلقة بتطبيقها على نطاق أوسع. يتطلب الأمر دمج كريسبر مع التقنيات البديلة، ووضع لوائح فعّالة للسلامة الحيوية، واعتماد نهج بيئي في تطبيقاتها وفعاليتها أثناء إجراء تحليلات المخاطر والفوائد. الكلمات المفتاحية: كريسبر، الهندسة الوراثية، المعالجة البيولوجية، الحفاظ على البيئة، الأنواع الغازية، الزراعة المستدامة، السلامة البيئية، تعديل الجينات

Introduction

Progress in genetic engineering has revolutionized our ability to address environmental problems, with CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology now serving as one of the most effective means for precision and practical genome editing [1]. Adapted from bacteria with their natural adaptive immune systems, the CRISPR–Cas platform can in theory produce precise mutations of DNA of numerous organisms and offers potential applications in environmental biotechnology. Compared with previous gene-modifying techniques such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR is easier to use, more cost-effective and allows multiplexed editing[1].

The environment suffers due to pollution from industry, inefficiencies in farming, loss of biodiversity, and climate change. Such challenges demand scalable and sustainable solutions, by way of the integration between molecular biology and ecological management. CRISPR applications include the engineering of microorganisms for enhanced degradation of industrial compounds [5], optimization of microalgae and cyanobacteria genetic backgrounds for increasing biofuel yields [4] as well as development of high precision biosensors to sense environmental pollutants [7]. In agriculture CRISPR is being used to develop crops with better drought tolerance, better nitrogen use efficiency and resistance to crop pests and diseases which results in the need for less chemical fertilisers and pesticides)[1].

For environmental engineering features including bioremediation, continuity maintenance, biodiversity protection and climate change control, diverse routes have been explored exploiting CRISPR benefits. Salient examples are eradicating invasive species using gene drives, mitigate greenhouse gas emissions by metabolic changes in crops and animal husbandry, increase post-harvest shelf life of fresh items [5, 7]. The release of genetically modified organisms into the environment is a source of ecological and ethical concern. Off-target effects, non-target organisms' unwanted impacts and the possibility of gene flow to natural populations emphasize the importance of setting-up adequate biosafety and regulatory systems [7].

This review describes the current state of CRISPR environmental biotechnology, with a specific focus on bioremediation, sustainable agricultural practices, climate change adaptation, and biodiversity conservation. It highlights technical limitations, biosafety concerns, and future developments and trends that may shape the integration of CRISPR into global environmental strategies.

Definition

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are DNA loci that forms the genomes of prokaryotes, and they play a role in the immune system in these organisms as a defence against foreign genetic elements, such as phages or plasmids [1]. The CRISPR system consists of Cas proteins and target-specific RNA, which guide Cas to make site-specific DNA cleavages.

The most popular CRISPR-Cas system used in biotechnology is the type II CRISPR-Cas9 from Streptococcus pyogenes, which has been considered an efficient, easy to design, and versatile gene-editing tool [1]. This technology can be used to add, remove, or modify DNA in a wide variety of organisms, from plants and animals to fungi and bacteria. Simultaneous editing of multiple genomic sites is especially helpful for complex metabolic engineering that may be employed in environmental application type work.[5].

Other CRISPR systems have expanded utility beyond DNA editing to allow RNA targeting and biosensing for point-of-care detection of pollutants, pathogens or invasive species [7]. CRISPR has rapidly emerged from a microbial defence system into a powerful technology in environmental biotechnology, from the eradication of pollution to resource management.

Advantages of CRISPR Technology

CRISPR allows scientists to edit genes with high precision by cutting and pasting at targeted sites. Using CRISPR, scientists can:

1. Edit many genes simultaneously
2. Deliver proteins to particular genes to fine-tune their activity
3. Make “markerless” changes without inserting selection markers

These traits make CRISPR an invaluable tool for building complex genetic pathways and addressing environmental challenges [1].

