Green opportunities for brown waters: potential of gene editing in wastewater bioremediation

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Key messages

- Gene edited organisms can make wastewater treatment more energy, time and cost efficient, and further reduce risk of environmental pollution and health hazards from untreated wastewater.
- Bacteria, white rot fungi, and algae are promising organisms for gene editing in wastewater treatment technologies, of which genome, traits and behavior must be further understood.
- Controversy around gene editing is mainly due to lack of standardized consensus on what gene editing is, and scandals and/or scepticism around the technology.
- Research must address uncertainties regarding unintended off-target gene editing, which will help clarify regulatory approaches and safety assessment procedures.
- Coordination of local governments, federal regulatory entities, trained local personnel and long-term planning are central when implementing new wastewater treatment technologies.
- Further research and implementation of gene editing technologies in wastewater treatment should not hinder construction of conventional wastewater treatment facilities.
- Gene editing technologies in wastewater treatment should be considered in the analysis of alternatives once it is consolidated and the capacities to implement and operate are set.

Wastewater treatment

Natural bodies of water around the world are becoming increasingly contaminated due to wastewater disposal, limiting availability of clean water[1]. Inadequately treated wastewater (WW) has caused significant hazardous impacts on human health and the environment [2–4] which could be addressed by implementation of novel technologies like gene edited (GE) organisms in WW treatment[4] (Fig 1). Currently, most commonly used WW treatments are expensive to install, maintain, and require large amounts of energy and/or space to run[5,6]. One of the biggest challenges is varying quality of incoming effluent into WW treatment facilities[7], requiring adaptable responses in WW treatment facilities operations[8,9]. GE technology has potential to make WW treatment facilities more efficient by tailoring treatment to influent source, potentially reducing treatment time and operational costs[10], and the need for physical and chemical treatments[2,3,11]. GE bacteria can also be used to recover heavy metals from WW, which can then be returned to the market[12,13].

Brief introduction to gene editing (GE)

GE is a trending technology in many industries, including health and agriculture, and is expanding to sectors such as bioremediation[11]. This genetic tool enables the switching on and off certain genes, a quicker and more precise process than extensive natural selection[14]. GE organisms differ from genetically modified organisms (GMO) in how the genome in edited, potential consequences of those edits, and how they are regulated within regions[15] (see A5). Briefly, GE uses only the existing genome of the organism, meaning that genetic material from other species is not added. Alternatively, in GMOs, genetic material from an unrelated species is added, in a way that does not occur naturally. However, there are differing, converging, and contradicting definitions of GE and GMO between countries that has led to confusion over how GE should be regulated[15] (see A5).

Potential risks when using GE include off-target mutations (unintended genome modifications), gene drive initiation (biased gene inheritance), and the ecological effects of those changes[15,16]. For example, if GE organisms encounter wild-type organisms and then alters the populations within an ecosystem. However, as GE precisely adds, deletes or modifies genes from the genome of the same organism[15], any features inserted or deleted will most likely disadvantage the organism compared to the wild-type outside of an enclosed treatment facility[13]. Questions surrounding safe and ethical use of GE technology preserves a continuous and necessary debate on how to maintain high levels of protection while enabling innovations that contribute to environmental and human wellbeing[17].

This brief investigates GE organisms that show potential in WW treatment processes to reduce water pollution and enhance efficiency. It will also consider selected countries that have potential and need for this novel WW treatment technology and discuss changes needed in governance for successful implementation of a GE pilot project.

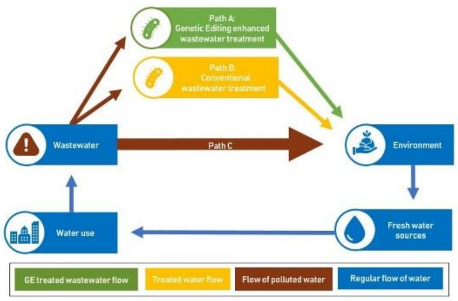

Figure 1. *A simplified water cycle, highlighting the importance of proper wastewater treatment. Untreated wastewater has negative effects on the environment and on freshwater sources.*

Brief introduction to the selected GE organisms

Three promising organisms with GE potential in WW treatment were selected to compare potential avenues of implementation: bacteria, white rot fungi, and microalgae. Bacteria are commonly used in existing WW treatment facilities via activated sludge and studied as model organisms[18–20]. White rot fungi are increasingly used in bioremediation of industrial waste or accidents with oil spills and can be engineered to increase those capabilities[21–23]. Microalgae are often used in bioreactors for WW treatment, farmed for various compounds, including biogas, and commonly edited for greater biogas yield[24–27]. For more details about these GE organisms and their potential use in WW treatment (see A6).

Analysis of GE organisms

A multi-criteria analysis (MCA) was conducted to assess GE bacteria, white rot fungi, and microalgae. Literature on their applications was compared to that of existing WW treatment technology and scored from 0-5 on five different criteria (Fig 2). Due to the novelty of GE technology, accurate evaluation of investment and implementation costs is not possible and were not considered for this MCA, though possible funding sources for implementation can be found in potential cases. Moreover, this analysis of GE technology is a look at current and short-term projections, and as such the way each organism can be edited to achieve a full score for all criteria may change as our understanding of them and how they can be edited increases.

