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Wastewater remediation via combo-technology

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3.1 Introduction

The availability of safe water has been an ongoing problem and is becoming worse with increasing urbanization and population density. Only about 30% of the fresh water total is available on earth, in groundwater and surface water, for drinking and other daily human activities as well as industrial and agricultural activities. However, only 2% of fresh water is available for usage due to its nonavailability (comes at inconvenient times and places) and contamination by human activities (Fig. 3.1). Developed countries have advanced technology for water treatment and are thus able to manage the problem of water scarcity to a large extent, but advanced technology is largely unprocurable in developing and undeveloped countries due to cost. Based on the current population growth rate, it has been estimated that over 3.5 billion people will be in a water scarcity condition by 2025. The problem has become exacerbated due to the introduction of various recalcitrant, nondegradable compounds by agricultural and industrial activities. These compounds cannot be removed completely by any current available technologies.

Contaminated water has a high concentration of heavy metals, phenolic compounds, pesticides, etc., in addition to a high microbial load. Children (0–8 years) are at major risk from consumption of contaminated water leading to various neurological diseases, weakening of the immune system, and arrested growth [1] (Table 3.1). Over 1.8 million (4.1% of total global deaths due to diseases) human deaths have been reported by WHO (World Health Organization) annually due to consumption of contaminated water. The number of deaths due to waterborne diseases is directly correlated with socioeconomic condition: the lower the socioeconomic condition, the higher the number of deaths due to waterborne diseases [2]. The available conventional water treatment technologies, like solvent extraction, activated carbon adsorption, chemical oxidation, or biological methods, are inadequate to effectively remove recalcitrant pollutants from water.
### FIG. 3.1

An overview of availability of fresh water in the world.

Adapted from [https://www.nanowerk.com/spotlight/spotid=4662.php](https://www.nanowerk.com/spotlight/spotid=4662.php).

### Table 3.1 Summary of Diseases Caused by Consumption of Polluted Water

| Diseases       | Microbial Agent          | Symptoms                                                                 |
|----------------|--------------------------|--------------------------------------------------------------------------|
| Amoebiasis     | *Schistosoma* sp.        | Abdominal discomfort, fatigue, weight loss, diarrhea, bloating, fever    |
| Cryptosporidiosis | *Dracunculus medinensis* | Flu-like symptoms, watery diarrhea, loss of appetite, weight loss, bloating, nausea |
| Cyclosporiasis | *Taenia* sp.             | Cramps, nausea, vomiting, muscle aches, fever, and fatigue               |
| Giardiasis     | *Fasciolopsis buski*     | Diarrhea, abdominal discomfort, bloating, and flatulence                 |
| Microsporidiosis | *Hymenolepis nana*      | Diarrhea and wasting in immunocompromised individuals                    |
| Schistosomiasis | *Echinococcus granulosus* | Blood in urine, fever, chills, cough, itchy skin                        |
| Dracunculiasis | *Ascaris lumbricoides*   | Allergy, rash, nausea, vomiting, diarrhea, asthmatic attack              |
| Taeniasis      | *Enterobus vermicularis* | Intestinal disturbances, neurological manifestations, loss of weight     |
| Fasciolopsiasis | *Fasciolopsis buski*     | Diarrhea, liver enlargement, obstructive jaundice, cholecystitis         |
| Hymenolepiasis | *Hymenolepis nana*      | Abdominal pain, severe weight loss, itching in the anus                  |
Table 3.1 Summary of Diseases Caused by Consumption of Polluted Water—cont’d

| Diseases                  | Microbial Agent                        | Symptoms                                                                 |
|---------------------------|----------------------------------------|--------------------------------------------------------------------------|
| Echinococcosis            | *Echinococcus granulosus*              | Liver enlargement, anaphylactic shock                                     |
| Ascariasis                | *Ascaris lumbricoides*                 | Fever, diarrhea, vomiting, underdevelopment                              |
| Enterobiasis              | *Enterobius vermicularis*              | Perianal itch, nervous irritability, hyperactivity, insomnia             |
| Botulism                  | *Clostridium botulinum*                | Dry mouth, blurred vision, difficulty in swallowing, muscle weakness, diarrhea, vomiting |
| Campylobacteriosis        | *Campylobacter jejuni*                 | Dysentery-like symptoms, high fever                                      |
| Cholera                   | *Vibrio cholerae*                      | Watery diarrhea, nausea, cramps, nosebleed, vomiting, hypovolemic shock, death |
| E. coli infection         | *E. coli*                              | Diarrhea, death in immunocompromised individuals                         |
| *M. marinum* infection    | *Mycobacterium marinum*                | Lesions on elbow, knee, hands, and feet                                  |
| Dysentery                 | *Shigella dysenteriae, Salmonella sp.* | Blood in the feces, vomiting of blood                                     |
| Legionellosis             | *Legionella pneumophila*               | Resembles acute influenza without pneumonia, muscle ache, diarrhea       |
| Leptospirosis             | *Leptospira*                           | Flu-like symptoms followed by meningitis, liver damage, renal failure    |
| Otitis externa            | Number of bacterial and fungal species | Ear canal swells, causing pain and tenderness                             |
| Salmonellosis             | *Salmonella sp.*                       | Diarrhea, fever, vomiting, abdominal cramps                              |
| Typhoid fever             | *Salmonella typhi*                     | Fever, sweating, diarrhea, sometimes death                               |
| Vibrio illness            | *Vibrio vulnificus, V. aiginolyticus, V. parahaemolyticus* | Tenderness, agitation, bloody stools, chills, hallucination, fatigue, weakness |
| Severe acute respiratory syndrome (SARS) | *Coronavirus* | Fever, myalgia, lethargy, cough, sore throat                             |
| Hepatitis                 | *Hepatitis A virus*                    | Fatigue, fever, nausea, diarrhea, weight loss, itching, jaundice         |
| Poliomyelitis             | *Poliovirus*                           | Headache, fever, spastic paralysis, sometimes death                      |
| Desmodesmus infection     | *Desmodesmus armatus*                  | Fever, diarrhea, vomiting, weakness                                      |
Nanotechnology can provide a solution to these water treatment issues by removal of many types of recalcitrant compounds in addition to microbial load, including viruses. Besides water treatments using nanomaterials, such as carbon nanotubes (CNTs), nanosorbents, dendrimers, etc., nanotechnology can also be used in water desalination, disinfection, and sensors (contaminants can be sensed even at sub-ppm concentrations). Four classes of nanomaterials are now being used for water treatment: metal-containing nanoparticles, carbonaceous nanomaterials, zeolites, and dendrimers. They can remove even toxic heavy metals like arsenic, organic material, salinity, nitrates, pesticides, etc. from surface water, groundwater, and wastewater. This is true because of their large specific surface area, high reactivity, high degree of functionalization, size-dependent properties, and high affinity for specific target contaminants. Most importantly, nanomaterials can be used to treat water without addition of chlorine (known to be controversial due to generation of carcinogenic compounds). The technology of water desalination, softening, and salt recovery as well as treatment of industrial and brackish water using nanostructured filters and nanoreactive membranes have made nanotechnology more in demand [3]. Electrosprun nanofibers have gained much attention in water treatment, as they also can inhibit the bacterial growth responsible for membrane biofouling. Nanomaterials used for water treatment can easily be merged into distributed optimal technology networks (DOT-NETs) being used in water treatment for small-scale housing subdivisions, apartment complexes, and commercial districts. Further, they can also be used in treatment plants like factory assembled, compact ready-to-use water treatment systems, point of entry (POE), and point of use (POU) treatment units.

The potential significance of nanotechnology in water treatment is huge, with several hundreds of publications and registered patents already. However, this technology has not yet reached the commercial market. The foremost reason for this is the potential toxicological risks for humans and the environment, which cannot be ignored for the sake of the many technological advantages. Genotoxicity and cytotoxicity studies have revealed that nanomaterials are effective agents in interacting with biological macromolecules present in living systems and thus are responsible for a number of diseases and clinical disorders [4]. Therefore a need exists for additional technology that can lower the toxicological impact of nanomaterials while retaining their abilities for water purification, disinfection, sensing, and monitoring.

An additional issue related to water purification is removal of biofilm (extracellular polymeric substance secreted by sessile bacterial communities) developed over a course of time. The development of biofilm is responsible for decreasing the efficiency of membranes derived from nanomaterials, with an additional risk of adding metabolic products and biological toxins that degrade water quality [5]. Biofilm is most frequently formed in water coming from the medical, paper, and food-processing industries. The available antimicrobials or chemicals are not efficacious in combating biofilm. Even mechanical methods have not helped in this respect, and they make the process costly and more labor intensive.

Enzyme technology, based on suitable enzymes being immobilized onto nanomaterials, can help in removing the toxicity of nanomaterials, as well as providing
efficacy in removal of biofilm by degrading its components containing polysaccharides and proteins. Enzyme technology has been found to complement nanotechnology by enhancing water-purifying capabilities by several-thousandfold, due to its excellent specificity and catalytic efficiency. Enzymes have further benefited this combination by increasing the stability and number of cycles of reusability. This chapter outlines water purification, disinfection, sensing, and monitoring using the combination of the two technologies, nanotechnology and enzyme technology.