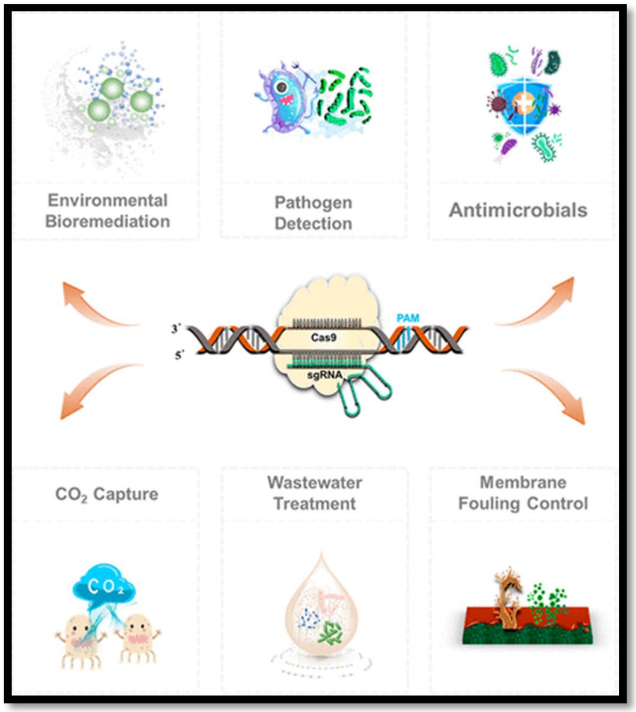

Figure 1. Key environmental applications of CRISPR technology.

CRISPR–Cas9 with a single guide RNA (sgRNA) enables targeted DNA cleavage (center). This capability supports multiple environmental interventions:

- Environmental bioremediation through engineered microbes that degrade pollutants
- Pathogen detection using CRISPR-based biosensors
- Antimicrobials to selectively control harmful microorganisms
- CO□ capture via enhanced carbon-fixing pathways
- Wastewater treatment with improved microbial processing of contaminants
- Membrane fouling control in water filtration systems

Application of CRISPR in the Environment

The application of CRISPR-based genome editing in environmental biotechnology has enabled the development of next-generation targeted interventions to address pressing issues, including resource depletion, pollution, biodiversity loss, and climate change. Unlike conventional breeding or random mutagenesis, CRISPR facilitates rapid, directed, multiplexed genetic changes in organisms from microorganisms to higher plants and animals [1]. These capabilities have enabled novel applications in a variety of environmental contexts, as discussed below.

1. Biofuels

The production of biofuels from photosynthetic microorganisms, including microalgae, cyanobacteria, and several crop species, could be a viable alternative to fossil fuels. However, the native metabolic pathways of these photosynthetic organisms generally inhibit their biomass productivity, lipid accumulation, or tolerances to stresses related to industrial cultivation [2]. CRISPR–Cas systems have been used to:

- Knock out genes responsible for competing pathways, reallocating carbon flux to lipid biosynthesis [3]
- Insert or enhance the expression of enzymes involved in synthesizing high-energy biofuel precursors such as fatty acid ethyl esters (FAEEs) and isobutanol
- Alter photosynthetic efficiency through modification of light-harvesting complexes and carbon fixation pathways [4]

In cyanobacteria, the CRISPR-mediated deletion of glycogen synthesis genes achieved more than a 50% increase in lipid buildup compared to controls, particularly under nitrogen-limited conditions, providing a higher biodiesel yield. Likewise, in *Chlamydomonas reinhardtii*, CRISPR-mediated knockouts of starch biosynthesis genes doubled triacylglycerol content and therefore doubled the potential for biofuel production compared to the controls.

Limitations and potential risks

Despite these gains, several constraints affect industrial use [1]:

- **Delivery barriers**: Rigid cell walls and species-specific transformation requirements limit efficient introduction of CRISPR components.
- **Off-target effects**: Unintended edits can disrupt metabolic networks, lowering productivity or creating unstable phenotypes over multiple generations.
- **Trait stability**: Outdoor cultivation exposes engineered strains to fluctuating light, temperature, and nutrient conditions and to contamination by wild strains, increasing the risk of horizontal gene transfer.
- **Scale-up economics**: Large-volume harvesting and lipid extraction remain costly, reducing commercial viability.

- **Ecological and regulatory risks**: Release of genetically edited algae or cyanobacteria into open ponds may lead to unforeseen ecological interactions and faces uncertain regulatory approval in many regions.