The analysis suggests that microalgae has most potential for implementation in WW treatment when compared to bacteria and fungi (Fig 2). Microalgae scored higher for (i) operational emissions and (ii) pathogen reduction, as it was determined that inclusion of GE bacteria and white rot fungi into WW treatment would not significantly change how WW treatment facilities work in those aspects. However, as microalgae is farmed in some WW treatment facilities for biogas it could act as an additional carbon sink by providing a source of energy and negating some emissions[25,27,28]. Additionally, microalgae showed potential to reduce pathogens in WW treatment at a higher rate than conventional treatment[29]. In terms of ability to treat pollutants in WW, bacteria and microalgae performed similarly but white rot fungi scored lower due to its restriction to specific pollutants (e.g., heavy-duty industrial pollutants)[21,30,31]. Bacteria and microalgae are used in many conventional WW treatment technologies, so their higher score reflects potential improvement on their existing use[26,32–34]. White rot fungi scored lower for use in general municipal waste but should be considered in specific cases where industrial waste is an issue [21,30,31].

Selected organisms scored low in ease of implementation and maintenance since they all require highly controlled environments. Bacteria requires slightly more maintenance due to greater sensitivity to changes in its environment, such as pH and salinity[9]. Finally, for implementation of all selected. GE organisms into WW treatment operations, secondary WW treatment infrastructure should be available[6].

Potential cases: addressing a need via implementation in promising countries

Potential areas for implementation were identified by using the SDG 6 Data Portal from UN Water[4]. Global maps of indicator 6.3.1 for wastewater flow treated and wastewater flow collection were used to find countries which need WW treatment technology (less than 50% of wastewater flow

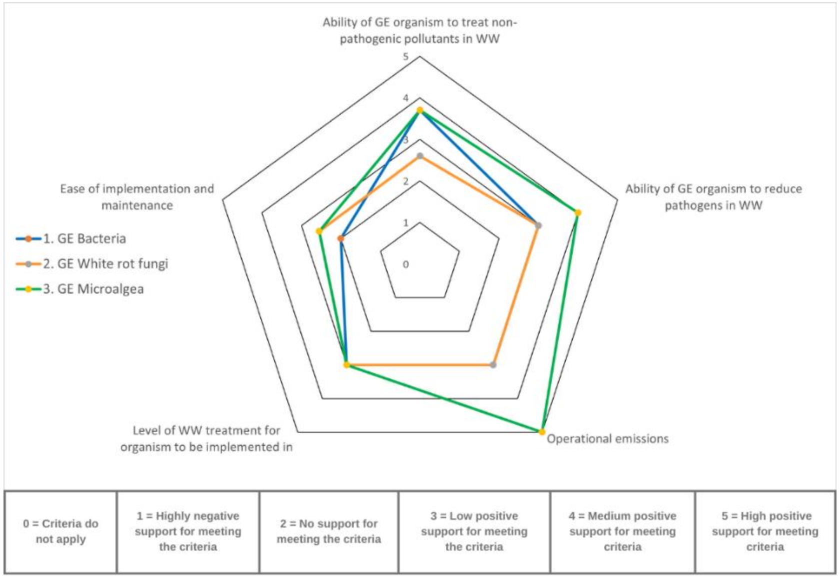

Figure 2. *Radar graph showing results of MCA comparing effectiveness of gene edited bacteria, white rot fungi and microalgae application in wastewater treatment compared to current wastewater treatment. Data from* [9,28–30,32,34–57] *was used for scoring (see A7 for comprehensive scoring analysis).*

treated) and have capacity for implementation of the technology (more than 50% of wastewater flow collected)[4].

Mexico, Brazil, and Egypt are countries that fit those conditions, meaning, they could benefit from wastewater treatment innovations. As introduction of novel technologies such as GE requires understanding and dialogue between regulators, NGOs (Non-Governmental Organizations), scientists, industry, and the public[11], this section aims to discuss (i) current state of WW treatment, (ii) actors involved in implementation of new WW treatment technology, and (iii) perceived openness toward GE technology in each country (A8 for results and A9 for further details).

Current state of WW treatment

A primary issue in current WW treatment operations in selected countries is lack of budget and trained technical personnel (A8, A9). Knowledge on operation and maintenance of WW treatment technologies is key to ensure continued running of WW treatment facilities[58–60]. Therefore, training of operators and clear communication between technology provider and implementor is vital for the application of new WW technologies. Additionally, local communities are often included in these projects through consultation, but further integration should be an aim, especially when indigenous communities are within project reach[41,59].

Main stakeholders involved in implementation

Implementation depends on ability of local government, federal regulation entities, and trained personnel to create a robust long-term project (see A8, A9). However, some systemic issues in selected countries need to be addressed. For example, Mexico and Brazil have issues with long-term planning due to personnel turn-over each election, and Brazil struggles with further ambiguity amongst governmental jurisdictions[61]. Another issue is pricing of sanitation service, for example in Mexico the budget required and the users' payment capacity is not necessarily consistent[61], and in Brazil, there is a lack of standardization of tariffs[62].

Openness toward GE technology

In selected countries, institutional, journalistic, and academic publications suggest interest in GE (specially on its applications for agriculture and medicine), either prospectively or by current applications[63–73]. However, controversy on use of GE remains, mainly due to lack of a standardized consensus on what the technology is [63,67,72,73], and scandals or scepticism over its use [69–71,74–76] (for further detail, see A8 and A9).