3.2 Overview on wastewater remediation across the world

Wastewater is the used water discharged from various sources including homes, businesses, industries, cities, and agriculture. There are various types of wastewater based on water uses: urban wastewater (blackwater coming from toilets and greywater coming from kitchens and bathing), industrial wastewater, commercial establishment wastewater (including hospitals), agricultural wastewater, etc. Further, collected wastewater in a municipal piped system (sewerage) is called sewage. Wastewater treatment usually follows three steps: primary treatment, secondary treatment, and tertiary treatment. Before proceeding through the various steps, wastewater is pretreated to remove grit, grease, and gross solids that could hinder the subsequent treatment stages. Primary treatment aims to settle down suspended solids (both organic and inorganic), followed by removing them. Primary settlers, septic tanks, and imhoff tanks are most commonly used for primary water treatment. Secondary treatment involves removal of soluble biodegradable organics through degradation by bacteria and protozoa, through aerobic or anaerobic biological processes. Aerated lagoons, activated sludge, trickling filters, oxidation ditches, etc. are used for secondary water treatment. Tertiary treatment polishes the effluent before it is discharged or reused, by removal of nutrients (mainly nitrogen and phosphorous), toxic compounds, residual suspended matter, or microorganisms, through disinfection with chlorine, ozone, ultraviolet radiation, or membrane filtration (micro-, nano-, ultra-, and reverse osmosis), etc. Tertiary treatment is the most costly of the three treatments, and is often not done in low-income countries. Finally, the treated water is released for various applications.

The need to provide accurate data on levels of wastewater generated annually has been emphasized by national policymakers, researchers, practitioners, and public institutions, as this data is necessary for developing national policy and action plans based on type of wastewater and contaminant levels. However, accurate information related to wastewater is not systemically monitored in most of the countries of the world, with significant data paucity in rural areas. In 2010, global annual domestic water withdrawals modeled by WaterGAP3 (global freshwater model that calculates flows and storages of water across the world) have found that there was approximately 450 km$^3$ of global production of wastewater coming from the domestic and manufacturing sectors, with 70% of wastewater (315 km$^3$) coming from the domestic sector. AQUASTAT (database that provides information on global water systems)
has reported that globally more than $330 \text{ km}^3 \text{ year}^{-1}$ of (mostly) municipal wastewater are produced [6]. Countries like the United States, China, Brazil, Japan, Russia, India, and Indonesia collectively produce over $167 \text{ km}^3$ of wastewater per year (half of global municipal wastewater production). It has also been reported by AQUASTAT that, on average, 60% of produced municipal wastewater is treated globally. These figures on wastewater treatment are much lower due to the poor treatment capacities of wastewater treatment plants, particularly in middle- and low-income countries. Further, data is generally not reported by some low-income countries with large urban populations (such as Nigeria). It has been found that most of the countries report only secondary and tertiary treated wastewater as “treated wastewater,” while some countries report only primary treated wastewater, which makes data aggregation and comparisons by country rather more difficult. In 2014, GWS (global water store) 2009 reported that globally $24 \text{ km}^3 \text{ year}^{-1}$ of wastewater generally passes all three phases of water treatment [7]. It has been found from wastewater treatment data that there is a strong correlation between treatments of wastewater and a country’s income [8]. High-income, middle-income, and low-income countries have reported an average of wastewater treatment as 70%, 28%, and 8%, respectively. More importantly, in the case of developing countries, most untreated wastewater is directly discharged into natural water bodies due to either dysfunctionality or limited capacity of water treatment plants.

Increasing water scarcity has been a factor in the growing realization of the necessity of wastewater treatment. However, the efficiency of wastewater treatment is directly related to physical, economic, social, regulatory, and political environments. It has been found that high-income countries follow complete protocols for wastewater treatment and the process is well regulated and planned (Fig. 3.2). The usage of wastewater is based on the economic status of the countries, as discussed in the following paragraphs.

- **Direct use of untreated wastewater**: This occurs in water-scarce areas having drier climates, usually in low-income countries. Lack of water sources, groundwater salinity, and unaffordability of fresh water are the major reasons behind usage of untreated wastewater. Most commonly, untreated wastewater is used for agriculture [9]. There are various examples worldwide, particularly in developing and undeveloped countries. A number of farms in Pakistan, for example, are using untreated wastewater due to the extreme salinity of the groundwater; farmers of the twin cities of Hubli-Dharwad (Karnataka, India), living in a semiarid climate, use untreated wastewater coming from open sewers and underground sewers for irrigation. Similarly, farmers from Cochabamba in Bolivia and Accra and Tamale in Ghana use wastewater from malfunctioning treatment plants or sewers. It has been found that in Haroonabad (Pakistan) and Hyderabad (India), wastewater is the only water flowing in the irrigation canals during the dry season. Even worse, in Nairobi (Kenya), Mali Saba (Kenya), Addis Ababa (Ethiopia), Bhaktapur (Katmandu Valley in Nepal), and Dakar (Senegal), farmers have removed sewage line inspection covers that block sewers, followed by the rising of raw sewage up to the manholes, which subsequently flows into their farmland [10].
The 10 largest wastewater treatment plants across the world.

Los Angeles
Capacity per day: 45-million-gallons
Hyperion sewage Treatment plant

Detroit
Capacity per day: 930-million-gallons
Detroit wastewater Treatment plant

Boston
Capacity per day: 1.27-billion-gallons
Deer island sewage Treatment plant

Cairo
Capacity per day: 449-million-gallons
Gabal el asfar Wastewater Treatment plant

Shanghai
Capacity per day: 528-million-gallons
Bailonggang Wastewater Treatment plant

Chicago
Capacity per day: 1.44-billion-gallons
Stickney water Reclamation Plant

Washington, DC
Capacity per day: 370-million-gallons
Blue plains Wastewater Treatment plant

Hong Kong
Capacity per day: 450-million-gallons
Stonecutters Island sewage Treatment works

Tokyo
Capacity per day: 406-million-gallons
Morigasaka Wastewater Treatment plant

Paris
Capacity per day: 449-million-gallons
Seine aval plant

FIG. 3.2
The 10 largest wastewater treatment plants in the world.

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• *Indirect use of untreated wastewater*: This is being practiced in low- and middle-income countries having drier and wetter climates. Here, untreated wastewater is directly discharged into freshwater streams for dilution and is subsequently used by farmers, in households, or by industries. Several examples of indirect use of untreated wastewater are found in sub-Saharan Africa, Nepal, India, and many cities in Brazil, Argentina, and Colombia that have inadequate sanitation facilities. It has been reported that in West Africa, farmers use highly polluted water in the vicinity of cities for irrigating their vegetables. It has been found that up to 90% of vegetables being consumed in the cities are grown through irrigation using polluted water [11].

• *Planned use of reclaimed water*: This occurs more frequently in higher income countries having well-established water treatment plants. They are much more environmentally aware of their wastewater, which is reclaimed and used to preserve freshwater ecosystems. Reclaimed water is directly used for agricultural irrigation, city landscaping, golf courses, toilet flushing, washing of vehicles, and groundwater recharge, as well as being a source of potable water supply. Windhoek in Namibia is one of the examples of this type of water reclamation and usage. Even industrial wastewater is purified to industrial standards and recycled within the system. In countries of the Middle East and North Africa, Australia, the Mediterranean, and the United States of America (AQUASTAT 2014; Global Water Intelligence 2010), planned use of reclaimed water is seen. In all of these cases, an effective sanitation and treatment technology supports water reclamation; however, the main challenge for water reuse is public acceptance [12].

Water treatment plants, besides producing clean water, also produce lots of sludge, which can be used for a variety of purposes. Even excreta collected from toilets either on-site (e.g., in a pit latrine or septic tank) or transported off-site in sewer systems are also termed sludge. Fresh and untreated sludge contains many pathogens, has a high proportion of water, has higher BOD (biochemical, or biological, oxygen demand), and is generally putrid and odorous. Additionally, it also contains essential nutrients like nitrogen and phosphorous, so it is a very beneficial fertilizer for plants. The organic carbon present in sludge is used as a soil conditioner, as it improves soil structure that helps in proper growth of plant roots. Further, the organic carbon can also be converted into energy through biodigestion or incineration. The composition of sludge depends on the source types; sometimes it is highly contaminated with harmful pollutants like heavy metals (in the case of sewage coming from industries). Sludge treatment is a must before using the sludge in various applications. The treatment involves reducing water content, BOD, pathogens, and obnoxious odor, followed by thickening, drying, and stabilization. Dewatering is an energy consumptive process, sometimes taking even weeks for complete sludge drying through incineration, pyrolysis, or gasification [13]. The most important step, responsible for making sludge fit for various applications, is stabilization, accomplished by aerobic and anaerobic processes. Aerobic stabilization uses composting at
higher temperatures (55°C), similar to the natural process that takes place in the forest leading to breakdown of organic material containing leaf litter, animal wastes, etc. and reducing volume by severalfold. In the case of anaerobic stabilization (cannot be used when contaminated with heavy metals), reduction of organic wastes takes place through bacterial decomposition under anaerobic conditions and produces a mixture of gases containing methane, carbon dioxide gas, and syngas. Properly treated sludge is called biosolids. These solids have a variety of beneficial uses like landscaping, energy recovery, generation of new soil, increasing soil fertility, etc. Sludge treatment is also dependent on the economic status of the country [14].

- **Informal use of untreated sludge**: Many low-income countries in West Africa and South Asia do follow the complete steps for sludge treatment. However, in these countries sludge is collected and air dried and directly used as fertilizer in the fields. In some cases, sludge fills vacant land, leading to anaerobic degradation by natural processes in an unplanned way. In other cases, sludge is discharged directly into water bodies, where it is diluted and finds its way back into the food chain when that water is used for farming.

- **Formal use of biosolids**: This occurs in developed countries with regulated and well-designed biosolid and septage programs. Properly planned sludge treatment leads to the generation of biosolids, which is very useful in producing soil nutrients and thus maintains soil fertility while reducing the pressure on the final disposal sites. Energy recovery from sludge through biodigestion and incineration has an additional advantage over reuse of sludge. However, there is the risk of contamination by heavy metals and toxic pollutants, so thorough studies on composition of the sludge must be done before sludge processing occurs.

