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Remediation by enhanced natural attenuation; an environment-friendly remediation approach

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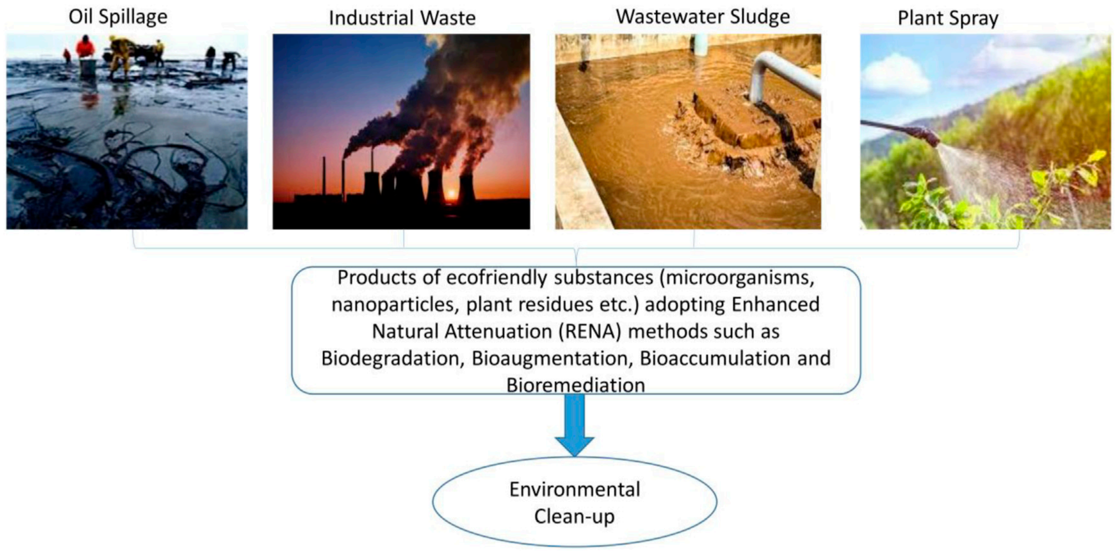
The uncontrolled use of chemicals, urban wastes, nuclear resources, mining, petrochemicals and disposal of sewage sludge only a few anthropogenic activities that have contributed to the rapid industrialization and severe heavy metal contamination of soils and waterways. Both inorganic and organic pollutants, such as heavy metals, pesticides, petroleum hydrocarbons, and polycyclic aromatic hydrocarbons, can impact the composition and functionality of soils. Soils and plants are affected by pollution, thus, pose a dire threat to food security. This directly renders the soil unuseful for agricultural purposes, destroys the beneficial microbes in the soil, reduces the soil organic matter content, causes the imbalance of soil nutrients, affects plant growth and the interaction between the plants and microbes, subsequently affecting the soil and crop productivity. In addition, environmental pollutants affect human health, leading to different illnesses such as headaches, allergies, coughs, depression, chest pain, nausea, diabetes, liver problems, cancers, eye problems, and so on. Remediation (physical, chemical or biological) is therefore necessary to reduce the impacts of these pollutants in the environment. Bioremediations involve using natural products from plants, microbes, and so on, to detoxify the environment and make it useful or productive again. A key type of remediation is the Remediation by Enhanced Natural Attenuation (RENA) which involves the turning of soil to promote microbial proliferation, aeration, nutrient availability, moisture and consequently, the degradation of pollutants. This review discusses the technology of RENA, the associated microbes, the mechanism of its action, challenges associated with its usage and recommendations to advance the use of RENA for a sustainable environment.

KEYWORDS

bioremediation, nanoremediation, phytoremediation, microbial enzymes, bioaugmentation



GRAPHICAL ABSTRACT

1 Introduction

The soil is often polluted by different agents, such as heavy metals from mining, agricultural, petrochemical and other industries, including radiological, nuclear and other anthropogenic pollutants (Li et al., 2019b; Chaudhary et al., 2022) (Table 1). These agents negatively affect the soil, plants, organisms and humans, and have necessitated the need for remediation (Tyagi and Kumar, 2021). Many compounds arising from different pollutants have been reported to be very dangerous to humans. Acetaldehyde causes lesions to the nervous system, benzaldehyde causes irritation to the respiratory system, eyes, and skin and reduces the functioning ability of the brain, while polychlorinated dibenzo-dioxin causes cancers, respiratory system, eye, and skin irritation (Alabi et al., 2019). In animals, plastic wastes have been reported to disrupt the digestive systems of cattle (Evode et al., 2021). Equally, plant growth and root development have been reduced as a result of hydrocarbon pollutants (Hussain et al., 2019). These have called for a need for remediation. Remediation is the removal or reduction of the effects of pollutants in the environment; remediation can be carried out physically, chemically or biologically (Ayangbenro et al., 2018) (Figure 1). The physical and chemical methods involve soil washing, chemical extraction, soil treatment, supercritical fluid oxidation, encapsulation, stream extraction, chemical treatment, thermal treatment and volatilization (Riser-Roberts, 2020). The biological method, referred to as bioremediation, is a process by which wastes and toxic materials are organically removed or rendered less harmful into the environment (Ojuederie and Babalola, 2017; Ayangbenro and Babalola, 2018). This utilizes agents such as plants, microbes and nanoparticles of biological sources (Figure 1), which are more cost-effective compared to the other methods (Tyagi and Kumar, 2021; Chaudhary et al., 2023b; Bhandari et al., 2023). Remediation by enhanced natural attenuation (RENA) is a type of remediation process used to control soil pollutants by turning the soil to promote microbial proliferation, aeration, nutrient, moisture and degradation (Okoye et al., 2019). RENA is mainly used to control pollution caused by crude oil through different microbes such as those belonging to the genera *Achromobacter*, *Azospirillus*, *Ochrobactrum*, *Bacillus*, *Alcaligenes*, *Lysinibacillus*, *Pusillimonas*, and *Proteus* (Chikere et al., 2017). These microbes reduce the environmental pollutants by using them as carbon sources or by immobilizing them, thus making them unavailable for plant uptake (Chikere et al., 2017). Different organic and inorganic substrates are applied to improve the ability of microbes used in RENA (Kumar et al., 2021b; Mafiana et al., 2021; Parveen et al., 2022). Microbes produce different enzymes such as lipase, hydrogenase, laccase, *etc.*, which help to degrade a wide range of pollutants (Bhandari et al., 2021). This is a very important mechanism of pollutants which should be well explored, especially in cases where an environment is polluted with more than one contaminant. The efficiency of RENA bioremediation can be altered by different factors which include the environmental pH, oxygen, temperature, and nutrient (Al-Hawash et al., 2018b). These factors affect the microorganisms directly and in cases where they are unfavorable the microbes that are expected to carry the bioremediation process die. RENA can be applied on both dry and swampy land areas, in cases where bioremediation is carried out in a swampy area, different steps are taken. Firstly, the stumps on the land has to be removed before the baseline studies are carried out (this is to ensure that the proper method of bioremediation is utilized (Orji et al., 2012). These procedures are followed by soil tillage, nutrient application and the monitoring of remediation. In cases

TABLE 1 Different pollutants and their toxicity effects.