Final considerations

This policy brief is based on literature and expert perspectives, not pilot projects, and as such does not make conclusions about feasibility or large-scale effectiveness of using GE organisms for WW treatment. **Further research on specific modifications and small-scale pilot projects**

should be conducted before large-scale implementation of GE technology.

Implementation of GE presents challenges regardless of organism, simply because **GE organisms require expertise, continued maintenance, and monitoring of WW treatment facilities.** Therefore, it is vital to ensure continued maintenance of conventional WW treatment facilities and train local personnel before implementing a more complex technology.

Recommendations

Three types of recommendations were built for (i) international organizations, (ii) national institutions, and (iii) technical suggestions for pilot projects.

For international organizations:

1. International organizations (e.g., UNCTAD-CSTD) should aim to standardize the definition of terms associated with gene technology such as GMO and GE, and subsequently set guidelines for their regulation.
2. Further research on behavior, traits, and risks of GE organisms needs to be conducted and communicated to the general public.

For national institutions:

1. Regulations surrounding GE should be updated to reflect its potential and risks:
    a. Local safety and operation standards should be in line with international standards based on research findings on possible use and risks of GE in WW treatment.
    b. Regulatory bodies should monitor implementation of gene technologies and have clear jurisdictions to avoid unintended institutional overlap.
2. Coordination of local governments and federal regulatory entities, availability of trained local personnel and establishment of a long-term management plan must be in place when implementing new WW treatment technology.
3. Potential implementation of GE technologies in WW treatment should not prevent construction of WW facilities by current technologies.

Technical suggestions for pilot projects:

1. Pilot projects should be undertaken to determine feasibility and management risks of using GE organisms in large-scale wastewater treatment.
2. Potential risks of implementing GE technology should be evaluated, monitored, and reported by determining: (i) comprehensive gene sequencing of target organism, (ii) precision of genetic edit and identification of unintended off-target edits, and (iii) safeguards to avoid gene drive initiation.
3. Containment within an enclosed system, kill switches, or other safeguard mechanisms must be employed to avoid unintended effects of organisms escaping into the natural environment.
4. Multiple pilot projects covering different conditions (e.g., source of WW, volume, flux, composition of WW, etc.) must be conducted to ensure feasibility of GE-enhanced WW treatment before any large-scale implementation can be considered.

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Appendices

A1. Glossary: Definitions, acronyms, and abbreviation

Term	Description
Enclosed treatment facility	A closed facility for treatment of wastewater to prevent the release of harmful wastewater into the environment during or after the treatment process.
GE	Genome or Gene Editing – "a technique that adds, deletes, or modifies precisely and site-specifically genes from the genome of an organism. The additions are from plants or animals with which the original subject can reproduce. The resulting organism could be obtained via conventional breeding, which uses natural hybrids" [77].
GMO	Genetically engineered/modified organisms – organisms "with DNA modified using genetic material from an unrelated species to confer some benefits" [77] in a way that does not happen in nature or through natural selection.
MCA	Multi Criteria Analysis.
SDGs	Sustainable Development Goals.
Untreated Wastewater	Wastewater discharged without any wastewater treatment.
Wastewater collection	Infrastructure and equipment used to collect and direct flow of wastewater to and from areas for treatment. The collection of wastewaters does not include treatment.
WW	Wastewater, both municipal and industrial.
WW treatment facilities	Wastewater Treatment Facility - a plant or premises used to treat industrial wastewater or domestic wastewater or any combination of industrial wastewater and domestic wastewater.

A2. List of subject matter experts interviewed

Table 1 - List of subject matter experts interviewed including date of interview, expertise, research field and country in which expert is currently conducting research.

Expert	Date	Expertise	Field/Prestige	Country
Alette Lagenhoff	09/11	Biological Wastewater treatment	PhD in WW treatment	Netherlands
Thomas Wagner	15/11	Wastewater Treatment by constructed wetlands	PhD in WW treatment	Netherlands
Maxime Mowe	23/11	Algal blooms	PhD in plankton and algal blooms	Singapore
Maria Eugenia de la Pena	17/11	Wastewater Treatment in Mexico and LATAM	Institutional expert on WW treatment	Mexico
Luis Alberto Arellano Garcia	02/12	Wastewater Treatment in Mexico	PhD in WW treatment	Mexico
Dominik Schild	25/11	Fermentation development, Biochemical Engineering, Process Engineering	Professor science and technology (Krems University of Applied Science)	Austria
John Van Der Oost	12/12	Biotechnology, Genetics, Genomics, Microbiology, Virology, Bacteria, Mutagenesis, Metabolomics, Archaea, Gene discovery	Professor – department of agrotechnology and food sciences	Netherlands
Raymond Staals	14/12	Microbiology	Professor – department of agrotechnology and food sciences	Netherlands

A3. Water pollution and wastewater treatment

Wastewater (WW) treatment is the process of removing contaminants from sewage or other used water so they can be converted into effluents and safely returned to the water cycle with minimal environmental impact[78]. Effective wastewater management helps protect freshwater systems, the oceans and human health, as harmful pathogens, nutrients, and other types of pollution are prevented from entering the environment. The quality of wastewater effluent is typically measured by physical and chemical parameters like chemical oxygen demand, dissolved organic carbon, levels of nitrogen and phosphorus, total dissolved solids, and coliforms. However there has been increasing awareness of microplastics and other emerging pollutants in water that could also have harmful effects.