### 3.3 Nanotechnology and water remediation

Nanomaterials (size \(<100\text{ nm}\)) are found to be effective in water treatment due to their high specific surface area, high reactivity, and excellent sorption [15]. Zeolites, CNTs, biopolymers, self-assembled monolayer on mesoporous supports, zero-valent iron nanoparticles, bimetallic iron nanoparticles, and nanoscale semiconductor photocatalysts are the most commonly used nanoparticles in water treatment [16] (Tables 3.2 and 3.3). Further, nanomaterial capabilities in desalination and their easier incorporation into existing technology has made them effective. Even industrial and brackish water can be made potable in a very short time span with little labor in a cost-effective way. Water treatment using membranes based on nanofibers have been widely accepted due to their efficacy and easier regeneration. However, the accuracy of the number of recycles using regeneration protocols has to be closely monitored, in order to nullify the probability of nanomaterials leaching into the treated water after a certain time. The following paragraphs provide brief details on water treatment using nanomaterials.
(i) Remediation and Desalinization

(a) Adsorption: Nanoparticles are excellent adsorbents due to their extremely large specific surface area (size: 10–100 nm), high selectivity, short intraparticle diffusion distance, tunable pore size, and adsorption kinetics. They can remove recalcitrant organic and inorganic contaminants, degrade organic compounds (chlorinated alkanes and benzenes, pesticides, organic dyes, nitro aromatics, dioxins, furans, etc.) and reduce toxic metal ions [Cr (VI), Ag (I), Pt (II)] [17]. Nanoparticles can easily be functionalized with various chemical groups depending on the contaminant types (making them more efficient). Commonly used nanoparticles for adsorbents are: TiO$_2$, Fe$^{0}$, Fe$^{3+}$/Pd$^{0}$, Fe$^{3+}$/Pt$^{0}$, Fe$^{0}$/Ag$^{0}$, Fe$^{0}$/Ni$^{0}$, Fe$^{0}$/Co$^{0}$, or hybrids (viz. Pt/TiO$_2$/Ru nanoparticles). Innovative nanosorbents prepared by hybridization of inorganic and organic nanosorbents (viz. sodium

| Applications | Nanomaterials | Enabled Technologies |
|--------------|---------------|---------------------|
| Adsorption   | Carbon nanotubes (CNTs), nanoscale metal oxides, nanofibers with core shell structure | Adsorption of recalcitrant contaminants, for adsorptive media filters and slurry reactors |
| Membrane processes | Nanozeolites, nano-Ag, CNTs, nano-TiO$_2$, aquaporin, nanomagnetite | Antibiofouling with stringent permeability capabilities |
| Photocatalysis | Nano-TiO$_2$, fullerene derivatives | For photocatalytic reactors, solar disinfection |
| Disinfection and microbial control | Nano-Ag, CNTs, nano-TiO$_2$ | POU water disinfection, antibiofouling surface |
| Sensing and monitoring | Quantum dots, CNTs, silica nanoparticles, magnetic nanoparticles, noble metal nanoparticles | Optical and electrochemical detection |

### Table 3.2 Summary of Nanomaterial Applications in Wastewater Treatment

### Table 3.3 Mechanisms Adopted by Nanomaterials for Effective Waterborne Pathogen Killing

| Nanomaterials | Antimicrobial mechanisms |
|---------------|--------------------------|
| Nano-Ag       | Release of silver ions, protein damage, suppress DNA replication, membrane damage |
| Nano-TiO$_2$  | Production of reactive oxygen species (ROS) |
| Nano-ZnO      | Release of zinc ions, production of H$_2$O$_2$, membrane damage |
| Nano-MgO      | Membrane damage |
| Nano-Ce$_2$O$_4$ | Membrane damage |
| Fullerol and aminofullerene | Production of ROS |
| CNTs          | Membrane damage, oxidative stress |
| Graphene-based nanomaterials | Membrane damage, oxidative stress |
dodecyl sulfate incorporated into magnesium-aluminum-layered double hydroxides) are found to have excellent sorption capacity for contaminants like tetrachloroethylene (PCE) and trichloroethylene (TCE) [18]. CNTs are used for sorption of metal ions like Pb (II), Cu (II), and Cd (II) with several thousand higher sorption capacities than the powdered or granular activated carbon. Further, they can adsorb volatile organic compounds and water-soluble dyes (acridine orange, ethidium bromide, eosin bluish, and orange G). Chitosan nanoparticles (40–100 nm) can easily be functionalized based on the application type, viz. phosphate functionalization used to adsorb Pb (II) [19]. Nanozeolites (viz. NaP1 zeolites: Na₆Al₆Si₁₀O₃₂, 12H₂O) are used for removal of heavy metal ions (Cr, Ni, Zn, Cu, Cd), even from acidic wastewater, due to their excellent metal ion exchanging capacities. Nanosorbents are available in various forms (pellets, beads, or porous granules), making them more easily incorporated into existing treatment processes. Further, there are well-established protocols for their regeneration, which makes them more desirable due to the increased reusability and cost reduction by severalfold. Arsen Xnp (hybrid nanoparticles of FeO and polymers) and ADSORBSIA (beaded TiO₂ nanoparticles with diameter from 0.25 to 1.2 mm) for arsenic removal have been commercialized on a pilot scale [20]. Following are brief details on nanosorbents:

- Carbon-based nanosorbents: The most commonly used carbon-based nanosorbent is the CNT. It is very effective in removal of bulky organic compounds, including antibiotics and various pharmaceutical compounds and heavy metal (Cu²⁺, Pb²⁺, Cd²⁺, and Zn²⁺) contaminants, more so than conventionally used activated carbon. This is attributed to the very large surface area and the number of interactions, including hydrophobic effect, π-π interactions, hydrogen bonding, covalent bonding, and electrostatic interactions [21]. It has fast adsorption kinetics due to the large number of accessible adsorption sites containing functional groups like carboxyl, hydroxyl, and phenol, as well as the small distance of intraparticle diffusion [22]. However, the frequent problem of CNT aggregation in aqueous media decreases its effective surface area. In terms of adsorption, there is little difference with CNT aggregation due to the presence of many interstitial spaces and grooves helping in adsorption [23]. For many specific applications, CNTs are coated with specific agents like sand granules, which helps in removal of toxic metal ions (Hg²⁺) and even removal of bulky dyes like rhodamine B [24]. Generally, carbon-based nanosorbents are used during the polishing step of water treatment due to their high cost and sensitivity.

- Metal-based nanosorbents: These are generally used for heavy metals (arsenic, lead, mercury, copper, cadmium, chromium, and nickel) and radionuclide removal from wastewater. Metal oxides (FeO, TiO₂, and Al₂O₃) are commonly used as adsorbents having excellent kinetics.
of metal adsorption due to the large surface area, short intraparticle distance of diffusion, and large number of reaction sites [25]. Metal oxide nanosorbents have the additional property of magnetism, giving them more efficiency in removal of heavy metals. It has been found that the smaller the size of nanosorbents, the greater is the magnetism and thus the higher the adsorption capacity. Lab experimentation has found that a decrease in particle size increases the volume of many atomic magnetic dipoles. However, if the size is less than 11 nm, the particle loses its own magnetism but responds to externally applied magnetism [26, 27]. Metal oxide nanosorbents can be easily compressed into porous pellets and fine powders using moderate pressure, based on the type of application. These nanosorbents are recommended for removal of arsenic and organic contaminants for POU applications. Further, their low cost and low toxicity have made them very popular.

- **Polymeric nanosorbents:** The most commonly used polymeric nanosorbents are dendrimeric nanosorbents for treatment of water containing organic compounds and heavy metals. Their inner shell is hydrophobic, which adsorbs organic compounds, while the hydrophilic exterior shell adsorbs heavy metals through its tailored branches. The phenomenon of adsorption is caused by complexation, electrostatic interactions, hydrophobic effect, and hydrogen bonding. Some dendrimer nanosorbents have been prepared for removal of specific contaminants; for example, polyamidoamine (PAMAM) dendrimer-NH$_2$ is used for removal of copper ions from wastewater. The main advantage of regeneration is desirable due to the significant cost reduction. Lab testing on dendrimer-based water treatment is in process; hopefully dendrimers will soon make it into the commercial market [28].

**b) Membranes and membrane processes:** Membranes and their related processes make use of nanomaterials like nano-Ag, CNTs, and polyvinyl-$N$-carbazole-SWNT (single walled nanotubes) nanocomposites. They carry out water treatment by providing a physical barrier using selectivity and permeability. They can remove organic and inorganic pollutants and heavy metals as well as biological contaminants (bacteria, virus) from groundwater and surface water. Membranes have wide credence due to their nontoxicity, reusability, minimal labor requirements for operation and maintenance, small space requirements, and ability to fit easily in any set-up. Further, they are mechanically and thermally stable, resistant to biofouling, and have excellent shelf-life [29]. Water purification and desalination are carried out by these membranes using ultrafiltration (UF), nanofiltration (NF), forward osmosis (FO), and reverse osmosis (RO). Only RO and FO are energy-driven processes, while the others work on the differences in the osmolalities. They have made it possible to even treat sea water and brackish water and make it fit for drinking [30]. Sometimes doping with suitable materials is required to prevent any nanomaterials leaching into the treated water; this also can
Nanotechnology and water remediation

3.3 Nanotechnology and water remediation

inactivate microbial pathogens [31]. The following paragraphs discuss different types of membranes and their applications.

- **Nanofiber membranes**: These are made of electrospinning ultrafine fibers derived from polymers, ceramics, and metals. They are highly porous and have very large surface area. Their physical properties, like diameter, composition, morphology, secondary structure, and spatial alignment, can easily be modulated, due to which they have wide applications. They work by UF, FO, and RO processes to purify water containing heavy metals, organic and inorganic pollutants, microbial pathogens, etc. They are also found to be useful in air filtration by removing all harmful gases. They are sometimes doped with materials like ceramics, TiO$_2$, to protect themselves from biofouling. They are in great demand due to their adaptability and ease of fabrication [32].