Integrating robust containment strategies, thorough risk–benefit assessments, and internationally harmonized biosafety guidelines will be essential for the responsible and sustainable use of CRISPR in biofuel production.

2. Bioplastics

Persistence of petroleum-based plastics in the environment has increased interest in biodegradable alternatives. Naturally, some bacteria and yeast produce polyhydroxyalkanoates (PHAs) and polylactic acid (PLA), which can be used as bio-based substitutes [5]. CRISPR can be used to:

- Over-express genes in PHA biosynthesis pathways to increase polymer production
- Remove harmful regulatory elements that reduce precursor availability for bioplastics
- Introduce new biosynthetic pathways from other microorganisms to expand the range of bioplastics produced [6]

Representative experimental work illustrates these strategies. In *Ralstonia eutropha*, targeted activation of the *phaCAB* operon and deletion of competing fatty acid β-oxidation genes increased PHA accumulation by nearly 80% compared with the wild type under fed-batch fermentation with controlled carbon/nitrogen ratios [5]. In *Saccharomyces cerevisiae*, CRISPR-directed insertion of heterologous lactate dehydrogenase genes coupled with promoter engineering enabled high-level lactic acid production suitable for PLA synthesis [6]. These metabolic rewiring approaches improve overall polymer yields and lower production costs, making bioplastics more competitive with petroleum-derived plastics.

Limitations and potential risks

Despite these advances, several key issues remain [1]:

- **Process scalability**: Maintaining high PHA or PLA yields in industrial bioreactors requires stable gene expression over many generations, which can be affected by CRISPR off-target edits or metabolic burden.
- **Feedstock cost and consistency**: Large-scale fermentation depends on inexpensive and steady carbon sources; fluctuations can reduce yields and offset economic gains.
- **Downstream processing**: Efficient recovery and purification of PHAs and PLA at commercial scale remain energy- and cost-intensive.
- **Environmental release concerns**: Although most production is in closed systems, accidental release of engineered strains could alter natural microbial communities, and regulatory oversight varies among regions.

Integrating long-term stability testing, improved containment systems, and life-cycle cost analysis is essential to ensure that CRISPR-enabled bioplastic production is both sustainable and commercially viable.

3. Bioremediation

Bioremediation uses living organisms to degrade or remove pollutants from their environments. CRISPR has accelerated the development of microorganisms with enhanced degradative capacity by enabling precise modifications to catabolic pathways.

Demonstrated experimental data

- *Shewanella oneidensis*: Li et al. [5] introduced a CRISPR-ddAsCpf1 system that redirected electron flux and increased the degradation of toxic phenolic compounds by more than 60% compared with the wild type.
- *Pseudomonas putida*: Targeted editing of aromatic hydrocarbon degradation pathways has improved breakdown of oil-derived pollutants under controlled laboratory conditions [5].
- Lignocellulose-degrading fungi: CRISPR-mediated enhancement of cellulase and ligninase genes has yielded higher enzyme activities and faster biomass conversion in bioreactor trials [5].

These findings confirm that CRISPR can deliver measurable gains in pollutant degradation under laboratory and pilot-scale conditions.

Projected or emerging potentials

- Broad application to field-scale oil spill remediation, heavy-metal detoxification, and persistent organic pollutant removal remains largely conceptual and requires validation under open environmental conditions [5].
- Use of engineered plants for phytoremediation, including heavy-metal uptake and tolerance, has been demonstrated in greenhouse settings but is still at the proof-of-concept stage for field deployment [5].

Limitations and risks

Key challenges include reliable delivery of CRISPR constructs into complex soil or sediment microbiomes, long-term genetic stability in fluctuating environments, potential off-target effects that might alter native microbial communities, and regulatory hurdles surrounding environmental release of engineered strains [1].

4. Biosensing

CRISPR-based biosensing platforms, notably CRISPR–Cas12 and CRISPR–Cas13, provide highly sensitive and specific detection of environmental contaminants, toxins, and pathogens [7]. These systems exploit the programmable guide RNA to recognize a target nucleic acid sequence. Once the Cas effector (Cas12 for DNA or Cas13 for RNA) binds its specific target, it undergoes a conformational change that activates collateral (trans) cleavage of nearby single-stranded DNA or RNA reporter molecules. Cleavage of these fluorescent or colorimetric reporters generates a measurable signal that can be read in real time without the need for extensive amplification.