Rapid urbanization has potential to accelerate water insecurity and water pollution, though medium-sized cities have been found to be most polluting as large cities have better environmental governance[79,80]. However, natural water around the world is becoming increasingly contaminated by wastewater disposal, increasing scarcity of clean water, especially in rapidly expanding urban areas[1]. On average, a person produces 400-500l of urine and 25-50kg of feces every year, which contain pathogens and parasites that are responsible for a variety of illnesses in developing countries near places where people and animals live and next to or into drinking water sources[3]. Furthermore, it is a common practice for WW treatment facilities to mix urban and industrial wastewater that contains toxic pollutants, which has led to evolution of bacteria with resistance to both antibiotics and heavy metals in the effluent[81]. Overall, this has led to an alarming situation for people's health and environment, especially in the Global South. Treated wastewater is also commonly used for agriculture in dry areas to overcome water scarcity, compounding potential risks of contamination[1].

Microplastic pollution has been a growing concern as scientists learn about their ability to absorb other pollutants and increase their toxicity, remain in the environment for long periods of time, and enter the food chain[82,83]. WW treatment facilities are considered an important pathway for microplastics to enter aquatic environments[83]. Although up to 99% of microplastics can be removed by conventional treatment, considerable amounts of microplastics are still released into aquatic environments after wastewater treatments due to sheer amount of discharge[82,83].

Emerging pollutants are a series of new contaminants that are considered dangerous at low concentrations. These include substances like pharmaceuticals, drugs, personal care products, and endocrine disruptors that may persist after treatment, be toxic, and bioaccumulate[84]. Pharmaceuticals and personal care products are of particular interest due to their current lack of regulation and capacity to induce physiological effects on humans at low doses[84]. Concentrations of emerging pollutants have been found to be increasing in aquatic environments due to bioaccumulation, causing potentially serious damage to ecosystems as well as increasing antibiotic resistance which could pose huge potential health risks[84].

A4. WW treatment steps

Wastewater contains pathogens and parasites that are responsible for a variety of diseases in developing countries[3] and causes millions of deaths every year[2]. Releasing large amounts of untreated wastewater causes significant hazardous impacts on human health and the environment.

Wastewater (WW) treatment is the process of removing contaminants like pathogens or pollution from sewage or other used water so they can be converted into effluents and safely returned to the water cycle with minimal environmental impact[78]. WW treatment is often very effective, but is also expensive to install, requires large amounts of energy to run and has high operational and management costs[5,6]. One of the biggest challenges of effective WW treatment is the quality profile of incoming effluent to a WW treatment facility, which can be impacted by weather, climate, rainfall, population, and socioeconomic activities in the area[7]. That is, different characteristics of influent require different and adaptable responses in WW treatment facilities operations[8]. Table 1 explains different levels of WW treatment that could be in a WW treatment facility.

Table 2 - Comparison of conventional wastewater treatment levels and examples of technology used at each level

Wastewater treatment levels		**Example**
Preliminary	Removal of coarse suspended solids	Grit Chambers, Grinders, Screens / Bar racks
Primary	Removal of sedimentary solids and organic matter by gravity	Flotation Systems, Primary sedimentation tanks, Neutralisation Tanks, Equalisation Tank
Secondary	Removal of particulate matter, etc	Aerobic processes (biological nitrogen and phosphate removal, activated sludge process, constructed wetland, waste stabilisation pond) Biological Treatment, Anaerobic processes
Tertiary	Removal of nutrients, soluble minerals, non-biodegradables and other micropollutants	Adsorption, membrane filtration, membrane distillation, Solvent extraction

A5. GE vs GMO

GE technology is distinct from GMO (genetic modification); however, it is often included under the GMO definition. Different authorities have different regulations regarding the two technologies: for example, US Department of Agriculture distinguishes GE from GMO provided that the GE technique does not introduce "novel" DNA into the organism, whereas the European Union does not distinguish between GE and GMO and has ruled all GE organisms fall under existing GMO directives[85]. Table 2 further explores differences between GE and GMO techniques and their associated risks.

Table 3 - Gene editing vs Genetic Modification, definition, environmental risks, type of modification and risk management. Information from [15,77].

	Gene editing	**Gene modification / engineering**
Definition	"A technique that adds, deletes, or modifies precisely and site-specifically genes from the genome of an organism. The additions are from organisms with which the original subject can reproduce. The resulting organism could be obtained via conventional breeding, which uses natural hybrids" [77].	A technique that modifies an organism "with DNA using genetic material from an unrelated species to confer some benefits" [77] in a way that does not happen in nature or through natural selection.
Main environmental risks	Off target mutations, gene drive	Gene leakage, horizontal gene transfer, vertical gene transfer

Type of modification / change to genome[15]	Transgene-driven change (stable or transient) - subtle mutation to native genes [15].	Ribonucleoprotein (RNP)-driven change: Precise mutation to native genes only[15].	Transgene - Stably integrated foreign gene[15].	Cisgene - Stably integrated gene: from same species or closely related[15].
Risk and management	“No foreign gene where gene editing nuclease is transiently expressed. Risk of spread of transgene or modified organism. Changes to native genes”[15].	“No foreign gene. Risk of spread of modified organism. More precise genetic changes than chemical, radiation, or UV mutagenesis”[15].	“Spread of foreign transgene or modified organism in the environment. Risk of harm of transgene product”[15].	“Spread of modified gene or organism in the environment. Risk of harm of cisgene product”[15].
Ruling of Non-GMO outside Europe[15]	Yes[15]	Yes[15]	No[15]	Yes[15]

A6. Detailed description of the organisms with GE potential for WW treatment

Wastewater facilities employ a wide range of natural organisms to decompose the inflowing material into its core elements. This miniature ecosystem is stimulated to be of the desired composition by controlling conditions present in the facility. Much measuring goes into assuring that this diverse bioreactor has right flux and decomposition rates. Specific groups of organisms with potential to be enhanced using GE have been identified through conducting interviews and reading academic literature. For clarity three classes of interest were identified, bacteria, white rot fungi and microalgae.