- **Nanocomposite membranes**: These are known for their increased efficacies with respect to various parameters such as membrane permeability, surface hydrophilicity, mechanical and thermal stability, and reduced fouling. A broad range of nanomaterials is used: hydrophilic metal oxide nanoparticles (Al$_2$O$_3$, TiO$_2$, and zeolite), antimicrobial nanoparticles (nano-Ag and CNTs), and (photo)catalytic nanomaterials (bimetallic nanoparticles, TiO$_2$). Thin film nanocomposite (TFN) membranes have been developed with enhanced membrane permeability and larger surface charge density (mostly negative) based on nanozeolites, nano-Ag, nano-TiO$_2$, CNTs, etc. Further, they have antifouling properties and increased membrane hydrophilicity, which helps in salt exclusion. Sometimes they are doped to gain additional properties like photocatalysis, higher versatility, etc. based on application type. Quantum Flux (the commercial name of a nanozeolite-based nanocomposite membrane) is used for desalination of sea water and makes it fit for use. It has been doped with nano-TiO$_2$ ($\leq$5 wt%), which prevents membrane fouling [33, 34]. TFN membranes are mostly desirous for their enhanced water permeability and reduced cost. However, they have not been very successful in water desalination due to various limitations [35].

- **Biologically inspired membranes**: These are derived from biological systems and have excellent selectivity and permeability. The most commonly used biological membrane is aquaporin-Z derived from *Escherichia coli*. It contains amphiphilic triblock-polymer vesicles with complete impermeability to small molecular weight solutes like glucose, glycerol, salt, and urea. For commercial applications, biological membranes are incorporated with nanomaterials, viz. CNTs. Aquaporins with aligned CNTs have increased water permeability with very high selectivity for small molecular weight solutes. They can be used in water desalination and for removal of various molecules. However, their efficacy is directly related to the uniformity of CNTs for reliable
salt rejection from seawater. Further, their chemical modification of CNTs helps in improving solute selectivity. For example, the carboxyl functional group rejects 98% of $\text{Fe(CN)}_6^{3-}$, 50% for KCl at 0.3 mM, and negligible when KCl is at 10 mM. There are various technical challenges that have impeded large production of aquaporins with aligned CNTs [36].

- **Nanostructured and reactive membranes:** These membranes are existing filtration membranes fabricated with various nanomaterials to enhance efficiency. For example, depositing nano-TiO$_2$ improves contaminant degradation in the presence of sunlight; metal retention capacity can be enhanced by depositing poly (l-glutamic acid) and poly (l-lysine); and fabrication with CNTs helps in removal of pathogenic microorganisms like *E. coli*, *Staphylococcus aureus*, and Poliovirus sabin 1 from contaminated water. These membranes are very useful in water treatment for removal of those recalcitrant contaminants that cannot be removed by any other known methods. Alumina membranes fabricated with several layers of poly(styrene sulfonate)/poly(allylamine hydrochloride) have high retention for divalent cations ($\text{Ca}^{2+}$ and $\text{Mg}^{2+}$) and anions like $\text{Cl}^-$ and $\text{SO}_4^{2-}$ while fabrication with A-alumoxanes nanoparticles (7–25 nm) leads to increased selectivity for synthetic dyes like Direct Red 81, Direct Yellow 71, and Direct Blue 71. Incorporation of bimetallic Fe$^{0}$/Pt$^{0}$ nanoparticles or zero-valent iron (nZVI) into a cellulose acetate membrane helps in reduction of chlorinated organic compounds like TCE (trichloroethylene), PCE (tetrachloroethylene), etc. More innovations are yet to come, with the additional advantage of wide acceptance due to the presence of the basic membranes, which are commonly used [37].

(ii) **Photocatalysis for Wastewater Treatment**

Photocatalysis (oxidation in the presence of sunlight) helps to kill pathogens using oxidation and degradation of recalcitrant organic compounds as well as hazardous nonbiodegradable contaminants. Nanomaterials like TiO$_2$, CeO$_2$ and CNTs have excellent photocatalytic properties due to their large surface area, which can be used for water treatment by completely degrading organic pollutants through fast catalysis. TiO$_2$ is an excellent photocatalyst nanomaterial, as it rapidly generates an ionic pair upon UV irradiation, which forms reactive oxygen species (ROS) responsible for contaminant degradation and pathogenic killing. It is very cost effective, not very toxic, and widely available. The smaller the size of the nano-TiO$_2$, the higher is the rate of photocatalysis due to the increased number of reactive facets. However, a size smaller than 11 nm does not further increase photocatalysis, as there is an increase in interfacial charge carrier transfer. Further, TiO$_2$ nanotubes are more efficient than TiO$_2$-nanoparticles due to the increased photocatalysis and higher degradation rate of organic compounds as provided by shorter diffusion paths inside the tubular wall. It has been found that additional doping with various novel metals, dye sensitizers, semiconductors, and anions can further increase the rate of photocatalysis by narrowing the bandgap, which allows excitation even by visible light. Crystallographic
studies on TiO$_2$ nanomaterials have revealed that {001} facets have the highest photocatalytic activity due to the small spatial separation between electron and holes as well as the strong adsorption [38]. Nano-TiO$_2$ has been recommended for water treatment on both small and large scales, using various-sized parabolic collectors. Nanomaterials like WO$_3$ and fullerene derivatives (aminofullerenes and fullerol) are also photocatalytic. However, they are very costly with respect to nano-TiO$_2$ but have a much narrower bandgap, which allows excitation by visible light. Nano-WO$_3$ has been commercialized as the Purifics Photo-Cat system to treat 2 million gallons of water per day containing recalcitrant organic compounds, with a power consumption of approximately 4 kWh m$^{-3}$.

Therefore, nanomaterials having photocatalytic properties could be an effective alternative for water treatment on an industrial scale. It has been recommended to use immobilized photocatalytic nanoparticles in commonly used reactors like slurry reactors, with optimized configuration and operation parameters (reactor design, reaction selectivity, pH, temperature, optimum light wavelength, and intensity). This has an additional advantage of reusability, which helps in lowering the cost and makes the process more effective and more eco-friendly [39].

(iii) Disinfection and Microbial Control

Conventionally, purified treated water is disinfected with strong oxidants like chlorine and ultraviolet (UV) irradiation before its release for consumption. These oxidants carry out pathogen (bacteria and virus) inactivation by blocking their metabolic activities, inhibiting synthesis of housekeeping gene products and disrupting the cell wall, leading to leaking of all cellular constituents. In the case of UV irradiation, DNA synthesis is additionally blocked, leading to disruption of all cellular activities and subsequently the death of the waterborne pathogens. These oxidants are cheap, efficacious, and easily accessible. However, they are also responsible for generation of toxic by-products (trihalomethanes, haloacetic acid, aldehydes), with some of them being carcinogenic in nature, including nitrosamines, bromates, etc. [40]. This condition becomes worse when high dosages are used for heavily pathogenic-loaded water and exceed the admissible range of general usage. Alternatives are being looked for, but all of them are at stage 1 of the Disinfection Byproduct Rule (1996), of the Safe Drinking Water Act amendment.

Nanomaterials like nano-Ag, nano-ZnO, nano-TiO$_2$, nano-Ce$_2$O$_4$, CNTs, and fullerenes are known for their effective antimicrobial properties and for leaving no by-products. They are recommended for controlling pathogens, biofilm formation, and microbial-induced corrosion present in storage tanks and distribution pipes. Different nanoparticles have varied modes of pathogen killing, with a commonality of a broad antimicrobial spectrum. Nano-Ag works by releasing Ag$^+$ which binds to thiol groups of cellular proteins and inactivates them, leading to death of the pathogens. Additionally, they prevent DNA replication, which inhibits synthesis of important cellular proteins. It has also been found that they can cause structural damage in the pathogenic cell envelope. Nano-Ag has been commercialized under the trade name MARATHON and Aquapure systems, used in POU treatment, microfilters, etc. Despite the low human toxicity, it is crucial to check the level of Ag$^+$ in the
released water. Factors like size, shape, coating, and crystallographic facets are significantly important in the release kinetics of Ag⁺. CNTs carry out pathogenic killing through their conductivity leading to cell membrane perturbation, oxidative stress, and disruption of cellular structures [41]. Further, they remove pathogens by size exclusion activity provided by their layered structure. Generally, multiwalled nanotubes (MWNTs) are the most effective due to their fibrous shape and layered structure, which helps in providing excellent conductivity. Mostly small-sized CNTs are preferred for POU devices, due to their higher toxicity than the larger-sized CNTs. Metallic nanoparticles (MgO) can kill Gram-positive and Gram-negative bacteria as well as their spores (e.g., Bacillus subtilis) by disrupting their membrane integrity. Nanoparticles like dendrons, dendrimers, hyperbranched polymers, and dendrigraft polymers obstruct pathogens by inhibiting their entry due to their small size (0.1–1.0 nm) provided by their architecture, containing core, interior branch cells, and terminal branch cell. They usually require pressure between 200 and 700 kPa, which makes the process highly energy consumptive. This can be circumvented by a tangential or cross-flow of water movement during purification.

Nanoparticles have been found to be an excellent substitute for water disinfection with respect to other conventional techniques, due to few or no by-products and their higher efficacies. However, they are a little costlier than other available techniques. Various solutions to this have been found, such as reusability of nanoparticles by coating with certain materials that help in their regeneration, and also avoiding any long-term side effects due to leakage. Further, this will also prevent biofouling caused by accumulation of microbes on their surface. Therefore, the nanoparticles could turn out to be the best substitute for present techniques for water purification and disinfection.

(iv) Sensing and Monitoring

Nanosensors are based on nanoparticles, used for checking water quality after their treatment by various methods. They have excellent sensitivity, selectivity, and photostability, and provide data within microseconds. Even harmful pathogens that remain undetected by conventional methods, like hepatitis A and E, coxsackie viruses, echoviruses, adenoviruses, Norwalk viruses, Legionella, Helicobacter, Cryptosporidium, Giardia, etc., can be easily detected in a very short time. This efficiency allows the detection of several thousands of samples in a short time period, which is generally not possible by any other available techniques. Thus, they can be used by municipal bodies for water-quality checks on a large scale. Further, they also ensure that even treated water coming from industrial uses can be used without suspicion.