Pollutant	Source	Impact	References
Polycyclic aromatic hydrocarbon	Crude oil	They are highly toxic and they have mutagenic and carcinogenic properties	Sakshi et al. (2019)
Pesticides	Agricultural activities	Soil toxicity	El Enshasy et al. (2017)
Heavy metals such as Cd, Cr, V, Cu, As, Zn and Pb	Industrial activities	Reduction in plant biomass, plant transpiration, nutrient uptake, photosynthesis, stomatal size, and ATP enzyme activity, changing	Long et al. (2021)
Chemical fertilizer	Agricultural activities	Renders the soil brittle, reduces soil nutrients, increases soil acidification, reduces the soil microbial population and alters the pH of the soil	Pahalvi et al. (2021)
Pyrites and pyrrhotites	Mining	Renders the soil unfit for agricultural activities	Havugimana et al. (2017), Agboola et al. (2020)
Sulfur dioxide	Burning of gasoline, and natural gas as well as refineries, coal and paper mill industries	It dissolves with water to form acid rain, leading to the destruction of stones, metals and forests	El Enshasy et al. (2017)
Radioactive wastes, accidental oil spillage	Industrial activities	Renders the soil unfit for agricultural activities	Havugimana et al. (2017)
Lead	Acid manufacturing companies	Slow down the rate of plant growth	El Enshasy et al. (2017)
cadmium, chromium, lead, arsenic, selenium	Sanitary wastes	Destroys the balance of the underground soil	Havugimana et al. (2017)

where baseline studies are not carried out on the soil, the extent of pollution might not be known. For instance, in cases of underground water pollution, which leads to an assumption that RENA is not an efficient bioremediation procedure (Adesipo et al., 2020). The aim of this review is to discuss the technology of RENA, the different microbes associated with its usage, its mechanism of action, challenges associated with its application and recommendations to advance its usage to promote a sustainable environment.

2 Environmental pollutants and their negative impacts

There are different types of soil pollutants which have different detrimental effects on the environment.

3 Types of bioremediation

To remove pollutants arising from heavy metals such ascadmium, copper, nickel, mercury, organic pollutants and hydrocarbons from the soil, different plants, microbes, and nanobiomaterials have been utilized (Masowa et al., 2022). Therefore, according to their agents, bioremediation can be classified as a plant, microbial and nanobiomaterial remediation.

3.1 Phytobioremediation

Phytobioremediation (phytoremediation), is a process by which plants and the microbes associated with them (the plants) helps to reduce toxic pollutants in the environment. This mechanism of pollutant reduction is a cost-effective and environment friendly, it stabilizes, immobilizes, degrades and uptake pollutants from the environment (Kafle et al., 2022). Plants are capable of removing antibiotics, heavy metals, pesticides, radionuclides and organic pollutants from the environment (Kafle et al., 2022). Different plants have been reported to be involved in phytoremediation, these include *Parthenium hysterophorus, Melilotus indicus, Cnicus benedictus, Anagallis arvensis, Verbesina encelioides, Dalbergia sissoo, Conyza canadensis, Lathyrus aphaca, Stellaria media, Xanthium strumarium, Chenopodium album, Medicago polymorpha, Amaranthus viridis, Mirabilis jalapa, Chenopodiastrum murale, Prosopis juliflora, Chrozophora tinctoria, C. tinctoria, Cerastium dichotomum* and *Arenaria serpyllifolia* (Naz et al., 2022).

Jiang et al. (2019) and Parsamanesh and Sadeghi (2019) reported that plant species, *Medicago scutellata* and *Mulberry* sp. were able to bioremediate cadmium successfully from the soil. Similarly, other plants have been reported to bioremediate heavy metals; for instance, *Cassia tora* was reported to remediate chromium (Patra et al., 2021), while *Polypogon monspeliensis* and *Rumex dentatus* remediated nickel from the soil (Samreen et al., 2021). The different types of phytoremediation that exist include phytoextraction, phytostabilization, phytofiltration and phytovolatilization (Shen et al., 2022). Phytoextraction is the process by which metalloids are extracted from contaminated soil, with the aid of accumulator plants (Yu et al., 2022). The phytostabilization process, uses plants that are resistant to metals to reduce he availability of pollutants in the environment (Kumar et al., 2023). Phytofiltration is the application of plants and the microbes associated with their roots in the removal of heavy metals from the environment (Akhtar et al., 2019). Phytovolatization is the process by which wastes are taken off the environment through plants and they are transformed into gaseous state, which is released into the atmosphere (Pidlisnyuk et al., 2021). These processes are affected by the bioavailability of heavy metals and biomass of plants (Shen et al., 2022). The efficiency of phytoremediation is affected by stomatal conductance, the species of the microorganism present, intensity of light, plant species

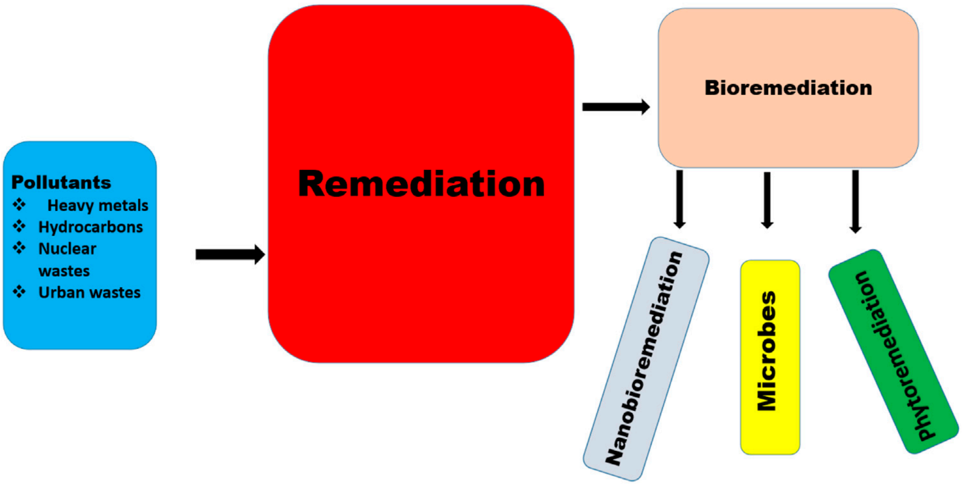


FIGURE 1
Types of bioremediation.

involved (its metabolism, photosynthesis and absorption rate) and temperature (Wei et al., 2021).