Bacteria - an example of bacteria typically found in bioremediation processes is *E. coli,* which, because of its simplicity, is a model organism for bioengineering. This simple bacterium is present in the lower gut and is therefore also present in fecal matter. This fast growing, easy to reproduce in vitro organism, is considered the best studied bacterium, and many efforts have been undertaken to change its genetic code[20,26,33,36]. Some examples of using GE bacteria for bioremediation include modifying *Deinococcus radiodurans* mercury resistance in its merA gene to treat radioactive waste sites from nuclear weapons[86], *E. coli* expressing EC20 for Cd and Hg resistance[87,88] and overexpressing ELP153AR for treating As in contaminated ground and drinking water[89], and *P. putida* expressing EC20 to treat Cd contamination[90].

White rot fungi - widely employed in bioremediation studies for their ability to produce peroxidases and other highly reactive enzymes that can break down very stable molecules. These fungi decompose lignin, the main component of woody material which is notoriously hard to degrade. Previous efforts have used white rot fungi in bioremediation on oil spills[30] and it has been studied for its effectiveness of breaking down novel resistant synthetic molecules [21,31,51,52]. Their genes are still being studied and sequenced, but there has been a study on the role of the CYP450S genes in *Phanerochaete* sp. for bioremediation of petrochemicals[91], pharmacochemicals[92–94], and endocrine-disrupting chemicals[95]. These studies provide basis for future research into increasing their bioremediation capacity through GE.

Microalgae - of interest because of the symbiotic relations they have with other organisms, enabling researchers to employ microalgae as the carbon source whilst other organisms biodegrade. Employing GE to manipulate microalgae into desired phenotypes has been undertaken [15,25,27], but limited efforts have been undertaken in employing this into bioremediation. Some examples of GE microalgae used in bioremediation include knockdown of CrPEPC1 gene in *Chiamydomas reinhardtii* to increase carbon uptake and lipid production[96] and overexpression of CrMTP4 gene in *Chiamydomas reinhardtii* to treat Cd contamination[97].

A7. Detailed description of the MCA

‘Ability of GE organism to treat pollutants in WW’ was divided into two criteria: ‘typical wastewater indicators’ and ‘microplastics and emerging pollutants,’ with ‘typical wastewater indicators’ having a weightage of 70% and ‘microplastics and emerging pollutants having a weightage of 30% to reflect current priorities in WW treatment needs. ‘Typical wastewater indicators’ was further subdivided into four measurements with equal weightage that reflected indicators typically measured for WW treatment: ‘BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) reduction’[38], ‘Nitrogen and Phosphorus reduction’ [39], ‘heavy metals’ [40], and ‘pathogens’ [41,42]. For ‘BOD and COD reduction,’ data from [30,32,34] was used for scoring. For ‘Nitrogen and Phosphorus reduction,’ data from [43,44] was used for scoring. For ‘heavy metals,’ data from[34,36,45,46] was used for scoring. For ‘pathogens, data from [47] was used to score microalgae. However, no data was found on the other two organisms for that measure, and it was assumed that there would be no change in effectiveness from existing WW treatment. ‘Microplastics and emerging pollutants’ was also divided into two measures: ‘microplastics’ [48] and ‘pharmaceuticals and other drugs’[54]. Other emerging pollutants were excluded as their high context specificity made scoring overly complicated. For ‘microplastics,’ data from [49,55–57,98] was used for scoring. For ‘pharmaceuticals and other drugs,’ data from [9,30,34,49–53] was used for scoring.

‘Operational emissions’ was divided into two criteria: ‘energy use’ for energy needed for the WW treatment technology used[38] and ‘direct emissions related to WW treatment’ relating to other emissions from the WW treatment process [38]. For bacteria and white rot fungi, no literature could be found, and it was assumed that there would be no change for these criteria compared to conventional WW treatment since existing research focuses on their treatment capability instead of emissions. For microalgae, data from [28,32,34] was used for scoring.

‘Pathogen reduction’ was scored according to reduction of known pathogens during WW treatment compared to conventional WW treatment [99]. For bacteria and white rot fungi, no literature could be found, and it was assumed that there would be no change for these criteria compared to conventional WW treatment since existing research focuses on their treatment capability for specific pollutants instead of pathogen reduction. Data from [29] was used for microalgae scoring.

‘Ease of implementation and operation’ is a measure of the change in infrastructure and amount of maintenance required to implement treatment using the specific organism[9]. ‘Level of WW treatment for organism to be implemented in’ is a measure of the change in WW treatment infrastructure required to implement treatment using the specific organism, based on [9].

Table 2 shows the rubrics used to score the different measures in the MCA and table 3 is a full breakdown of the scoring for each type of GE organism. Figure 3 is a different visualization of the MCA scores, showing the score variability for each criterion.