The sensors contain four units: recognition agent, amplifier, processor, and output. Nanomaterials are important components in the last three units due to their electrochemical, optical, and magnetic properties, while recognition is generally handled using antibodies, aptamers, carbohydrates, antimicrobial peptides, etc. Nanomaterials can be used in the first unit of the sensor due to having a very large specific surface area, which can easily be functionalized by various chemical groups for modulating their affinity towards a variety of water contaminants. For example,
quantum dots (QDs) modified with TiO$_2$ are used to detect polycyclic aromatic hydrocarbons at pM, while QDs modified with CoTe are used for bisphenol A at 10nM, etc. Commonly used nanomaterials for sensors are magnetic nanoparticles, noble metals, silica nanoparticles, dye-doped nanoparticles, CNTs, and QDs. Metal nanoparticle detection is based on changes in absorption at specific wavelengths, while novel metal nanomaterials detect change in Rayleigh scattering due to their localized surface plasmon resonance (LSPR). In the case of silica nanoparticles, surface modification with organic or inorganic luminescent dyes is used to enhance their sensitivity and protect from photobleaching [42]. CNTs are used in the electrode of the sensor, as they have high conductance, which facilitates faster electron transfer in addition to the amplifier unit of the sensor. However, the preparation of homogenous CNTs is the most challenging aspect. They are used for detecting trace metals and organic pollutants in the water. Dynabead is a commercially used nanosensor to detect pathogens. It is based on a magnetic nanocomposite made of CdSe, which is a semiconductor, having excellent electronic characteristics, that detects based on changes in fluorescent spectra. Nanomaterials as sensors can easily enter the market as they are not placed in direct contact with users. However, aspects such as cost effectiveness, regulatory and public acceptance, water solubility, long-term performance and stability, and testing in real natural and wastewater must be researched thoroughly, to bring about their ultimate success in the commercial market. The previously mentioned annoying aspect of aggregation has impeded the use of nanomaterials in nanosensors, as it impairs sensitivity and reproducibility. Work is in progress to obstruct aggregation by surface functionalization, but currently there is no hard-core protocol claiming to resist aggregation completely.

Nanomaterials have provided solutions to water desalination, removal of recalcitrant toxic contaminants and toxic heavy metals, and development of chlorine-free water treatment. Further, their significance in water quality checks makes them desirable for use in various types of water purifiers. The Freedonia group has released a forecast of nanomaterials demand by 2020 by classifying nanomaterials into metal oxides, clays, metals, polymers and chemicals, nanotubes, and dendrimers in various applications besides water treatment. It has been anticipated that nanomaterials like silica, TiO$_2$, clays, metal powders, and polymers will be in great demand in the coming years. The role of CNTs, fullerenes, dendrimers, and membrane-derived nanomaterials for processes like RO, NF, and UF in wastewater treatment is formidable, with their advantages of flexibility, easier operation and maintenance, and scalability [43]. However, besides the many expectations, various challenges remain to be met, to bring them into the commercial market. The crucial aspects that must be thoroughly taken care of are: commercial availability of nanomaterials, extent of compatibility with the existing infrastructure, and associated health risks (such as leaching of nanomaterials into treated water). It has been found that the water generated directly from various sources (household and industrial) is rich in suspended particles that impede the treatment, particularly removal of soluble recalcitrant contaminants. It has been suggested to use nanotechnology-based water treatment methods followed by conventional treatment methods, which will lower the loads of suspended
particles and help in efficient water treatment in a cost-effective way. Further, using nanotechnology either in the primary or secondary stages rather than in the final stages of water treatment would lower the risk of any nanomaterials leaching into the treated water and coming in direct contact with humans and the environment. Various other measures have been under consideration to make nanotechnology-based water treatment a complete success.

3.4 Enzyme technology and water remediation

Enzymes have high specificity (even a hundred or a thousand times higher than nanoparticles), high catalytic turnover, cost effectiveness, nontoxicity, and are nonerosive. They can degrade recalcitrant water pollutants in an ecofriendly manner, requiring no toxic chemicals for their action. They work under mild physical conditions, which makes the process energy saving. Some enzymes require additional factors for their action, particularly metal ions like Cu, Ni, Mg, Mn, Zn, etc. Enzymes can work in the presence of foams, sprays, lotions, and even detergents, which has made them even more in demand for water treatment. Generally, enzymes are extracted from microbial or plant sources. However, recombinant enzymes are also prepared when the source has insufficient enzyme concentrations. Most importantly, enzymes have been proven to degrade those recalcitrant water contaminants that cannot be degraded by oxidation (chemical, photochemical, electrochemical), ozonation, photolysis using \( \text{H}_2\text{O}_2 \) and \( \text{O}_3 \), corona process, \( \text{TiO}_2 \) photolysis, radiolysis, and or even adsorbed or filtered by known nanoparticles in some cases (Tables 3.4 and 3.5). There are various reasons for contaminant recalcitrance, the foremost being the presence of unusual substitutions with halides (\( \text{Cl}^- \) or \( \text{Br}^- \)), azo (\( -\text{N} \equiv \text{N} \)) linkage, very large molecular size, and the presence of unusual bonds or highly condensed aromatic rings or presence of tertiary and quaternary carbon atoms. Enzymes can act specifically on the unusual bonds or substitutions and produce degradation. Further, enzymes do not increase BOD or chemical oxygen demand (COD) of water while causing degradation of water contaminants. The following paragraphs give a brief outline of the various aspects of enzymes being used during water treatment.

(i) Water Decontamination

Water can be decontaminated using a variety of enzymes from plants or microbial sources. A brief summary of various enzymes used in removal of pollutants is given in the following paragraphs.

- Phenolic contaminants and related compounds: These are generated during treatment of coal, petroleum refining, synthesis of resins and plastics, metal coating, wood preservation, textiles, dyes and other chemicals, mining and dressing, and pulp and paper, etc. [44]. Their usage has been strictly regulated in most developed countries. Various classes of enzymes being used in their degradation are:
  - Peroxidases: They are used for hydrolyzing aromatic contaminants with their action dependent on the presence of peroxides (\( \text{H}_2\text{O}_2 \)). They can be obtained from a number of microorganisms as well as plants. Different peroxidases
Enzyme technology and water remediation

(based on substrate or sources) that have been used for treatment of water pollutant treatment are listed here:

1. Horseradish peroxidase (HRP): It can hydrolyze toxic aromatic compounds like phenols, biphenols, anilines, hydroxyquinoline, arylamine, carcinogens (benzidines and naphthylamines), and related heteroaromatic compounds. Further, compounds that are not the substrates of HRP can also be treated, as the enzyme helps in forming insoluble precipitates while acting on its substrates followed by sedimentation and filtration of precipitates [45]. The enzyme has a broad range of physico-chemical parameters (pH, temperature, ionic strength, etc.), and thus it is one of the most effective enzymes in treatment of wastewater highly contaminated with pollutants.

Table 3.4 Water Pollutant Degrading Enzymes

| Enzyme              | Source                 | Pollutants                                                                 |
|---------------------|------------------------|-----------------------------------------------------------------------------|
| Peroxidase          | Horseradish            | Phenol, chlorophenol, anilines: degradation, phenol determination, Kraft effluent: decontamination |
|                     | *Atromyces ramosus*    | Phenol, polyaromatics, herbicides: degradation, humic acid polymerization   |
|                     | Plant materials        | Water decontamination                                                       |
| Chloroperoxidase    | *Caldariomyces funago* | Oxidation of phenolic compounds, chlorophenol detection                     |
| Lignin peroxidase   | *Phanerochaete chrysosporium* | Phenol, aromatic compounds: degradation, Kraft effluent: decontamination     |
| Manganese peroxidase| *Phanerochaete chrysosporium* | Phenol, lignin, pentachlorophenol, dyes: degradation                         |
| Tyrosinase          | *Agaricus bisporus*    | Catechol oxidation                                                          |
| Laccase             | *Trametes hispida*     | Dye decoloration                                                             |
|                     | *Pyricularia oryzae*   | Azo-dye degradation                                                          |
|                     | *Trametes versicolor*  | Textile effluent, chlorophenol, urea derivatives: degradation               |
|                     | Plant materials        | Chlorophenol degradation, xenobiotic binding to humus                        |
| Catechol dioxygenase| *Pycnoporus cinnabarinus* | Benzopyrenes degradation                                                     |
|                     | *Comamonas testosteroni* | Chlorophenol oxidation, diuron degradation                                   |
|                     | *Pseudomonas pseudoalcaligenes* | Polychlorinated biphenyls, chlorothanes: degradation                        |
| Phenol oxidase      | *Trametes versicolor*  | Chlorinated compounds: degradation                                           |
|                     | *Thermoascus aurantiacus* | Kraft effluent decontamination                                               |
2. Lignin peroxidase (LiP): It is also called ligninase and diarylopropane oxygenase. It is mainly produced by white-rot fungus (*Phanerochaete chrysosporium*). This enzyme has lower stability towards extreme physico-chemical conditions than HRP; however, it can hydrolyze a wide range of aromatic recalcitrant compounds like polycyclic aromatic and phenolic compounds in addition to its natural substrate (lignin) [46, 47].

3. Other peroxidases: Chloroperoxidase produced by *Caldariomyces fumago* is used to oxidize several phenolic compounds through oxygen transfer reactions. Manganese peroxidase produced by *P. chrysosporium* catalyzes oxidation of several monoaromatic phenols and aromatic dyes in the presence of Mn$^{2+}$ [48, 49].

4. Use of plant material: Plant material containing peroxidases is being directly used in mineralizing phenolic compounds present in water as well as soil. Generally, plants’ roots are best suited for mineralization of phenolic compounds. Peroxidases from tomato and water hyacinth plants have broad specificity for a variety of phenolic substrates while that from minced horseradish, potato, and white radish are specific and act only on 2, 4-dichlorophenol and related compounds [50].