3.2 Nanoremediation

Nanoremediation is the application of nanomaterials in the treatment of environmental pollutants, especially on the soil and in water (El-Ramady et al., 2020). The different types of nanoparticles used in bioremediation, they include zinc nanoparticles, iron nanoparticles, aluminum nanoparticles, gold nanoparticles, titanium nanoparticles, carbon nanoparticles, and silver nanoparticles (Alazaiza et al., 2021). Nanoparticles, especially iron nanoparticles, are very active in soil bioremediation. The mechanism of nanoremediation include catalysis, adsorption, photodegradation and filtration (Mukhopadhyay et al., 2022). In a study carried out by Ji et al. (2023), diatomaceous earth nanoparticles were combined with polyethyleneimine nanoparticles to remove copper pollutants arising from acid mine drainages. Equally, nano biosurfactants have been reported to be capable of cleaning up toxic wastes from the soil arising from fertilizers, pesticides, herbicides, insecticides and heavy metals (Boregowda et al., 2022). Research carried out by Cao et al. (2022) showed that iron oxide nanoparticles could bioremediate cadmium and lead. These nanobiomaterials have been proven to be very efficient in the removal of toxic chemicals and heavy metals from the environment, particularly, the soil (Torimiro et al., 2021 (Chaudhary et al., 2023a)). However, a few drawbacks like adverse effects on soil microorganisms and the reduction in the activity of these nanoparticles as they age have been reported (Cecchin et al., 2017). Since the reduction in the efficiency of these nanoparticles with age can impact negatively their shelf life when made into commercial products, it is necessary that more research should be channeled toward strategies to increase their stability and lessen their harmful impacts on beneficial soil microbes to enhance their applicability as agents of bioremediation. The efficiency of nanoparticle as agents of bioremediation can be improved by fortifying them with polymers, zeolites, biochar, activated carbon and clay minerals (Mukhopadhyay et al., 2022).

3.2.1 Microbial bioremediation

Microbial remediation is the use of microbes to reduce the concentration of heavy metal pollutants in the environment (Jin et al., 2018). Microorganisms such as fungi, algae and bacteria have been used to bioremediate polluted soils (Ndeddy Aka and Babalola, 2016; Karthika et al., 2017; Ndeddy Aka and Babalola, 2017; Thesai et al., 2021; Kumar et al., 2022) (Table 2). The efficiency of these organisms is affected by the temperature of the environment, substrate where the microbes are getting their nutrient from and the pH of the environment (Jin et al., 2018). A report by Ghosh et al. (2021) revealed that generally, bacteria species like *Brevibacterium iodinum*, *Pseudomonas aeruginosa, Pseudomonas florescens* and *Alcaligenes faecalis,* as well as the fungi species like *Saccharomyces cerevisiae* are very active in the bioremediation of the soil; the researchers equally reported the effectiveness of *Anaeromyxobacter* sp., *Comamonas* sp., *Saccharibacteria* sp., *Desulfomicrobium* sp., *Acinetobacter* sp., *Zoogloea* sp., *Sphingobiun* sp., *Terrimonas* sp. and *Thiobacillus* sp. in the bioremediation of organic compounds such as pyridine, indole and quinolone. El-Ansary and Ahmed-Farid (2021) as well reported the ability of algae species such as *Scenedesmus obliquus*, *Nostoc muscorum*, *Chlorella vulgaris* and *Anabaena oryza* to degrade oxyl nematicide. These organisms usually use these pollutants as carbon and energy sources and convert them into water, microbial biomass, metabolites and carbon dioxide, which are generally not as toxic as the initial pollutants but could be useful to the soil health and plants alike (Tyagi and Kumar, 2021).

Some microbes utilize pollutants as their energy source during remediation, while some may immobilize or transform them and make them unavailable for plant uptake. RENA has been reported to be effective in the remediation of coal tar; Telesiński and Kiepas-Kokot (2021) reported that there was a decrease in the contents of phenol and naphthalene by 98%–100%, when the RENA technology was used.

TABLE 2 Different microorganisms used in bioremediation.

Organism	Species	Pollutant removed	References
Bacteria	*Achromobacter, Lysinibacillus* sp., *Azospirillus* sp., *Ochrobactrum* sp., *Proteus sp, Bacillus* sp., *Pusillimonas* sp. and *Alcaligenes* sp.,	Hydrocarbons	Chikere et al. (2017)
	Cellulosimicrobium sp	Chromium	Bharagava and Mishra (2018)
	Klebsiella sp.	Lead	Wei et al. (2016)
	Organisms from the genera *Cupriavidus, Burkholderia*, *Paenibacillus* and *Ensifer*	Chromium and Cadmium	Minari et al. (2020)
	Enterobacter asburiae, Stenotrophomonas sp., Enterobacter cloacae, Brevibacillus reuszeri, Acinetobacter junii, and *Enterobacter aerogenes*	Lead, chromium, and nickel	Sarma et al. (2019)
	Staphylococcus pasteuri	Phenanthrene pyrene, and fluoranthene	Anawar (2015)
	Bacillus amyloliquefaciens, Bacillus aerius, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, and *Chryseobacterium* sp.	Lead, nickel and cadmium	Su (2016)
	Modicisalibacter sp., *Idiomarina sp* and *Brevibacterium* sp.	Pyrene, benzopyrene, phenanthrene, naphthalene, phenol, hexadecane	Gomes et al. (2018)
	Bacillus megaterium	Cadmium, boron and lead	Esringü et al. (2014)
	Bacillus thurigiensis, Rhodococcus hoagii and *Bacillus pumilus*	Petroleum hydrocarbon	Viesser et al. (2020)
	Enterobacter sp.	Cadmium	Mitra et al. (2018)
	Arthrobacter ureafaciens	Simazine	Viesser et al. (2020)
	Pseudomonas aeruginosa	Cadmium	Chellaiah (2018)
	Morganella morganii	Chromium	Princy et al. (2020)
	Cupriavidus sp. strain Cd^{+2}	Cadmium	Li et al. (2019a)
	Rhodopseudomonas sp.	Crude oil and hydrocarbon	Mai et al. (2021)
	Enterobacter asburiae KUNi5	Nickel	Paul and Mukherjee (2016)
	Erythromicrobium ramosum and *Erythromonas ursincola*	Selenite and tellurite	Maltman and Yurkov (2018)
	Bacillus sp. KL1	Nickel	Taran et al. (2019)
	Halomonas sp. and *Marinobacter* sp.	Phenanthrene	Wang et al. (2020)
	Bradyrhizobium sp. and *Rhizobium metallidurans*	Lead and zinc	Sujkowska-Rybkowska and Ważny (2018)
	Lactobacillus plantarum	Lead and cadmium	Ameen et al. (2020)
	B. cereus, B. licheniformis and *B. subtilis*	Copper, lead and chromium	Shameer (2016)
	Cupriavidus metallidurans	Chromium	Alviz-Gazitua et al. (2019)
	Shinella sp.	Arsenic and antimony	Nguyen et al. (2021)
	Escherichia coli, Acinetobacter lwoffii, Bacillus thuringiensis, Enterobacter ludwigii, Vitreoscilla sp., *Pseudomonas fluorescens, Klebsiella pneumoniae*, and *Enterobacter asburiae*	Nickel, lead and zinc	Mosharaf et al. (2018)
	Bacillus aryabhattai	Paraquat	Inthama et al. (2021)
	Bacillus subtilis	Nickel and lead	Igiri et al. (2018)
	Staphylococcus sp. and *Pseudomonas* sp.	Phenanthrene	Mnif et al. (2017)
	Pseudomonas sp.	Arsenic	Satyapal et al. (2018)
	Pseudomonas sp.	Nickel, chromium, lead, and copper	Naz et al. (2016)
	Gemella sp. and *Micrococcus* sp.	Lead, chromium and cadmium	Marzan et al. (2017)
	Bacillus cereus and *Pseudomonas aeruginosa*	Lead and cadmium	Nath et al. (2018)
	Arthrobacter aurescens	Trifluralin pesticides	Lara-Moreno et al. (2022)
	Corynebacterium vitarumen, Bacillus macerans and *Bacillus megaterium*	Arsenic	Tyagi et al. (2018)