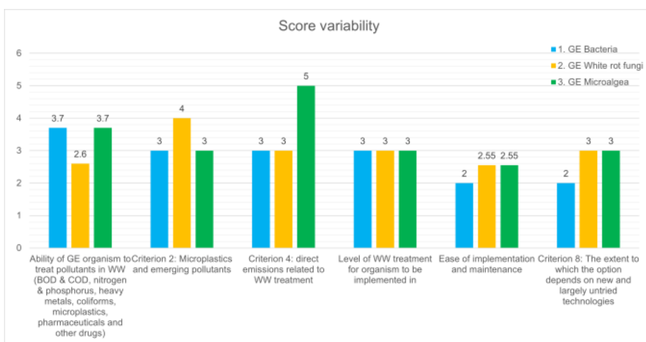
Table 4 - Criteria for MCA scoring

Objective	Criteria	Measures	Score				
			1	2	3	4	5
Ability of GE organism to treat pollutants in WW	Criterion 1: typical wastewater indicators	BOD and COD reduction	No significant reduction after treatment	Reduction less than existing conventional WW treatment	Reduction at same level of existing WW treatment (85-99% BOD, 76-91% COD)	Greater reduction than existing WW treatment	Undetectable levels of pollutant after treatment
		Nitrogen and Phosphorus reduction	No significant reduction after treatment	Reduction less than existing conventional WW treatment	Reduction at same level of existing conventional WW treatment (60-95%)	Greater reduction than existing WW treatment	Undetectable levels of pollutant after treatment
		heavy metals	No significant reduction after treatment	reduction less than existing conventional WW treatment	reduction at same level of existing conventional WW treatment (23-63%)	greater reduction than existing WW treatment	Undetectable levels of pollutant after treatment
		pathogens	No significant reduction after treatment	reduction less than existing conventional WW treatment	reduction at same level of existing conventional WW treatment (0.11-3.53 log reduction)	greater reduction than existing WW treatment	Undetectable levels of pollutant after treatment
	Criterion 2: Microplastics and emerging pollutants	Microplastics	No significant reduction after treatment	reduction less than existing conventional WW treatment (<35%)	reduction at same level of existing conventional WW treatment (35-99.9%)	greater reduction than existing WW treatment (>99.9%)	Undetectable levels of pollutant after treatment
		Pharmaceuticals and other drugs	No significant reduction after treatment	reduction less than existing conventional WW treatment	reduction at same level of existing conventional WW treatment (25-80%, depending on specific drug)	greater reduction than existing WW treatment	Undetectable levels of pollutant after treatment
Operational emissions	Criterion 3: energy use	amount of energy required	very high energy requirements	more energy required compared to existing WW treatment	energy requirements at same level of existing conventional WW treatment	less energy required compared to existing WW treatment	no outside energy source needed
	Criterion 4: direct emissions related to WW treatment	greenhouse gas (GHG) emissions	very high amounts of GHG produced during treatment	WW treatment produces more pollution than existing WW treatment	GHG production during treatment at same level of existing conventional WW treatment (20075.24)	WW treatment produces less pollution than existing WW treatment	no GHGs produced during treatment or all GHGs produced mitigated
Pathogen reduction	Criterion 5: projected impact on disease spread or prevention	disease spread or prevention	WW treatment actively worsens local waters much more than existing WW treatment facilities	WW treatment produces more pollution than existing WW treatment	WW treatment produces same amount of pollution as existing WW treatment facilities	WW treatment produces less pollution than existing WW treatment	WW treatment greatly improves the water quality of local ecosystems
Level of WW treatment for organism to be implemented in	Criterion 6: level of WW treatment at which technology can be implemented	Change in WW treatment infrastructure	Significant change in infrastructure is required (tertiary)	Change in infrastructure is required (secondary)	Change in infrastructure is required (primary)	Change in infrastructure is required (preliminary)	Infrastructure is not required
Ease of implementation and maintenance	Criterion 7: Level of experience delivering the works specified under each option	Level of experience delivering similar works	Significant lack of relevant experience	Clear lack of relevant experience	Insignificant level of relevant experience	Clear level of relevant experience	Significant level of relevant experience
	Criterion 8: Extent to which the option depends on new and largely untried technologies	Extent to which the option depends on new and largely untried technologies	Project depends for at least some critical components on innovative and largely untried technologies and techniques	Clear role for more innovative technologies and techniques	Some lesser roles for new technologies that will need to be managed	Relies on tried, tested and low risk technology and techniques but there are specific implementation risks that need to be managed	Relies exclusively on tried, tested and low risk technology and techniques with a low level of implementation risk

Table 5 - Objectives and criteria weighting for MCA

Objectives and criteria	Weight	GE Bacteria	GE White rot fungi	GE Microalgae
Ability of GE organism to treat pollutants in WW (BOD & COD, nitrogen & phosphorus, heavy metals, coliforms, microplastics, pharmaceuticals and other drugs)	**100%**	**3.7**	**2.6**	**3.7**
Criterion 1: typical wastewater indicators	70%	4	2	4
Criterion 2: microplastics and emerging pollutants	30%	3	4	3
Operational emissions	**100%**	**3**	**3**	**5**
Criterion 3: energy use	50%	3	3	5
Criterion 4: direct emissions related to WW treatment	50%	3	3	5
Ability of GE organism to reduce pathogens in WW	**100%**	**3**	**3**	**4**
Criterion 5: ability of GE organism to reduce pathogens in WW	100%	3	3	4
Level of WW treatment for organism to be implemented in	**100%**	**3**	**3**	**3**
Criterion 6: level of WW treatment at which technology can be implemented	100%	3	3	3
Ease of implementation and maintenance	**100%**	**2**	**2.55**	**2.55**
Criterion 7: level of experience delivering the works specified under each option	45%	2	2	2
Criterion 8: extent to which the option depends on new and largely untried technologies	55%	2	3	3