**(b)** Polyphenol oxidases: These are helpful in decontamination of phenolic pollutants present in wastewater.

1. Tyrosinase: It is also called polyphenol oxidase, phenolase, and catecholase [51]. It removes phenols by its hydroxylation followed by production of o-quinones, which are insoluble in water hence easily collected through filtration [52]. The enzyme usually works in the concentration range from 0.01 to 1.0 g dm$^{-3}$ of phenol present in wastewater.

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**Table 3.5 Summary of Enzymes Used in Biosensors for Detecting Various Water Contaminants**

| Pollutants       | Enzymes                                |
|------------------|----------------------------------------|
| *Heavy metals*   | Urease                                 |
| Mercury, cadmium, arsenic, copper, lead |                                       |
| *Phenolic compounds* | Laccase and tyrosinase                |
| Binary mixtures like phenol/chlorophenol, catechol/phenol, cresol/chlorocresol, and phenol/cresol | Polyphenol oxidase |
| Phenol, p-cresol, m-cresol, catechol |                                       |
| *Pesticides*     | Peroxidase                             |
| Simazine         |                                        |
| Parathion        | Parathion hydrolase                    |
| Paraoxon         | Alkaline phosphatase                   |
| Carbaril         | Acetyl cholinesterase                  |
| *Herbicides*     | Acetyl cholinesterase                  |
| 2, 4-dichlorophenoxyacetic acid |                                      |
2. Laccase: It is produced by many fungi, but *Rhizoctonia pratica*ola produces a larger amount of the enzyme than any other known sources. This enzyme is not very specific towards its substrate, but it can act on a number of similarly looking phenols. During its action, it generates anionic free radicals, which help in precipitation of many recalcitrant pollutants present in wastewater [53].

- **Pulp and paper wastes**
  (a) Peroxidases: During wood pulping, 5%–8% (w/w) of residual modified lignin is produced by the Kraft process, which imparts a brown color to the pulp. This brown color is removed by bleaching agents (chlorine and chlorine oxides) at commercial scale. These chlorinated compounds are highly toxic and mutagenic and are released into the effluents, creating a major environmental hazard. Horseradish peroxidase and lignin peroxidase (from *P. chrysosporium*) are helpful in treating Kraft mill effluents, particularly in color removal and degradation of lignin by oxidizing aromatic units to cation radicals, which can be easily decomposed [54, 55].

  (b) Laccase: This enzyme is useful in treating effluent coming from bleaching industries and helps in removal of chlorophenols and chlorolignins [56]. It is an intracellular enzyme produced by the fungus *Coriolus versicolor*.

  (c) Cellulolytic enzymes: These include cellobiohydrolase, cellulase, and β-glucosidase, helpful in treatment of sludge generated from pulping and deinking operations. They are able to convert cellulolic sludge (60 kg ton⁻¹ of pulp) generated from pulping into ethanol (higher commercial value). Further, they are not inhibited by high ink content (present in pulp) and their efficiency can be increased by adding surfactants [57].

- **Pesticides:** They are chemicals used against herbs, weeds, insects, and fungal pathogens for crop protection. The worst part is not their recalcitrance towards degradation but their usage in amounts several times higher than are actually required, due to ignorance. This leads to their enhanced concentration not only in the growing crop but also in the water table deep inside the soil. Further, pesticide-producing industries have other serious concerns due to their continuous disposal of chemical wastes into surface waters. Physical (such as incineration) and chemical treatment of pesticides are not effective and they produce hazardous by-products and are expensive [58]. Enzyme-based treatment of pesticides has given some hope; presently only an enzyme has been identified to be useful in pesticide detoxification, which is parathion hydrolase [59].

  (a) Parathion hydrolase (phosphotriesterase): It is produced by bacteria like *Pseudomonas* sp., *Flavobacterium* sp. and a recombinant *Streptomyces* [60]. It can hydrolyze organophosphate pesticides (major proportion of agricultural pesticides with the highest toxicity) like methyl and ethyl parathion, diazinon, fensulfothion, dursban, and coumaphos. Hydrolysis of the mentioned pesticides using the enzyme helps in their solubilization followed by their degradation using UV ozonation [61]. The enzyme parathion hydrolase has temperature and pH stability at 50°C and 5.5–10.0°C, respectively [62].
Cyanide wastes: These are produced during industrial processes like production of synthetic fibers, rubber, chemical intermediates, pharmaceuticals, ore leaching, coal processing, and metal plating, as well as from food and feed production, due to the presence of cyanogenic glycosides in various crop materials. Further, they are produced during natural biological processes through enzymatic action by plants, microbes, and insects. Over 3 million tons of \( \text{CN}^- \) is being discharged by various processes yearly in drinking water throughout the world. \( \text{CN}^- \) is extremely toxic, as it is the metabolic inhibitor of crucial respiratory enzymes.

(a) Cyanidase: It is produced by *Alcaligenes denitrificans* having \( K_m \) of 0.02 mg dm\(^{-3} \) for \( \text{CN}^- \) with optimum pH range from 7.8 to 8.3 [63]. It can convert \( \text{CN}^- \) present in wastewater into ammonia and formate without being affected by common ions like \( \text{Fe}^{2+} \), \( \text{Zn}^{2+} \), and \( \text{Ni}^{2+} \) or organic substrates (acetate, formamide, acetamide, and acetonitrile) present in wastewater. This enzyme has been found to be very useful in treating waste coming from the food industry based on debittering apricot seeds.

(b) Cyanide hydratase (formamide hydrolyase): It is produced by fungi like *Gloeocercospora sorghi* and *Stemphylium loti* for the treatment of industrial waste rich in \( \text{CN}^- \) [64].

Food-processing wastes: These are produced by the food-processing industry in the millions of tons every year. They are a major cause of the increase in water BOD and make the water unfit for drinking and other applications.

(a) Proteases: They can be obtained by *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas marinoglutinosa*, and *Acromonas hydrophila* [65]. They are used in solubilizing waste streams coming from the food industry processing of meat, fish, and livestock [66]. They are also helpful in processing waste coming from poultry slaughterhouses, particularly in processing of feathers. However, before protease treatment there is a pretreatment of waste feathers by using NaOH, followed by mechanical disintegration and then enzymatic treatment. The process produces lots of protein as end products, which can be used as a feed constituent.

(b) Amylases: They are used in reducing BOD of wastewater and treatment of wastewater through saccharification and fermentation. They are found to be useful in the synthesis of bioplastics (photodegradable and biodegradable) using waste containing cheese whey, potato waste, etc. [67]. They are also used in the preparation of mulch films, compost bags, and in programmable fertilizer and pesticide delivery systems (protect from excessive pesticide runoff) using food wastes present in water [68, 69].

(c) Other enzymes: Pectinesterase from *Clostridium thermosulfurogenes* and pectin lyase from *Clostridium beijerinckii* are used to degrade pectin (cell wall component of most fruits) present in wastewater into butanol (commercially valuable). The enzyme \( l \)-galactonolactone oxidase, from *Candida norvegensis*, is used to convert galactose (present in whey contaminated water) into \( l \)-ascorbic acid (commercially valuable) [44].
The billions of kilograms of whey (responsible for increasing BOD of wastewater) produced annually by dairy industries can be treated by the lactase enzyme [70]. Chitinase from Serratia marcescens QMB1466 is used for shrimp pretreatment, leading to its size reduction, deproteination and demineralization, followed by production of single cell protein (SCP). Further, it is used in treatment of shellfish waste rich in chitin content [71].

- **Solid waste and sludge treatment:** Solid waste and sludge are generally accompanied with lignocellulosic and cellulosic waste, which can be treated with an enzyme system containing endoglucanase, cellobiohydrolase, and cellobiase and turned into useful products like sugars, ethanol, biogas, and other energetic end products [72]. This enzyme system has been found to be useful in treating the organic fraction present in municipal solid wastes (MSW) into fermentable sugars like ethanol or butanol under anaerobic conditions, which can then be collected for various other applications [73]. The enzyme system econase (endo-1, 4-β-d-glucanase, cellobiohydrolase and exo-1, 4-β-d-glucosidase) is also helpful in treating MSW [74]. Sludge dewatering is done using carbohydrase, lipase, and proteinase to reduce the volume of sewage sludge [75]. Peroxidases are also helpful in dewatering MSW sludge as well as in sedimentation of slimy material (very difficult to settle) by removal of water molecules and enhancement of mechanical binding of slime particles. Further, they also promote growth of microbes like algae and molds, which helps in additional aggregation and increased viscosity, leading to effective sedimentation of slimy material [76].

- **Removal of heavy metals:** Various industrial processes like mixing, smelting, electroplating, synthesis of pigment, nuclear power, defense, and fuel reprocessing produce a number of heavy metals like arsenic, copper, cadmium, uranium, lead, chromium, strontium, etc. discharged into water. Phosphatase containing cells of Citrobacter sp. are found to be useful in remediation of wastewater containing heavy metals. The remediation of heavy metal-containing wastewater by the enzyme phosphatase involves production of insoluble phosphate, followed by precipitation of almost all types of heavy metals, and collection. This enzyme has optimal pH stability in the range from 5 to 9, with temperature stability from 10°C to 40°C. The enzyme can severely be affected by the presence of high concentrations (>5 mM) of ions like Cl⁻ and CN⁻ [77–79].

(ii) **Water Hardness**

Hard water contains metallic ions like calcium, magnesium, aluminum, barium, iron, manganese, strontium, and zinc, which make it unfit for drinking and other daily activities. Further, it is also responsible for precipitate deposition in hot water pipes, heaters, boilers, kitchens, bathtubs, and other units. The two most common methods used for hard water treatment are lime soda and ion exchange softening. They are popular due to their cost effectiveness and faster action. Other methods include distillation, nanofiltration, electrodialysis, CNTs, capacitive deionization, and reverse osmosis. Lime soda and ion exchange softening methods also have drawbacks. In the case
of lime soda, there is production of lots of sludge, which requires additional treatment by harsh chemicals followed by acidic treatment. In the case of lime soda, it generates lots of sodium content in the treated water, which is responsible for health issues like stroke, high blood pressure, hypertension, etc. [80].