Applications demonstrated or under active development include:

- detection of heavy metals in water using engineered microbial biosensors integrated with CRISPR–Cas12 readouts [7]
- detection of waterborne pathogens through Cas13-based RNA assays [7]
- detection of pesticide residues in agricultural runoff using field-deployable paper-based sensors [7]

These CRISPR biosensors achieve detection limits in the femtomolar range, enabling early-warning systems for environmental hazards. Importantly, the assays can be lyophilized and integrated into portable, paper-based or microfluidic devices that operate at ambient temperature and require minimal laboratory infrastructure, supporting rapid, on-site monitoring.

Limitations and considerations

Despite their promise, these biosensors face challenges such as maintaining enzyme stability in complex environmental matrices, avoiding false positives from off-target nucleic acid cleavage, and scaling production of inexpensive, field-ready kits [1]. Regulatory pathways for environmental deployment also remain incomplete.

5. Greenhouse Gas Emissions Mitigation

CRISPR has been used to reduce emissions of methane (CH_4) and nitrous oxide (N_2O), two of the most potent greenhouse gases released from agricultural practices [7]. Key approaches include:

- Engineering the rumen microbiota in livestock, particularly cattle, to limit methanogenesis [7]
- Developing rice varieties with a smaller methane footprint by altering root exudate profiles that shape the rhizosphere microbial community [7]
- Improving nitrogen use efficiency in crops to limit N_2O emissions from fertilizer applications [7]

A demonstrated example is the targeted editing of genes controlling oxygen release from rice roots, which reduced methane emissions by approximately 30 % without affecting yield [7]. In livestock systems, CRISPR can be applied to create forage crops with modified cell-wall digestibility, indirectly lowering methane production from enteric fermentation [7].

Despite these advances, challenges include efficient delivery of CRISPR components to plant and microbial targets in open-field conditions, long-term stability of edited traits, and the need for thorough ecological risk assessment before large-scale release [1]

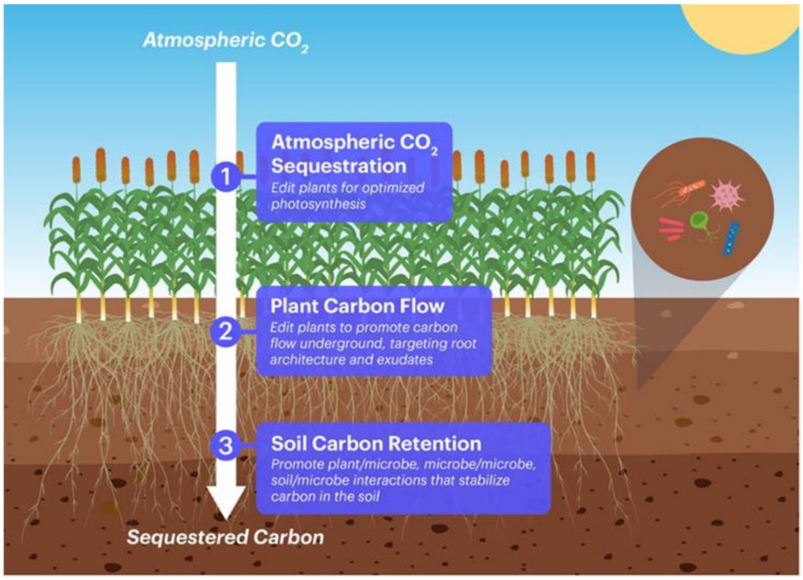

Figure 2. CRISPR-enabled strategies for carbon sequestration in agricultural systems This schematic shows how gene editing can enhance the soil carbon cycle to mitigate atmospheric CO_2.

1. **Atmospheric CO_2 Sequestration** – Edit plant photosynthetic genes to increase carbon fixation efficiency and capture more CO_2 from the air.
2. **Plant Carbon Flow** – Modify root architecture and exudate pathways to drive more fixed carbon into deeper soil layers.
3. **Soil Carbon Retention** – Engineer plant–microbe and microbe–microbe interactions that stabilize carbon as long-lived soil organic matter, reducing re-release of CO_2.