Figure 3 - Score variability for MCA



A8. Key factors of WW treatment technology in selected countries

Table 6 - Key factors of current state of WW treatment technology, how implementation of new WW treatment technology would look and openness for GE techniques to be applied in WW in Mexico, Brazil and Egypt

Current state of WW treatment technologies	Implementation of new WW treatment technology	Openness for GE technologies
	Mexico	
Most common WW treatment technologies: stabilization ponds, activated sludge, and UASB (Upflow Anaerobic Sludge Blanket) reactors [100]. **State**: several of the facilities have been abandoned, due to lack of long-term planning for operation, maintenance, and monitoring, and -by extension- lack of budget, equipment, and trained personnel [59,101]	**Probable implementor**: The State Commission of Water and the municipalities, in collaboration with private companies hired to carry out execution [59,101]. **Opportunities**: Carefully handled infrastructure innovation is viable, either via (i) research and/or development proposal for a new WW treatment from a research centre or (ii) a pilot-project from a development bank [59,101]. **Funding:** can be obtained via (i) self-funding or (ii) credit loans by international organisms (e.g., the World Bank) [102].	**From institutions**: legislation uses GMO interchangeably with Modified Living Organism, and Genetically Engineered Modified Organism [63]. **Current use**: GE can be used for food production, public health, or bioremediation, having prior authorization from the Federal Commission of Protection for Sanitary Risks (COFEPRIS), and regulated by the Intersectoral Commission on Genetically Modified Organisms Safety (CIBIOGEM) [63,64]. **General perception**: Local publications mention interest in the potential of GE for medicine and agriculture [65,66], whilst many highlight concerns over it [74–76].

Brazil		
Most common WW treatment technologies: anaerobic ponds, facultative ponds, UASB reactors, activated sludge, maturation ponds, septic tanks, and anaerobic filters [58]. **State:** the use of ponds and activated sludge is being phased out by the adoption of UASB reactors followed by some form of post-treatment, mainly due to reduced costs and capacity for upgrade [58].	**Probable implementor**: the municipalities and the National Water and Basic Sanitation Agency (ANA) [61,62]. **Opportunities:** Although it is variable among service providers, implementors tend to be more conservative[103]. Therefore, new WW treatment technologies are plausible, but take time[103].	**From institutions:** legislation describes GMO as an umbrella term (i.e., any "organism whose genetic material, DNA/ARN, has been modified by any genetic engineering technique") [67]. **Current use:** Governmental institutions allow implementation in industry with prior authorization from the National Technical Commission on Biosafety (CTNBio) [68]. **General perception:** GE is seen with potential but also with caution in Brazil, especially in medicine [69,70] and agriculture [71].
Egypt		
Most common WW treatment technologies: trickling filter, activated sludge, oxidation ditches, stabilization ponds, constructed wetlands, rotating biological contactors, sequencing batch reactors, UASB reactors, and septic tanks [60]. **State**: many WW treatment facilities do not meet the expected capacity, due to insufficient funds and technical capacities for operation and maintenance [60].	**Probable implementor**: Local agencies, public/private companies, or governorates, in coordination with the Holding Company for Water and Wastewater (HCWW), the Egyptian Water Regulatory Agency (EWRA) [60]. **Opportunities**: Public-Private partnerships are promoted within Egypt, providing opportunities for international collaboration [60,104].	**From institutions**: Egypt's legislation describes GE organisms as an umbrella term (i.e., "any organism modified by techniques referred to as biotechnology, gene technology, genetic modification or gene manipulation") [72,73]. **Current use & General perception**: Publications show interest in using GE technologies, mainly for medical purposes [105–107], and GE crops are currently in use [72,73].

A9. Detailed description of selected countries

Currently, the main WW treatment facilities in selected countries are UASB reactors, ponds, and activated sludge, and the main issue with their current operation is lack of budget and trained technical personnel.

In Mexico, the most common WW treatment facilities are stabilization ponds, activated sludge, and UASB reactors[100]. Nevertheless, several facilities have been abandoned, as highlighted by experts, due to lack of long-term planning for operation, maintenance, and monitoring, and -by extension- lack of budget, equipment, and trained personnel[59,101]. In Brazil, the most common WW treatment facilities are anaerobic ponds, facultative ponds, UASB reactors, activated sludge, maturation ponds, septic tanks, and anaerobic filters[58]. However, use of ponds and activated sludge is being phased out by adoption of UASB reactors followed by some form of post-treatment, mainly due to reduced costs and capacity for upgrade[58]. Lastly, the main WW treatment technology used in Egypt are trickling filter, activated sludge, oxidation ditches, stabilization ponds, constructed wetlands, rotating biological contactors, sequencing batch reactors, UASB reactors, and septic tanks[60]. However, due to insufficient funds and technical capacities for operation and maintenance, many treatment plants are not able to meet expected capacity[60].