Enzymes have provided solutions for removing water hardness without generating any side effects, and in a cost-effective manner. However, they have not yet landed in the commercial market, as large-scale testing is still in process. The laboratory set-up (diagrammatically shown in Fig. 3.3) contains an electrolytic cell containing an anode (immobilized glucose dehydrogenase) onto Nafion membrane and a cathode of Pt/C. A porous ion exchanger membrane is between the anodic and cathodic chambers. There is a central chamber with an inlet of hard water and an outlet for treated water without metallic ions. A solution of glucose prepared in phosphate buffer (pH 7.1) in addition to NAD$^+$ fills the anodic chamber while there is only a phosphate buffer with the same pH in the cathodic chamber. During hard water softening, the cathodic chamber exchanges cations (such as Ca$^{2+}$, Mg$^{2+}$) while the anodic chamber exchanges anions (such as Cl$^-$). This is carried out by oxidation of glucose into gluconic acid with the reduction of NAD$^+$ to NADH$^+\cdot$H$^+$ by

![Diagram of enzymatic water softener](image)

**FIG. 3.3**
Schematic of an enzymatic water softener (AEM, anion exchange membrane; CEM, cation exchange membrane).

Adapted from Nigam VK, Shukla P. Enzyme based biosensors for detection of environmental pollutants: a review. J Microbiol Biotechnol 2015;25:1773–1781.
the enzyme in the presence of oxygen being sparged along with the hard water. In the anodic chamber, production of H\(^+\) ions leads to removal of Cl\(^-\) from hard water as HCl, while gluconate in the cathodic chamber combines with divalent (Ca\(^{2+}\) and Mg\(^{2+}\)), thus freeing the hard water from the ions responsible for its hardness. It has been found that Ca\(^{2+}\) can be removed more easily than Mg\(^{2+}\) as the latter has a larger hydrated radius. This set-up has an efficiency of 80\%, which is highly dependent on the amount of Mg\(^{2+}\) in the hard water. The performance of this softerner is dependent on the enzyme stability and efficiency of cofactor cycling. This type of enzyme softener has been recommended for residential use. Work is in process to enhance the enzyme stability by various methods, of which immobilization has been given utmost priority. The other enzyme substitute has also been looked into, to make the process NAD\(^+\) independent, namely glucose oxidase [81].

(iii) Enzymes are Best Suited for Biofilm Removal

Biofilm is an extracellular polymeric substance (EPS) secreted by microbial species like Leuconostoc mesenteroides, Pediococcus sp., Streptococcus salivarius, Sulfolobus acidocaldarius, Bacillus subtilis, etc. [82]. It helps in cells’ attachment to the surface, channeling of water and oxygen as well as nutrients, serving as a barrier against hostile environmental conditions, and providing resistance against antimicrobial agents. It can be either hydrophilic or hydrophobic, depending on environmental conditions. It contains polysaccharides (homo or heteropolysaccharides containing sugars like glucose, fructose, mannose, galactose, pyruvate, mannuronic acid, glucoronic acid, etc.), proteins (10–200kDa with ~40%–60% of hydrophobic amino acids), nucleic acids, lipids, and humic substances [83]. However, polysaccharides are the most important component as they provide adhesion to microbes on the surface. The composition of biofilm depends on microbial strain, stage of growth phase, limiting substrate (carbon, nitrogen, and phosphorus), oxygen limitation, ionic strength, temperature, and presence of shear force. A biofilm can be capsular (tight microbial binding) or slimy (loose microbial binding) depending on the constituents of the water. A biofilm is generally formed in water highly contaminated with pollutants rich in organic waste.

Biofilm has been a nuisance for water treatment and creates enormous problems for water treatment and drinking water distribution systems (increased concentration of contaminants), with reduced quality of potable water and increased rate of pipe corrosion. It leads to fouling of various instruments used during water treatment, followed by accelerated corrosion. Various methods have been tried for efficient biofilm removal including chemical (antibiotics, chemical biocides, detergents, surfactants, disinfectants like chlorine, chlorine dioxide) and physical methods (UV irradiation, scraping, sonication, freezing and thawing). None of them are completely effective due to increased rates of genetic exchange, altered biodegradability, and increased secondary metabolite production. Further, these methods are time consuming, have poor penetrative ability, and are costly.

Enzymes have brought solutions due to their effective degradative capabilities, through direct action onto biofilm. The type of enzyme application requires complete information on the composition of the biofilm, which then determines the type
of enzyme, its concentration, temperature, pH, ionic strength, etc. Enzymes act by destroying the physical integrity of biofilms by weakening of bonds present in their constituents like polysaccharides, proteins, lipids, nucleic acids, etc. It has been found that degradation of polysaccharides by polysaccharases has been very effective in degradation of biofilm, as it is the dominating factor for biofilm attachment and proliferation [84]. Enzymes like cellulase, α-amylase, and β-glucanase are effective biofilm removal agents, regardless of enzyme source. However, enzymes like protease, β-glucosidase, aminopeptidase, β-galactosidase, lipase, phosphatase, and lipase assist in biofilm degradation. Generally, bacterial and fungal sources are preferred due to ease of enzyme isolation and purification. The best recipe for enzyme preparation obtained A. naeslundii, N. subflava, L. rhamnosus, P. gingivalis, S. oralis, S. mutans, V. dispar and S. sanguinis [Patent CA2001539A1, Patent US4936994, Patent EP0388115A1] containing α-amylase, lipase, β-glucanase and protease enzymes, which have been found most effective with respect to any other combinations [85]. Protease present in the combination helps in protein degradation and makes it highly labile to be easily acted on by other polysaccharases, most importantly α-amylases. [86] Enzymatic action has been found to be more effective, more time saving, and less laborious in removal of biofilm than any other methods known currently. However, this method has not been yet commercialized due to lack of technical set-up and the presence of lower-cost biocides prevailing in the market. Enzyme technology through enzyme immobilization has been suggested, which would be a help in commercializing enzyme-based biofilm removal at large scale in water treatment plants.

(iv) Sensing and Monitoring

Enzymes are an excellent sensor in monitoring various water pollutants in treated water due to their catalytic specificity [87, 88]. The most commonly used enzyme-based biosensors are being used to monitor pollutants like carbamates, phosphates, organophosphates, alcohols, ammonia, cyanide, formaldehyde, organonitriles, phenol, zinc, and BOD as well as contamination by sewage. Enzymes are generally immobilized onto the transducer tip or entrapped inside a polymeric membrane made of poly-(2-glucosyloxyethyl methacrylate)-concanavalin, polyethyleneimine, polyvinylferrocenium, etc. Sometimes, inorganic carriers like clay, porous silica, and alumina powder are used for enzyme immobilization, to provide better thermal and mechanical stability to the enzyme. They are preferred as they are nontoxic with respect to other reported carriers.

3.5 Combo-technology: water purification, disinfection, sensing, and monitoring

Enzymes are best suited for water remediation due to their ability to act on a vast variety of recalcitrant compounds. However, the soluble state of enzymes cannot be directly introduced into wastewater due to rapid inactivation and loss of catalytic efficiency [89]. A number of delivery systems can be used to introduce enzymes into wastewater, including:
• **Enzyme delivery by direct use of biological source:** Enzymes containing a source (microbial cells, plant tissue, or cells, etc.) are introduced directly into water effluent, rather than extracting soluble enzyme from the source. In the case of a plant source, either plant tissue or sometimes whole plants are used, depending on the enzyme secretion being used for degradation of pollutants. For example, degradation of dyes like malachite green, methyl orange, and brilliant blue R4 uses the enzyme peroxidase secreted from the roots of *Typhonium flagelliforme*. However, the major disadvantage of introducing plant parts or a complete plant is the increase in BOD and COD of the water effluent, which complicates the complete water remediation process. Enzymes from microbial cells are much more effective in water remediation, as enzymes can be induced to be synthesized by microbial cells depending on the type of pollutant. However, induction requires modification of the growth media with the addition of essential nutrients, which is generally not possible with in situ water remediation by enzymes. For example, a pure bacterial strain *Staphylococcus arlettae* can degrade azo dyes like CI Reactive Yellow 107, CI Reactive Red 198, CI Reactive Black 5, and CI Direct Blue 71 by 97%. However, degradation requires the presence of an active carbon source for effective pollutant degradation [90]. Further, enzymes containing microbial cells for pollutant degradation do not initiate as soon as microbial cells are introduced into the water. They need time for acclimatization to recalcitrant pollutants present in wastewater, which makes complete water remediation more time-consuming and less effective.

• **Enzyme delivery as cell-free enzyme extracts:** Cell extract containing the enzyme required for pollutant degradation is prepared through source lysis using chemical or physical methods. Here, the complexity of the growth medium as well as the time duration for acclimatization is not the issue being raised, thus making water remediation faster, simpler, and more cost effective. However, crude enzymes have lower catalytic efficiency than pure enzymes, in addition to the fact that pure enzymes are more susceptible to degradation by slight changes in the physico-chemical environment, which is not possible to control, particularly in wastewater heavily laden with recalcitrant pollutants [91].