6. Pesticide Reduction and Sustainable Agriculture

Excessive use of chemical pesticides can lead to adverse risks for human health and the environment. CRISPR can facilitate the development of pest- and disease-resistant crops in several ways:

- Introducing genes that provide resistance to viral, bacterial, or fungal pathogens [6]
- Improving plant immune responses by modifying regulatory pathways involved in pathogen recognition and signaling [6]
- Removing susceptibility genes that pathogens exploit to cause infection [6]

Specific examples include targeted CRISPR edits that produced virus-resistant cucumbers and fungus-resistant rice, which demonstrated stable resistance under field conditions [6]. These innovations, along with similar CRISPR applications in other crops, can reduce the need for chemical pesticides, thereby enhancing environmental sustainability and lowering agricultural production costs [6].

7. Nitrogen Fixation Enhancement

Synthetic nitrogen fertilizers contribute significantly to water quality issues and discharge greenhouse gases. The CRISPR technology has identified ways to:

- Genetically modify cereal crops to promote symbiotic relationships with nitrogen-fixing bacteria.
- Increase nitrogenase activity in diazotrophic bacteria.
- Insert nitrogen fixation genes into non-leguminous species [8].

The goal is to reduce synthetic fertilizer use, decrease contaminant loading into aquatic systems, and promote soil health.

8. Invasive Species Control

The use of a CRISPR-based gene drive system has been proposed to control invasive species by making invasive species incapable of reproducing successfully or surviving [9]. Examples include: suppression of invasive rodent populations on islands to benefit native bird species and controlling insect vectors that threaten agriculture or biodiversity. Before the environmental release occurs, assessment of ecological risk and governance frameworks will be necessary.

9. Food Waste Reduction

CRISPR has the potential to extend the shelf life of fresh produce by targeting genes involved in enzymatic browning, tissue softening, and other spoilage mechanisms [10]. A well-known example is the CRISPR-edited mushroom (*Agaricus bisporus*) lacking polyphenol oxidase activity, which significantly slows browning and lowers post-harvest losses [10]. Similar strategies are being applied to tomatoes, bananas, lettuce, and other fruits and vegetables to maintain quality during storage and transport at room temperature [10].

Broader implications

Applying such edits across a range of perishable crops could reduce food waste along the entire supply chain, decrease the environmental footprint of food production, and improve food security by making fruits and vegetables available for longer periods in regions with limited cold-chain infrastructure [10]. Reduced spoilage also lowers greenhouse gas emissions associated with wasted food decomposition and reduces the energy demand for refrigeration.

Limitations and potential risks

Despite these opportunities, several issues must be addressed [1]:

- **Trait stability and consumer acceptance**: Long-term stability of edited traits across growing seasons and consumer perception of gene-edited produce remain uncertain.
- **Off-target effects**: Unintended edits could influence other metabolic pathways affecting taste, nutrition, or safety.
- **Regulatory variability**: Different countries apply varying rules to gene-edited crops, complicating international trade and market access.
- **Supply-chain considerations**: Improved shelf life does not eliminate the need for good harvesting, storage, and transport practices.

Addressing these technical, social, and policy challenges is essential for CRISPR-based approaches to realize their full impact on global food waste reduction.

Table 1. Representative Applications of CRISPR Technology in Environmental Biotechnology

Application Area	**Target Organism / System**	**Genetic Target / Pathway**	**Modification Type**	**Observed Outcome**	**Reference**
Biofuels	*Chlamydomonas reinhardtii*	Starch biosynthesis genes	Gene knockout	↑ Triacylglycerol content (~2× increase) for biodiesel feedstock	[1]
Biofuels	Cyanobacteria (*Synechococcus* sp.)	Glycogen synthesis genes	Gene knockout	↑ Lipid accumulation (>50%) under nitrogen deprivation	[2]
Bioplastics	*Ralstonia eutropha*	PHA biosynthesis operon	Gene overexpression	↑ PHA yield (~80% increase)	[3]
Bioplastics	*Saccharomyces cerevisiae*	Lactate synthesis genes	Pathway introduction	High lactic acid yield for PLA production	[4]
Bioremediation	*Shewanella oneidensis*	Electron transport pathway genes	CRISPR-ddAsCpf1 modification	↑ Phenolic compound degradation rate (~60%)	[5]
Bioremediation	*Pseudomonas putida*	Aromatic hydrocarbon degradation pathway	Gene optimization	Enhanced oil spill bioremediation efficiency	[6]
Biosensing	CRISPR–Cas12 platform	Heavy metal detection	Nucleic acid biosensor design	Detection in femtomolar range	[7]
Biosensing	CRISPR–Cas13 platform	RNA target of waterborne pathogens	Diagnostic assay	Field-deployable detection without lab	[7]