(Continued on following page)

TABLE 2 (*Continued*) **Different microorganisms used in bioremediation.**

Organism	Species	Pollutant removed	References
	Sphingomonas sp.	Hydrocarbon	Song et al. (2021)
	Bacillus sp.	Chromium	Ramírez et al. (2019)
	Bacillus safensis and *Pseudomonas fluorescens*	Chromium	Kalaimurugan et al. (2020)
	Bacillus subtilis, Bacillus licheniformis, and *Bacillus cereus*	Lead, chromium, and copper	Syed and Chinthala (2015)
	Halomonas sp.	Chromium	Kalola and Desai (2020)
	Sphingomonadales sp	Hydrocarbon	Bastida et al. (2016)
Fungi	*Arthrographis* sp., *Aspergillus* sp., *Mucor* sp., *Trichophyton* sp., *Fusarium* sp., *Rhizomucor* sp.,	Hydrocarbon	Okoye et al. (2019)
	Rhodotorula sp., *Penicillium* sp., *Candida* sp., *Rhizopus* sp., *Acremonium* sp., *Sporothrix* sp. *and Geotricum* sp.		
	Aspergillus sp.	Crude oil	Zhang et al. (2016)
	Ulocladium sp., *Penicillium* sp., *Fusarium oxysporum, Penicillium crysogenum, Ulocladium atrum, Aspergillus terreus, and Aspergillus parasiticus*	Hydrocarbon	Medaura et al. (2021)
	Aspergillus sp.	Crude oil	Al-Hawash et al. (2019)
	C. tropicalis and *T. asahii*	Hydrocarbon	Gargouri et al. (2015)
	Penicillium sp.	Petroleum hydrocarbon	Al-Hawash et al. (2018a)
	Monilinia sp.	Crude oil	Wu et al. (2008)
	Penicillium sp.	Hydrocarbon	Sari et al. (2019)
	C. tropicalis	Engine oil	Mbachu et al. (2016)
	T. versicolor	Toluene and benzene	PM Tavares et al. (2017)
	A. oryzae	Crude oil	Asemoloye et al. (2020)
	Aspergillus flavus	Surfactants and dyes	Ghosh and Ghosh (2018)
	Aspergillus sp. and *Penicillium* sp.	Crude oil	Sari et al. (2019)
Algae	*Scenedesmus quadricauda, Selenastrum capricornutum, Chlorella vulgaris* and *Scenedesmus platydiscus*	Petroleum hydrocarbon	Fu and Secundo (2016)
	Spirulina platensis and *Nostoc punctiforme*	Crude oil	El-Sheekh and Hamouda (2014)
	Gracilariacorticata sp.	Nickel	Raju et al. (2021)
	Chlorella vulgaris	Crude oil	Kalhor et al. (2017)
	Gelidium amansii	Lead	El-Naggar et al. (2018)
	Sargassum filipendula	Nickel, chromium and zinc	Costa et al. (2020)
	Ulva lactuca	Zinc	Senthilkumar et al. (2019)
	Spirulina platensis	Copper	Anastopoulos and Kyzas (2015)
	Synechocystis sp.	Pyrene	Patel et al. (2015)
	Turbinaria ornata	Lead	Al-Dhabi and Arasu (2022)
	Ulva lactuca	Zinc	Senthilkumar et al. (2019)
	Sargassum filipendula	Cadmium	Nishikawa et al. (2018)
	Chlorella vulgaris	Heavy metals	Alhumairi et al. (2021)
	Oscillatoria pranceps, Phormidium mucicola, Westiellopsis prolific, Lyngbya digueti and *Anabaena variabilia*	Hydrocarbon	Al-Hussieny et al. (2020)
	Dunaliella salina, Nannochloropsis oculata, Platymonas subcordiformis and *Phaeocystis globose*	Nonylphenol	Wang et al. (2019)

(Continued on following page)

TABLE 2 (*Continued*) **Different microorganisms used in bioremediation.**

Organism	Species	Pollutant removed	References
	Osmundea pinnatifida, Fucus vesiculosus, Ulva intestinalis, Fucus spiralis, Gracilaria sp. and *Ulva lactuca*	Mercury	Fabre et al. (2020)
	Caulerpa scalpelliformis	Zinc	Jayakumar et al. (2021)
	Sargassum filipendula	Chromium	Moino et al. (2017)
	Sargassum polycystum	Zinc and cadmium	Jayakumar et al. (2022)
	Cystoseira barbata and *Cystoseira crinite*	Chromium	Yalçın and Özyürek (2018)

3.2.1.1 Bacterial bioremediation

Bacteria are important agents of bioremediation, they are capable of removing polyaromatic, aromatic and aliphatic hydrocarbons (Table 2). The optimum conditions required for bacterial remediation include a temperature ranging between 30°C and 40°C, a carbon/nitrogen and phosphorus ratio of 100; 20; 1, a pH range of between five to eight and an oxygen level of between 10 and 40 percent (Kebede et al., 2021). Bacteria involved in bioremediation can live in a cooperative or competitive relationship, when in a cooperative relationship, the biodegradation process is enhanced; however, when they are in a competitive relationship, the biodegradation process is reduced (Kebede et al., 2021). Resident bacteria are more competitive in hydrocarbon degradation compared to the introduced species, especially in a long term (Kaminsky et al., 2019). Bacteria undergo genetic modifications to maximally remediate hydrocarbons, otherwise, they produce enzymes. Therefore, more research should be carried out to focus on the discovery of more enzymes that can successfully bioremediate complex pollutants.