Implementation of new WW treatment technology in selected countries mostly depends on local governmental implementors, federal regulation entities, availability of trained personnel and robustness of a long-term execution plan.

Implementation of GE in WW treatment technologies in Mexico would be overseen by the State Commission of Water and the municipalities, which are often careful on infrastructure investments and hire private companies to carry out execution[59,101]. Therefore, presentation of WW treatment technology should pay special attention to these stakeholders[101]. Due to implementors' carefulness on infrastructure innovation, in (i) research and/or development proposal for a new WW treatment brought up by a robust proposal from a research centre or (ii) a pilot-project from a development bank[59,101]. An issue with this management lay out is lack of long-term planning (due to personnel turn-over each election) and of local resources (budget, trained operators)[101]. Funding for new WW treatment facilities can be obtained via (i) self-funding or (ii) credit loans by international organisms (e.g., World Bank, InterAmerican Development Bank)[102]. In either case, it is expected that cost of implementation and operation are covered by tariffs over operation of projects, meaning, to recover investment costs by charging users. However, issues can occur given that the budget required, and the users' payment capacity do not necessarily match[61]. Additionally, consultation with local population (including indigenous communities) is expected, especially when compliance with the standards of international loaning organisms is needed[59].

Often, jurisdiction over sanitation in Brazil falls under the municipalities or regional companies, which must comply and coordinate with regulatory environmental agencies[62]. Nevertheless, most of the management is being attributed to ANA (former National Water Agency, now called National Water and Basic Sanitation Agency) because of the number of environmental regulatory agencies (1 national, 34 municipal, 13 inter-municipal and 25 state) and lack of support from these agencies to municipalities (only 52% of municipalities are supported)[62]. In turn, role redundancies or ambiguities can allow for state centralization to increase, rather than increasing managerial capacity at the municipal level. Additional issues may arise from lack of long-term planning due to personnel turn-over in elections, as seen in Mexico, regulatory voids (e.g., lack of standardization of tariffs and subsidies arrangements) and enforcement voids (linked to the gap in institutional coordination)[62]. Another common characteristic in Mexico is that although openness to innovation is variable among service providers, implementors of WW treatment technologies tend to be more conservative[103]. For example, anaerobic technology (now mainstream in Brazil) took several years to be accepted[103].

On the other hand, Egypt went through a privatization process in the 1990s which entailed that utilities were established for O&M of water and sanitation services[60]. Later, this enabled the foundation of the Holding Company for Water and Wastewater (HCWW) in 2004, responsible for financial and technical sustainability to local utilities, and of Egyptian Water Regulatory Agency (EWRA) in 2006, which regulates utilities managed by the HCWW[60]. Furthermore, the government of Egypt hires private companies to finance, design, build, and operate public infrastructure for sanitation, usually in long-term contracts that last

20 years[60]. Overall, management of WW treatment facilities is led by local agencies, public/private companies, or governorates, in coordination with forementioned entities[60]. Adding to the three listed stakeholders, international partnerships could be useful for implementing new WWT technologies[104]. This stands out by comparison with Mexico and Brazil, since it could be used to deflect the issue with severe implementor changes resulting from each electoral election. Nevertheless, other concerns would need to be addressed at the sight of a new WW treatment technology, such as interinstitutional coordination, supply of skilled staff, robust execution plans, availability of data[60]. Overall, these conditions are set for introduction of new WW treatment technologies, but GE technologies propose an additional challenge due to controversy on its use.

As considered in the MCA, GE is seen as a tool that should be handled carefully: not only from a technical perspective, but also from a social point of view. In the selected countries, institutions and publications show interest GE, but general controversy on its use remains.

Mexico's legislation uses GMO (OGM in Spanish) as an umbrella term, interchangeable with Modified Living Organism (OVM in Spanish), and Genetically Engineered Modified Organism (MIG in Spanish)[63]. All these terms refer to organisms altered by genetic engineering tools for improving a certain trait that can be used for food production, public health, or bioremediation, having prior authorization from Federal Commission of Protection for Sanitary Risks (COFEPRIS in Spanish), and regulated by the Intersectoral Commission on Genetically Modified Organisms Safety (CIBIOGEM in Spanish), which abide to GMO Safety Law and Regulation[63,64]. Apart from having this legal framework for GE use in Mexico, it is worth noting that few local publications mention interest in potential of GE for biomedical and agroengineering uses[65,66], whilst many highlight concerns and caution over use of GE[74–76], especially since He Jianku's confession on using gene editing in treating human embryos.

Brazil presents a similar case to that of Mexico. Brazil's governmental institutions also allow their implementation in industry with prior authorization from National Technical Commission on Biosafety (CTNBio in Portuguese)[68], as per stated in the Law and Regulations for GMOs published in 2005[67]. This law also describes GMOs as any "organism whose genetic material, DNA/ARN, has been modified by any genetic engineering technique," which would classify within our scope of technologies. As in Mexico, GE is seen with potential and caution in Brazil, especially in medicine[69,70] and agriculture[71].

Publications from Egypt show interest in using gene editing technologies, mainly for medical purposes[105–107], and already is engaged in using gene-edited crops[72,73]. In Egypt, GE organisms are considered as any organism modified by techniques referred to as biotechnology, gene technology, genetic modification or gene manipulation and their regulation is overseen by the National Food Safety Authority (NFSA), where application must be approved by the government[72,73].