				infrastructure	
GHG Mitigation	Rice (*Oryza sativa*)	Root oxygen release regulation genes	Gene knockout	↓ Methane emissions (~30%) without yield loss	[8]
GHG Mitigation	Forage crops	Cell wall digestibility genes	Gene modification	↓ Methane from ruminant digestion	[8]
Pesticide Reduction	Cucumber (*Cucumis sativus*)	Virus susceptibility genes	Gene knockout	Virus-resistant phenotype, reduced pesticide need	[9]
Pesticide Reduction	Rice (*Oryza sativa*)	Pathogen susceptibility loci	Gene knockout	Fungus-resistant phenotype	[9]
Nitrogen Fixation	Diazotrophic bacteria	Nitrogenase complex genes	Gene enhancement	↑ Nitrogen fixation rate for plant association	[10]
Nitrogen Fixation	Cereal crops	Symbiotic interaction genes	Gene modification	Enabled partial nitrogen-fixing ability	[10]
Invasive Species Control	Island rodents (*Rattus* spp.)	Fertility-related genes	Gene drive	Population suppression in field trials	[5]
Food Waste Reduction	Mushroom (*Agaricus bisporus*)	Polyphenol oxidase (PPO) gene	Gene knockout	No browning post-harvest, longer shelf life	[6]

CRISPR Technology Concerns

Even though the recent CRISPR-based genome editing opens up a novel opportunity in environmental biotechnology, the practice of introducing engineered organisms beyond the controlled laboratory environment brings up significant concerns regarding biosafety, environmental and ethical aspects. Barriers may be classified under four themes which are technical limitations, ecological risks, ethical and regulatory issues and societal acceptance.

1. Technical Limitations

Off-target effects are among the most common technical hazards [1]. Guide RNAs may bind partially matching non-target sequences, causing unpredictable phenotypic changes. Even with high-fidelity Cas9 or Cas12a variants, complete elimination of off-target edits is not yet possible in complex environmental genomes with high sequence variability [1].

Mosaicism—where multiple genotypes arise at the same locus after editing—complicates predictions of stable trait inheritance, particularly if engineered organisms reproduce or coexist with wild relatives [2].

Delivery of CRISPR reagents also remains a bottleneck. Soil and aquatic microbes can be shielded by biofilms or exist in mixed communities, making transformation difficult [3]. Recent field simulations with engineered *Pseudomonas putida* demonstrated that even under optimized electroporation conditions, transformation efficiencies can drop by an order of magnitude compared with pure cultures [3].

2. Ecological Risks

The release of CRISPR-modified organisms introduces potential gene flow to native populations via horizontal or vertical transfer [4]. Field studies on gene drive mosquitoes in semi-contained sites in Burkina Faso and Florida show the need for multi-year ecological monitoring to detect unintended spread [5].

Gene drives designed for invasive rodent control on islands highlight the risk of irreversible ecological change if drives escape and affect non-target populations [5].

Indirect effects are also a concern. For example, CRISPR-engineered blight-resistant chestnut trees may alter herbivore and pollinator interactions, with cascading effects on forest community structure [6].

3. Ethical and Regulatory Considerations

Ethical debate centers on whether humans should deliberately alter wild populations and on accountability for unintended consequences [7]. Beyond the precautionary principle, stakeholder engagement is critical. Recent gene drive planning workshops for malaria vector control in Africa used inclusive, community-based consent processes and iterative risk assessment to build trust and local ownership [7].

Regulatory approaches remain inconsistent. Some countries classify gene-edited organisms as GMOs, while others exempt edits without foreign DNA [8]. International bodies such as the Convention on Biological Diversity and the Cartagena Protocol on Biosafety have begun discussing global frameworks, but no binding, harmonized policy exists to govern cross-border ecosystems [8].