3.2.2 Fungal bioremediation

Different fungal species have been successfully used as agents of bioremediation (Table 2). Fungi degrade pollutants by intracellular compartmentalization, organic acid precipitation, metal-binding proteins, active transport, metabolite, and inorganic acid precipitation (Li et al., 2020). When different metabolites and compounds are released by fungi, they help to immobilize and mobilize metal pollutants in the soil. In addition, fungi produce melanin and polymers which have oxygen groups which include carbonyl, carboxyl, phenolic hydroxyl, methoxyl, and alcoholic hydroxyl which are used to clean up pollutants in the environment (Li et al., 2020). They are capable of degrading a wide range of substrates such as pesticides and hydrocarbons, due to their ability to tolerate and survive in adverse environments (Mostafa et al., 2022).

3.2.2.1 Algal bioremediation

Algaebioremediation happens majorly through two different mechanisms, the first is adsorption, while the second is adsorption (Dwivedi, 2012). Adsorption involves the adherence of pollutants to the surface of algae, the process takes place very fast and is independent of the cell metabolism. Adsorption is a two-way process, initially, the pollutants get adsorbed to the surface of the cell, and subsequently, they are moved into the cytoplasm, in a process called chemisorption (Liu et al., 2021). Interestingly, algae can bioremediate pollutants both in their living and dead states, but the living cells have the ability to bioremediate pollutants better than the dead ones (Salama et al., 2019). The ability of algae in bioremediation is affected by different factors such as pH, the effect of counter ion, temperature, ionic strength and contact time (Salama et al., 2019). If these factors are optimized, the maximum potentials in the bioremediation process of algae would be tapped into.

4 Mechanism of RENA

RENA is a modern concept of microbial-assisted remediation, and it is gaining more insights because it enhances the function and the ability of the microbes in bioremediation. It is a more affordable method when compared to other remediation methods which include washing of soils and burning and helps to ensure maximum remediation as the microbes involved are able to completely break down pollutants such as hydrocarbons (Kanwal et al., 2022). Pollutants in the soil can be degraded by the RENA technique through different mechanisms, which include biodegradation, biotransformation (bioaugmentation) and bioaccumulation (bioassimilation) (Oghoje et al., 2021) (Figure 2).

4.1 Bioassimilation

Bioassimilation, which is also referred to as bioaccumulation is the process by which microbes accumulate heavy metals in their body, making them unavailable for uptake by the plant; for instance, the sorption of chromium, cadmium and lead by *Panteoa species* and *Pseudomonas koreensis* have been reported (Ayangbenro and Babalola, 2017; Ayangbenro et al., 2019; Oghoje and Ukpebor, 2020) (Table 3). Bioaccumulation is a bioremediation process that consumes energy and also serves as a basis for methylation and redox in microbial remediation (Yin et al., 2022). When bacterial cells are used, bioaccumulation includes transport mediated by carriers, ion pumps, lipid infiltration and endocytosis (Yin et al., 2022). The pollutants removed from the environment through this method is attached to the cellulose derivatives, chitin and polysaccharides active sites of microorganisms through the physical and chemical binding with biofunctional groups (Tarfeen et al., 2022). This method involves forces such as the ion exchange processes, electrostatic attraction, covalent bonding, Van der Waal's forces, and microprecipitation, while the functional groups involved include the sulfhydryl, hydroxyl, phosphonate, carboxyl and amine on the active cell component (Tarfeen et al., 2022).

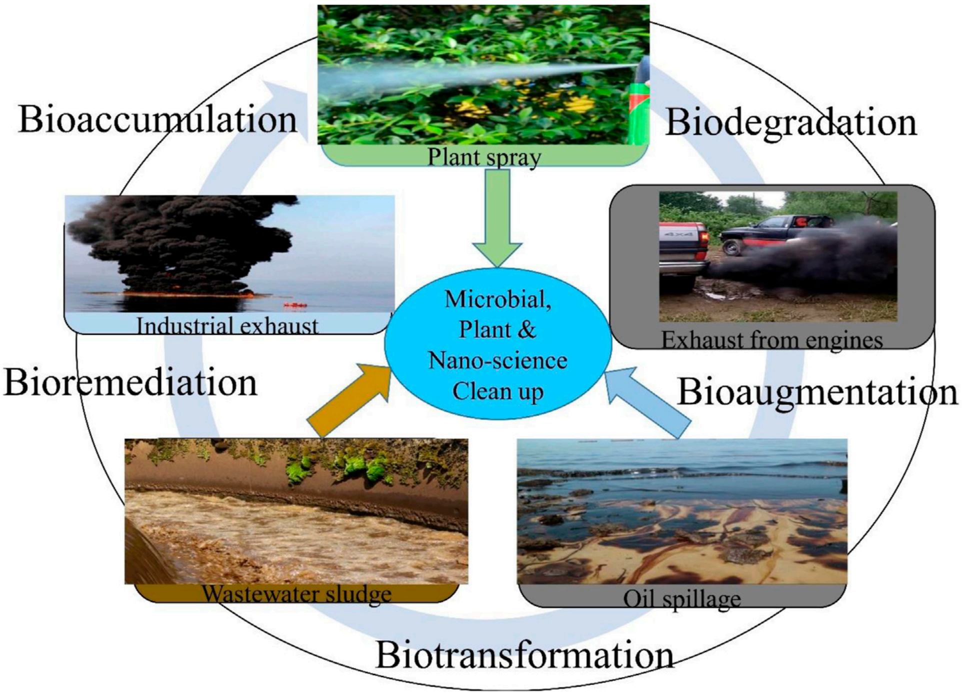


FIGURE 2
Mechanism of RENA.

TABLE 3 Mechanism of microbial bioremediation.

Microbe	Waste remediated	Mechanism	References
Monodictys pelagic and *Aspergillus niger*	Chromium and lead	Bioaccumulation	Sher and Rehman (2019)
Pseudomonas putida IRN22, *Acinetobacter pittii* IRN19, *Micrococcus luteus* IRN20	polyethylene	Biodegradation	Montazer et al. (2019)
Bacillus cereus M116	Nickel	Bioaccumulation	Naskar et al. (2020)
Bacillus cereus	Lead	Bioaccumulation	Utami et al. (2020)
Streptomyces rimosus	Iron and lead	Biosorption	Sahmoune (2018)
Staphylococcus hominis	Lead and cadmium	Biosorption	Rahman et al. (2019)
Cronobacter muytjensii KSCAS2	Different heavy metals	Biosorption	Saranya et al. (2018)
Bacillus sp. and *Paenibacillus* sp	Plastic	Biodegradation	Park and Kim (2019)
Serratia liquefaciens	Kraft lignin	Biotransformation	Singh et al. (2019)
Stenotrophomonas sp	Poly (butylene adipate- co -terephthalate)	Biodegradation	(Jia et al., 2021; Shilpa et al., 2022)
	(PBAT)		