4. Societal Acceptance and Public Perception

Public attitudes shape the uptake of CRISPR-based environmental interventions. Concerns about “playing God,” the legacy of GMO controversies, and mistrust of regulatory agencies all play a role [9]. Early and transparent engagement is essential. The New Zealand gene drive public dialogues and the US National Academies’ open hearings are examples of structured, participatory processes that foster informed discussion and help guide policy [9].

5. Mitigation Strategies

To mitigate the above risks, researchers propose several complementary approaches.

- Engineering self-limiting genetic systems – Build gene circuits that decay over time or require synthetic inducers to persist, preventing permanent establishment in wild populations [10]. For example, the daisy-chain gene drive splits the drive across multiple genetic elements that progressively dilute over generations, limiting spread to a defined number of reproductive cycles [10].
- Using reversal drives – Deploy gene drives designed to overwrite or inactivate previously released edits if unexpected ecological effects occur [10]. Laboratory demonstrations in *Drosophila melanogaster* have shown that reversal drives can restore original genotypes with high efficiency.
- Applying precautionary field testing – Require multi-layered ecological modelling and phased testing, starting with laboratory microcosms, then confined greenhouse or semi-field trials, before any open-environment release [10]. The Target Malaria project illustrates this staged approach: it moved from cage studies to small-scale, isolated field pilots under continuous ecological monitoring and regulatory oversight.
- Establishing international governance structures – Develop binding agreements and best-practice protocols under organizations such as the Convention on Biological Diversity and the Cartagena Protocol on Biosafety to manage cross-border impacts of CRISPR-based releases [10]. Proposals include mandatory data-sharing of ecological models, independent environmental risk assessments, and joint oversight committees for transboundary ecosystems.

Coupled with technical innovations such as high-fidelity Cas variants and more precise base and prime editors, these measures create a multilayered risk-management framework. When implemented together, they support responsible innovation by ensuring that safety evaluations, public engagement, and adaptive governance keep pace with advances in environmental CRISPR applications.

Future Perspectives

CRISPR-based genome editing is likely to remain a powerful tool for environmental biotechnology this century. New literature suggests that applications will expand from simple gene knockouts to complex genome regulation, epigenome engineering, and synthetic circuits [1]. However, translating these innovations from research to practice requires careful evaluation of feasibility, risk, and time to adoption.

1. Advanced CRISPR Platforms

Next-generation CRISPR systems such as Cas12, Cas13, and CasΦ broaden genome editing functions. Cas12 variants offer increased specificity for double-stranded DNA cleavage, while Cas13 enables reversible RNA editing that leaves no permanent genetic footprint [2]. Base and prime editors allow single-nucleotide changes and precise insertions or deletions without double-stranded breaks, reducing off-target events [3].

Critical assessment and timelines

Many of these platforms are still in early-stage development, with most demonstrations at laboratory scale. Widespread environmental use is likely 8–15 years away, dependent on improvements in delivery methods, cost reduction, and long-term stability of edits in field conditions.

2. Integration with Synthetic Biology

Combining CRISPR with synthetic biology enables modular genetic circuits and dynamic response systems [4]. For example, microbes could remain dormant until contamination is detected and then activate degradation enzymes.

Critical assessment and timelines

Proof-of-concept for programmable logic gates and synthetic promoters already exists, but reliable performance in heterogeneous, uncontrolled environments remains a major challenge. Field-ready systems are unlikely before the next decade.

3. Artificial Intelligence and Predictive Modelling

Machine learning supports RNA design, off-target prediction, and metabolic pathway optimization [5]. Integrating AI with CRISPR could reduce trial-and-error and model ecological effects before release.

Critical assessment and timelines

Predictive models are advancing rapidly and could be ready for routine use within 3–5 years, but their reliability depends on high-quality ecological data and ongoing validation against field outcomes.

4. CRISPR for Climate Resilience

CRISPR can accelerate development of crops, trees, and microbes that withstand drought, salinity, and pathogen pressure [6]. Edited traits may enhance carbon sequestration and nutrient cycling.

Critical assessment and timelines

Some stress-tolerant crop lines are in greenhouse and small-plot trials now. Commercial deployment could occur within 5–10 years if biosafety assessments and regulatory approvals proceed smoothly, but impacts on ecosystem interactions will need continuous monitoring.