5 Biotransformation

Biotransformation, also called bioaugmentation, is the conversion of a pollutant from one oxidation state to another. In bioaugmentation, microbes that are capable of degrading hydrocarbons but are not residents in the soil are introduced from external sources to supplement the resident organism, mostly bacteria and fungi (Essabri et al., 2019; Sayed et al., 2021) (Table 3). Sulfur-reducing bacteria such as *Sulfolobus* sp., *Acidiphilium cryptum*, *T. thioparus*, *A. cryptum*, *S. acidophilus*, *Citrobacter* sp., *Thiobacillus denitrificans*, *Acidithiobacillus ferrooxidans*, *Sulfobacillus thermosulfidooxidans*, *At. caldus*, *Gallionella ferruginea*, *At. thiooxidans*, *Ferroplasma acidiphilum*, *Clostridium* sp., *Acidianus brierleyi*, and *Leptospirillum ferrooxidans* have been reported to be potent in the mobilization and elimination of heavy metals from mines by dissolution, precipitation and retrieving safer metals from them (Schippers et al., 2010; Martins et al., 2011; Anawar, 2015; Ayangbenro et al., 2018). The bioaugmentation procedure is a fast, easy and effective method of RENA using different agents such as bacteria, algae, archaea and fungi (Ghosal et al., 2016; Kong et al., 2018).

6 Biodegradation

Biodegradation is the process by which microbes use the carbon in petroleum pollutants as a source of energy to disintegrate the hydrocarbon compounds into an environment-friendly form, such as carbon dioxide and water, with the aid of genes and enzymes produced by the microbes (Arumugam et al., 2017; Ahmed et al., 2018; Arumugam et al., 2018; Masowa et al., 2018; Karthika et al., 2020; Masowa et al., 2021; Priya et al., 2022) (Table 3). Adlan et al. (2020) reported the biodegradation of crude oil paraffin wax using *Anoxybacillus geothermalis*, *Geobacillus kaustophilus*, *Parageobacillus caldoxylostilyticus*, *Geobacillus jurassicus*, *Geobacillus stearothermophilus* and *Geobacillus thermocatenulatus* where around 70% degradation efficiency was observed in crude oil; the organisms involved in the degradation produced enzymes such as esterase, alkane monooxygenase, lipase and alcohol dehydrogenase.

When microbes use pollutants as carbon sources, they do so through three different techniques, namely, biostimulation, bioaugmentation and bio-facilitation (Azubuike et al., 2016; Oghoje et al., 2021). Bio-facilitation is a process by which the physiochemical content, and oxygen level, among others, in the soil are altered to make the inherent soil microbes more active to access pollutants. An example of this technique is land farming which involves the spread of evacuated soil and the tilling of soils where pollutants are found to increase oxidation (Oghoje et al., 2021). For instance, nutrients from organic wastes, synthetic fertilizers, humic acids, nanomaterials and so on, can be added to enhance microbial growth (Bianco et al., 2020). Bianco et al. (2020) carried out research to assess the ability of fresh organic municipal solid wastes, combined macro and micronutrients and digestate to increase the metabolism of microbes in the remediation of pyrene, anthracene, fluoranthene and phenanthrene. Other agents of biostimulation include blood meal which is a form of fertilizer that releases nutrients slowly. It is highly rich in nutrients such as tryptophan, lysine, histidine, leucine and valine, and can be used in the degradations of dichlorodiphenyltrichloroethane and polycyclic hydrocarbons (Wang et al., 2017). Biostimulation can be carried out using composting; it is cost friendly as it employs agents like manures, activated sludge and maple leaves, which will increase the population of microbes in the soil; compost from green forest debris and sewage sludge was able to reduce polycyclic hydrocarbons pollution (Guo et al., 2020).

7 Enzymes used in RENA

Scanty information is available on the microbial enzymes used in RENA; however, enzymes like laccase, hydrolase, cytochrome P450, protease, dehalogenase, lipase, dehydrogenase and so on, have been reported to be useful (Adlan et al., 2020). Proteases enhance the disintegration of peptide bonds in a protein. Their ability to degrade a wide range of substrates and their unique catalytic mechanism makes them a very effective method of bioremediation (Bhunia and Basak, 2014). Examples of microbes that produce proteinases are *Aspergillus* sp., *Cladosporium* sp., *Trichoderma* sp., *Bacillus* sp. and *Penicillium* sp. (Kumar and Jain, 2020). Cytochrome P450 enzymes are monooxygenases (haem) that can bioremediate pollutant compounds by reducing an atom to water and inserting an atom from molecular oxygen into the substrate (Behrendorff, 2021). White rot fungi have recently been used extensively in degrading pollutants, owing to the cytochrome enzyme produced by them (Lin et al., 2022); cytochrome P450 degrades xenobiotics using chemical transformation techniques such as dehalogenation, epoxidation, dealkylation and aliphatic hydroxylation (Bhandari et al., 2021). Laccases are enzymes that oxidize compounds in pollutants, leading to degradation and bioremediation. They are majorly observed in plants and fungi but lately, researches are emerging about their production by bacteria (because of their ability to survive at high temperatures and in different organic compounds), although their efficiency is determined by their substrate tolerance, selectivity and the center of their catalysis (Zhang et al., 2020). Dai et al. (2020) reported the ability of laccase from bacteria to degrade pollution resulting from heavy oil by 66.5% in 100 days. Remediation using dehydrogenase happens through reductive, oxygenlytic and hydrolytic mechanisms; oxygenlytic mechanism occurs when one or two atoms of oxygen is incorporated into a substrate, hydrolytic mechanism occurs with the water molecule acts as a cofactor when the hydroxyl group replaces the halogen substituent in SN reaction. In contrast, in the reductive mechanism, under aerobic conditions, hydrogen substitutes the halogen using the organohalides as the terminal electron acceptor (Bhandari et al., 2021). Li et al. (2019c) unraveled a novel hydrolase enzyme from *Bacillus amyloliquefaciens* to degrade soil and food polluted by carbendazim. Equally, Ugochukwu et al. (2008) carried out a research and reported that *Bacterium aliphaticum, Candida tropicalis, Bacillus megaterium, Pseudomonas maltiphilia, Bacillus cereus, Botryodiphodia thiobroma, Edwardsiella tarda, Fusarium vertiaculloide, Cryptococcus neofomas, Aspergillus niger* and *Fusiarum oxysporum* can bioremediate crude oil pollution due to the production of lipase.