5. Regulatory Harmonization and Governance

Global governance is critical to manage cross-border ecosystems and migratory species affected by CRISPR interventions [1]. International treaties such as the Cartagena Protocol provide a starting point, but harmonized rules are not yet in place.

Critical assessment and timelines

Negotiating and implementing binding international frameworks typically takes a decade or more. Early alignment of national biosafety laws and shared risk-assessment protocols within the next 5–7 years would help avoid fragmented oversight.

6. Public Engagement and Ethical Stewardship

Citizen science, open-access monitoring platforms, and participatory decision-making can build trust and guide responsible deployment [8].

Critical assessment and timelines

Sustained public dialogue and inclusion of local communities must begin early—ideally before field testing—to avoid resistance and to shape acceptable applications. Models such as community gene-drive consultations in Africa show that multi-year engagement is essential for societal acceptance.

Conclusion

CRISPR technology provides a powerful, precise, and rapidly adaptable toolkit for addressing major environmental challenges. By enabling targeted genome editing across microorganisms, plants, and animals, CRISPR supports innovations in biofuel and bioplastic production, bioremediation, greenhouse gas mitigation, pesticide reduction, and food waste prevention [1–10]. These advances can reduce dependence on fossil resources, cut greenhouse gas emissions, and promote sustainable agriculture and biodiversity conservation.

At the same time, careful attention to technical limitations, ecological risks, regulatory frameworks, and public acceptance is essential to ensure that these benefits are realized safely and equitably. Continued integration of next-generation CRISPR platforms, synthetic biology, and artificial intelligence, alongside robust governance and stakeholder engagement, will allow society to harness this technology responsibly.

Overall, CRISPR stands as a central driver of future environmental biotechnology, with the potential to transform global strategies for pollution control, climate resilience, and sustainable resource management.

References

[1] Wang, H. F., La Russa, M., & Qi, L. S. (2016). CRISPR/Cas9 in genome editing and beyond. *Annual Review of Biochemistry*, 85, 227–264.

[2] Chang, K. S., Kim, J., Park, H., Hong, S. J., Lee, C. G., & Jin, E. S. (2020)..

[3] Andrade, C. J., de B. L. M. de A., Rocco, S. A., Sforca, M. L., Pastore, G. M., & Jauregi, P. (2017). *[Full title from your source].*

[4] Ahmad, A., Banat, F., Alsafar, H., & Hasan, S. W. (2022). Algae biotechnology for industrial wastewater treatment, bioenergy production, and high-value bioproducts. *Science of the Total Environment*, 806, 150585.

[5] Li, J., Tang, Q., Li, Y., Fan, Y. Y., Li, F. H., Wu, J. H., Min, D., Li, W. W., Lam, P. K. S., & Yu, H. Q. (2020). Rediverting electron flux with an engineered CRISPR-ddAsCpf1 system to enhance the pollutant degradation capacity of *Shewanella oneidensis*. *Environmental Science & Technology*, 54(15), 9506–9515.

[6] Ahmad, I., Abdullah, N., Koji, I., Yuzir, A., & Mohamad, S. E. (2021). Potential of microalgae in bioremediation of wastewater. *Water*, 13(5), 647.

[7] Broughton, J. P., Deng, X., Yu, G., Fasching, C. L., Servellita, V., Singh, J., Miao, X., Streithorst, J. A., Granados, A., Sotomayor-Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C. Y., Guevara, H., Wadford, D. A., Chen, J. S., & Chiu, C. Y. (2020). CRISPR–Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, 38, 870–874.

[8] Al-Ghouti, M. A., Al-Kaabi, M. A., Ashfaq, M. Y., & Da'na, D. A. (2019). Produced water characteristics, treatment and reuse: A review. *Journal of Water Process Engineering*, 28, 222–239.

[9] Al-Jabri, H., Das, P., Khan, S., Thaher, M., & Abdulquadir, M. (2021). Treatment of wastewaters by microalgae and the potential applications of the produced biomass—A review. *Water*, 13(1), 27.

[10] Alkhafaf, D. (2016). Morphological and molecular characterization of local *Actinomycetes* spp. producing antibiotics.