Six Esterases which are produced by *Bacillus sp*. are useful in degrading polyesters, plastics and polyurethane (Bhatt et al., 2019). Nitrilases were used to remediate cyanide and can be produced by different yeast, bacteria and fungi (Gong et al., 2012; Park et al., 2017). Similarly, peroxidases produced by *Sphingobium sp*. are used to degrade phenols, lignin, methoxybenzenes and manganese oxide (Wang et al., 2009; Sharma et al., 2019). Dehydrogenase which is an active enzyme utilized for respiration by microbes was observed to be very helpful in the measurement of the total oxidation activity during the bioremediation of diesel (Lee et al., 2011). Manganese peroxidase which was sourced from fungi was reported to be positively correlated with the complete biodegradation of petroleum hydrocarbon (Košnář et al., 2019). Out of all the enzymes produced by fungi, oxidoreductases and hydrolases have a broad spectrum, with the ability to degrade a wide variation of pollutants (Kumar et al., 2021a). In their natural environment, microbes produce enzymes which are very active in the degradation of environment pollutants, however, the further utilization of these microbes has proved to be very challenging (Sharma et al., 2018). Sometimes, compaenzyme production in the lab can be complicated, as microbes tend to act differently in the lab red to the when they are in their natural environment. In situations where they are able to produce the enzymes in the laboratory, technologies such as enzyme immobilization, nanozymes and recombinant technology are used to multiply and/or stabilize the enzymes so that they can be further used on the field (Meng et al., 2019; Kumar et al., 2021a). Practical application of RENA at the field level.

On the field scale, RENA is used to remove pollutants from the soil and water, using methods such as stabilization, chemical transformation, dilution, volatilization dispersion and biodegradation (Naeem and Qazi, 2020). RENA has been used to clean up different oil spillage in groundwater and soil. In Nigeria, RENA was reported to be used in the remediation of oil spillage in Emohua community, Rivers State by adding top soils to the polluted site and frequent aeration (Chikere et al., 2019). A significant reduction was observed in the petroleum hydrocarbon from 8,635.68 mg/kg to 677.2 mg/kg after 56 days on this site. The bacteria involved were *Pseudomonas* sp., *Xenorhabdus* sp., *Bacillus* sp., *Myroides* sp., *Proteus* sp., *Staphylococcus* sp., *Pectobacterium* sp., and *Providencia* sp., while the fungi species which include *Fusarium* sp., *Penicillium* sp., *Meyerozyma* sp., and *Candida* sp. were used. Generally, topsoil contains many bacteria and fungi species and nutrients irrespective of organic and inorganic nutrient amendments. Hence, mixed plowing of nutrient-rich topsoil with contaminated soil can increase soil nutrient parameters necessary for microbial growth (Celestina et al., 2019) Similarly, in the Ikarama community, Bayelsa state, Nigeria RENA was used to clear oil spillage in a period of 60 days, by burning the polluted site's vegetation and plowing with a tractor (Ezekoye et al., 2018). This process utilized different organisms such as *Acremonium* sp., *Phoma* sp., *Candida* sp., *Scopulariosis* sp., *Aspergillus* sp., *Sepedonium* sp., *Cladophialophora carrionii, Gliocladium* sp., *Paecilomyces variotii, Trichophyton tonsurans* and *Geotrichum cardidum* (Ezekoye et al., 2018). The effective usage of RENA on the field has been ascertained; however, the technology might be ineffective when practices such as fertilizer application, tilling and windrow are used, this is as a result of the presence of non-biodegradable residues in the soil beyond where there is aeration. Other challenges with this technology include a delayed response to oil spillage emergencies, which enhances the penetration of oil to a region beyond the soil which can be reached by turning (Mafiana et al., 2021).

Chicken manure digestates have been reported to serve as a source of nutrients when RENA technology is used in the removal of diesel on farmland (Oghoje et al., 2021). When 10% and 20% of the chicken manure digestate were used, about 50% and 58% of the diesel were removed from the environment. Spent mushroom substrate also have been reported to be used to serve as a source nutrients for four different fungal species, namely, *Agaricus bisporus*, *Pleurotus eryngii, Pleurotus ostreatus*, and Lentinula edodes, which were used to remediate petroleum hydrocarbon. The remediation process was evaluated for 40 days and the aliphatic and aromatic hydrocarbon were observed to reduce from C_{10} to C_{35} (Antón-Herrero et al., 2022).

7.1 Molecular mechanism of RENA

Identification of the microbes involved in RENA bioremediation is very necessary as it will help to understand better, the mechanism and enzymes used in the remediation of different heavy metals, organic pollutants, and hydrocarbons and also enhance the remediation process. Different molecular methods are used to study the microbes involved in the bioremediation processes such as RENA, as the methods (proteomics, transcriptomics, metagenomics and metabolomics) help to elucidate non-culturable organism, and also reveals the genes involved in bioremediation process (Rawat and Rangarajan, 2019). Each of these methods have an advantage of others, for instance, in a case where the quantity of the total mRNA is required, transcriptomics is used; however, this method does not reveal other expressed protein as well as their biological activity and expressed protein. Metagenomics is a method used to study the taxonomic and functional structure of different microbes (Pande et al., 2020; Hualpa-Cutipa et al., 2023). Proteomics is the study of the entire protein that are produced or modified by different microorganism (Nascimento et al., 2022). Transcriptomics deals with use of the total set of mRNA and the noncoding RNA transcripts which are produced by microbial cells, it controls the physical expression and acts as a connector between protein and DNA (Bogati and Walczak, 2022). Metabolomics is the study of all the primary and secondary metabolites produced by microbes, and in RENA, it involves microbes that are involved in RENA remediation (Wu et al., 2022). Hence, to have a detailed knowledge of the microbes present during RENA remediation, it would be helpful to combine different omics processes instead of just one approach.

8 Factors affecting the efficiency of RENA

The application of RENA to biodegrade pollutants is hindered by several factors ranging from the availability of microbes capable of degrading the pollutants to pH, temperature, nutrients and oxygen. When the diversity of microbes that can degrade microbes in the soil is limited, the biodegradation process is limited (Al-Hawash et al., 2018b). This is why sometimes nutrients are added to the environment, which will promote the growth and activities of the desired organism.

Bioremediation using RENA can be carried out in the presence and absence of oxygen, while in the presence of oxygen, it is referred to as aerobic condition the lack of oxygen, it is termed anaerobic condition; in the latter, microbes utilizes iron, carbon dioxide, sulfate and nitrate to exchange electron, thereby, forming methane and carbon (Patel et al., 2020). An oxygen percentage of 10%–40% was reported to be optimum for the biodegradation of hydrocarbon because, at this temperature, microbial activity and degradative enzymes are promoted (Kebede et al., 2021).

The mobility of pollutants (e.g., oil) can be affected by the moisture content in the soil, which consequently affects the allocation, existence, movement and activities of microbes in the soil (Al-Hawash et al., 2018b). Some pollutants are more soluble in aqueous solutions, while some are not, and this affects their remediation (Fu and Secundo, 2016). The chemical constituent of hydrocarbons or waste to be degraded is an important factor to be considered when hydrocarbons are degraded, and specific microorganisms are just capable of degrading a single chemical compound, while some are capable of degrading multiple hydrocarbons or pollutants (Fu and Secundo, 2016).

The pH of the soil is paramount in the survival of microbes in the soil, unfavorable pH can be dangerous to the survival of microbes in the soil. Those that survive in acidic pH are termed acidophiles. Those that live in alkaline pH are called alkaliphiles, while those that survive neutral pH are called neutrophils (Schröder et al., 2020). The optimum pH observed when *A. niger* was used to bioremediate Hg, Cu, Co., Zn, and Ag was between 4–5.5 (Acosta-Rodríguez et al., 2018). On the other hand, Pawar (2015) reported that a pH of 7.5 was optimum for the degradation of hydrocarbons when *Penicillium freii* and *A. niger* were used.

Microbes required for the degradation of toxic heavy metals can die in the absence of optimum nutrients. Hence the presence of desired nutrients is needed for them to metabolize and remediate pollutants effectively. For example, *Aspergillus sydowii* bioremediated heavy metals and pesticides when mineral salt was used as the medium, as the nutrient was optimum for the growth and metabolism of the fungi (Zhang et al., 2019).

Some organisms are capable of surviving in extreme conditions; some organisms which live in extreme temperatures are termed extremophiles; those capable of living in cold regions are termed psychrophiles, and those who live in high temperatures are termed thermophiles (Malakootian et al., 2018). Research carried out by Acosta-Rodríguez et al. (2018) where *A. niger* was used to remove heavy metal pollutants in the environment revealed that the optimum remediation process occurred at a temperature of 28°C (Acosta-Rodríguez et al., 2018). Hence, it is important to carry out more research to understand the different pH levels, temperature, nutrient and oxygen requirements which will enable the survival of other microbes that can degrade various pollutants in the environment.

9 Challenges associated with the adoption of RENA

RENA, as technology has proven useful in the degradation of pollutants in the soil, and in ensuring the effective bioremediation of soil, but, the RENA process comes with some challenges which will be highlighted in this section. First, the RENA technology cannot degrade all waste. For example, RENA is highly specific in nature, hence, if there is the presence of more than one waste on the site, different microbes must be recruited, which probably might not be compatible to survive together (Sharma, 2020). Second, the biodegradation process of RENA sometimes brings about new products that may be more toxic compared to the initial pollutant, and the process is often time-consuming (Sharma, 2020). Third, the introduction of external nutrients or the turning of the normal soil structure of the soil during RENA to enhance the activity of microbes from another perspective can be seen as a negative move, as the normal eco-balance of the soil could shift and the soil structure can be destroyed by the movement of the soil (Chikere et al., 2017). Still, the addition of nutrients, especially from synthetic sources can lead to air and water pollution during rainy seasons (Chikere et al., 2017). Turning of the soil during RENA can as well promote the leaching of soil pollutants, as the pollutants which were at the surface are moved downwards, leading to underground water pollution (Chikere et al., 2017). Fourth, when the environment is altered, such as in the case of RENA, the expression of the gene by microbes might be altered (Smith et al., 2018). This may eventually affect the activity of the microbes either positively or negatively. It would not be appropriate to leave this to a game of chance, more research should be done to optimize the alteration in gene expression to be favorable to the microbes.

Lastly, the inhabitants of many communities do not trust the RENA technology, owing to the fact that in cases of severe pollution, a rapid method is always preferred. Since RENA is most times slow, it is assumed that it is not effective (Council, 2000). As a buttress, in some cases where a soil sample is taken from the environment to the laboratory or greenhouse to demonstrate the RENA technology, a false positive result might emerge because other factors might favor the technique in the greenhouse and might be otherwise on the field, resulting in lack of trust from the populace (O'Brien et al., 2021).

10 Conclusion and future prospects

RENA is an adequate, sustainable, non-toxic, cost-effective process which can be carried out at the site of pollution without posing any major health threat to the land, microbes and humans. This method removes the pollutants permanently, and not just transfer them to another environment (Sharma, 2020; Nuhu et al., 2022). Methods of RENA, such as the introduction of aeration and moisture to the soil which does not involve the movement of the soil, should be encouraged. Also, in cases where external nutrients would be added to the soil, organic nutrients in optimum quantity should be added as they are non-toxic to the environment, and if applied in optimum quantity, they will help to reduce the risk of eutrophication in water bodies.

RENA technology has proven to be effective and has successfully bioremediated some wastes, it comes with some drawbacks. This includes the inability to degrade some pollutants and the fact that RENA occurs mainly at the topsoil (0–5 m). Hence pollutants that go beyond this depth are leached into underground water (Ebuehi et al., 2005; Orji et al., 2012; Bolade et al., 2021)); therefore, it is recommended that research should be intensified to unravel organisms with strong abilities to bioremediate complex wastes. Also anaerobic microorganisms can be utilized through a deep injection technique to give microbes' access to wastes that are beyond 5 m where microbes used in RENA cannot access. Alternatively, RENA should be combined with other safe bioremediation techniques such as phytoremediation and nanoremediation to ensure a safe and more sustainable environment.

Since remediation using RENA has come with some challenges, it is therefore advised to carry out more research to beat the challenges associated with it to improve the utilization of the technology. For instance, when using RENA, the microbes used are highly specific, that is, they remove one compound at a time from the environment which makes the process complicated in a situation where different compounds pollute a particular environment. Hence, it is recommended that more studies should be carried out to discover different microbes that have no antagonistic effects on each other and are capable of remediating different organic compounds and pollutants in a favourable manner. In addition, more studies should be conducted to recruit more microbes, especially from untapped resources such as the endophytes, rhizosphere and rhizoplane of underutilized legumes, since the underutilized legumes can survive in extreme conditions and the microbes capable of surviving in such environments could possess the same ability as well. Furthermore, not much work has been done on the different microbial enzymes and their ability to produce enzymes in different environments. An insight on this could help develop these enzymes into stable products for commercial purposes, which could be applied directly on the soil for bioremediation. To promote the use and acceptability of RENA, it has become important that more attention should be paid to local communities. Informal and formal education in secondary schools on the benefits of a sustainable environment should be conducted. Hesitancy and other cultural quirks that could slow the adoption of RENA technology should be broken through proper enlightenment. This will in turn, help to foster homegrown technological expertise, researchers, stake holders, investors, and grant donors, thereby, further reducing the cost of bioremediation in the long run.

Experiments which have been tested in the laboratory or greenhouse should be well tested on the field in different environments to ascertain their functionality and effectiveness across diverse soil conditions, to earn the trust of the populace. Governmental policies could also be amended to promote the use of bioremediation techniques like RENA, compared to the physical and chemical methods, which are more disadvantageous and environmentally unsustainable. Finally, it is necessary to understand the mechanism of RENA when combined with other bioremediation methods, as this would help to improve these two technologies and make them more sustainable.

Author contributions

MA and OB conceived the idea, wrote and revised the manuscript. BA, MA, SA, CF, FO, UA, LG, RO, RJ, and ME contributed to the writing of the manuscript and the final version of the work was approved by all authors for publication and production. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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