• **Enzyme delivery in immobilized form:** This is the method best suited for introducing enzymes into wastewater without affecting enzyme conformation, an important factor in their catalytic activity. Enzymes are protected from extreme physico-chemical conditions like high temperature, very low or very high pH, high ionic strength, presence of inhibitors, etc., commonly found in polluted water. Further, this technique provides the additional benefit of increased reusability, making the complete process more cost effective with enhanced adaptability, easier handling, and higher specificity than any other known water treatment. Enzyme immobilization can be carried out by various means, such as adsorption, covalent binding, chemical coupling, etc. Enzyme matrices chosen for immobilization are well characterized with respect to their mechanical and hydrodynamic properties, filter characteristics, and biocidal properties, in addition to enzyme stability, reusability, and catalysis [44, 92–94].
Nanoparticles are best suited for acting as matrices for enzyme immobilization, due to their very large surface area to volume ratio, high reactivity, and sequestration properties (Fig. 3.4). Generally, enzymes used for water treatment are immobilized either by chemical coupling or covalent binding, as they provide increased reusability and more stability with minimal restrictions being imposed by pollutant diffusivity. However, there is a chance of enzyme inactivation when active sites are involved in enzyme attachment. In those cases, optimization is carried out with respect to the modifiers being used, and the conditions during enzyme immobilization are chosen with utmost care. Further, the enzyme immobilized by nanoparticles can be systematically moved from bench scale testing, followed by statistical analysis for lower volume systems, to commercialization for water treatment at pilot scale [95]. Thus complementing enzyme technology with nanotechnology would be very effective in water decontamination, due to removal of several drawbacks faced when used individually.

Several reports have been published on enzymes being immobilized onto nanoparticles for water pollutant degradation, sensing and removal of biofilm. Chymotrypsin (protease) has been immobilized inside a silicate shell-like cage (allowing pollutants to permeate) for biofilm degradation [96]; peroxidases, laccases, tyrosinase, dehalogenases, and organophosphorous hydrolases have been immobilized onto CNTs for degradation of various recalcitrant organic contaminants like phenols, polyaromatics, dyes, chlorinated compounds, and pesticides [97–101]. It has been found that nanoparticles in the form of nanosponges (made of nanopolymers) containing

**FIG. 3.4**
An overview of various nanomaterials used for enzyme immobilization during effective water treatment.
microscopic particles with nanosized cavities are useful in enzyme immobilization by encapsulation, increasing the catalytic efficiency by severalfold [102]. Enzymes like peroxidase and laccase (used for water remediation containing waste coming from dyeing and textile-processing industries) have been effectively immobilized inside nanosponges [103]. Immobilized enzymes using nanoparticles have been very useful due to various abilities: hydrolysis of all types of recalcitrant and xenobiotic compounds, adaptability at high and low concentrations of contaminant, treatment can be done over a wide range of physico-chemical parameters (pH, temperature, salinity, etc.), high selectivity (can easily be used even in diluted water), negligible shock loading effects, negligible time for acclimatization, easier process control, less sludge volume due to the absence of biomass as well as inhibition by substances generally toxic to living organisms, cost effectiveness, and smaller retention times with respect to other treatment methods.

The most commonly used nanoparticles for enzyme immobilization are:

- **Magnetic nanoparticles:** These nanoparticles are much preferred over other nanoparticles for enzyme immobilization due to the presence of magnetism, which helps in easier separation of immobilized enzymes after water treatment. Most importantly, the superparamagnetic property of magnetic nanoparticles acts to retain magnetism in the presence of a magnetic field, while retaining no magnetism once the magnetic field is removed. Magnetic nanoparticles do not agglomerate and remain suspended in solution due to their supermagnetism, an important feature responsible for the excellent efficiency of immobilized enzymes. Further, the enzyme can be positioned accurately onto nanosized magnetic particles with the help of magnetic resonance imaging. In addition to providing enzyme stability and easier separation from the reaction mixture, magnetic nanoparticles are also used in sensing and monitoring various contaminants present in water. Generally, magnetic nanoparticles like FeO and Fe$_2$O$_3$ are used for enzyme immobilization due to size uniformity and excellent electrical, optical, magnetic, and chemical properties. Sometimes, magnetic nanoparticles are surface modified with polymers (dextran, polyvinyl alcohol; PVA, diethylaminoethyl; DEAE-starch), silica, etc. based on the enzyme type and application. Water-remediating enzymes like cellulase, catalase, $\beta$-glucosidase, lipase, peroxidase, agarase, $\kappa$-carrageenanase, and laccase have been immobilized onto magnetic nanoparticles. Enzymes like alkaline phosphatase and glucose oxidase have been immobilized onto magnetic nanoparticles and are used for sensing and monitoring water contaminants [104–107].

- **Gold (Au) and silver (Ag) nanoparticles:** Besides providing a large surface area for enzyme immobilization, Ag and Au nanoparticles exhibit excellent electronic, optical, and thermal properties and act as conduction centers to facilitate transfer of the electrons. These nanoparticles are highly versatile with respect to their size and structure, which makes them best suited for enzyme immobilization in water treatment as well as in the synthesis of biosensors. It has been found that they provide favorable orientation of
enzyme immobilization (particularly redox enzymes) due to the presence of conducting channels between enzymes and the surface of nanoparticles. Thus they are helpful in imparting maximum stability and reusability to immobilized enzymes. However, they have a tendency of aggregating, due to having very high surface energy. Therefore, surface passivation of Ag and Au nanoparticles has been very important during enzyme immobilization. It can be done using citrate and thiol-functionalized organics, by self-assembling into monolayers, by encapsulation inside water pools of reverse microemulsions, etc. Generally, enzymes are immobilized onto Ag and Au nanoparticles using physical and covalent (direct and through cross-linker) adsorption. The covalent method is the more preferred, as it imparts better stability and versatility of enzyme immobilization. However, the covalent method often leads to enzyme inactivation as compared to the physical method. The covalent method involves functionalization of Ag and Au nanoparticles using cysteamine or cetyl trimethylammonium bromide (CTAB), followed by either direct enzyme addition or after addition of a spacer (glutaraldehyde). Spacer-based enzyme immobilization is more effective, as there is less chance of enzyme inactivation and this technique imparts better catalytic efficiency. There should be intensive optimization, particularly the ratio of functionalization reagent and spacer, to minimize excessive aggregation and enzyme inactivation. CTAB-based enzyme immobilization has been generally used for water-treating enzymes; however, it requires a larger amount of enzyme for immobilization. Water-treating enzymes that are immobilized onto Ag and Au nanoparticles are: lipase, peroxidase, keratinase, α-amylase, esterase, urease, endoglucanase, carboxypeptidase, and laccase. Further, sensing and monitoring enzymes immobilized onto Ag and Au nanoparticles are: choline oxidase, glucose oxidase, and tyrosinase [108–111].

**Chitosan nanoparticles:** Chitosan is a hydrophilic, positively charged biopolymer with uniform dispersion, molecular mobility, relaxation behavior, and adaptable thermal and mechanical properties. These nanoparticles have size versatility ranging from a nanometer to a millimeter, which is helpful in immobilizing variety of enzymes as well as also helpful in types of applications. However, the smaller the size of the chitosan nanoparticle, the greater is its immobilization efficiency. Further, they do not have any magnetic properties and have higher fragility with respect to any nanoparticles being used for enzyme immobilization. Enzyme immobilization onto chitosan nanoparticles is much easier without requirement of any additional chemical reagent due to presence of number of active functional groups. There is proper distribution of enzyme molecules onto chitosan nanoparticles due to regular arrangement of active functional groups onto the surface of chitosan nanoparticles. Enzymes immobilized onto chitosan nanoparticles are highly stable towards adverse physicochemical conditions (pH, temperature, ionic strength), resistant to proteases and various denaturing compounds. Chitosan nanoparticles provide an ideal microenvironment for enzyme catalysis in water treatment. Various water-treating enzymes immobilized onto chitosan nanoparticles are: lipase, laccase,
peroxidase, invertase, cellulase, esterase, phytase, neutral proteinase, keratinase, glucoamylase, and α-amyrase. A sensing and monitoring enzyme immobilized onto chitosan nanoparticles is alkaline phosphatase [112–116].

- **CNTs**: These form one of the excellent matrices for enzyme immobilization due to their cylindrical shape, with the length in micrometers while the diameter is 100 nm. The surface of the nanotubes can be single layered (SWCNT) or multilayered (MWCNT), which provides better versatility to enzyme immobilization. However, SWCNTs are preferred, as they provide a larger surface area for enzyme interaction, while MWCNTs are cost effective and have easier dispersibility. CNTs provide high mechanical strength, are chemically inert, have excellent thermal conductivity, and are environmentally stable. Enzyme immobilization requires chemical functionalization by oxidizing sidewalls using $\text{HNO}_3$, $\text{H}_2\text{SO}_4$, $\text{KMnO}_4$, $\text{O}_3$ which helps in introducing functional groups like carboxylic acid, hydroxyl, phenol, carbonyl and quinone groups onto the nanotube’s surface. These functional groups are used for covalent enzyme binding through esterification, thiolation, alkylation, and arylation. In some cases, enzyme molecules are trapped inside the hollow channel of the nanotube by simple adsorption. CNTs can also be modified with magnetic nanoparticles or complex (chitosan and magnetic nanoparticles) to attain their additional qualities, and it is also helpful in preventing nanoparticle aggregation after enzyme immobilization inside reaction mixtures. Water-treating enzymes that are immobilized onto CNTs are: lipase, laccase, peroxidase, and cellulase. Further, a sensing and monitoring enzyme immobilized onto CNTs is glucose oxidase. Sometimes a specific protocol has to be designed for a particular enzyme immobilization, for example, enzyme peroxidase can be actively immobilized onto MWCNTs by covalent linkage onto a functionalized nanotube surface with cross-linker (glutaraldehyde), followed by blocking of nonbound functional groups using $N$-hydroxsuccinimide (NHS) or $N$-ethyl-$N'$-3-dimethylaminopropylcarbodiimide hydrochloride (EDC). Immobilized peroxidise onto MWCNTs are found to be highly stable and catalytically active for the degradation of recalcitrant compounds present in waste water. Treated water can be monitored with immobilized enzymes (glucose oxidase and peroxidase) onto complex chitosan-SWCNTs [117–121].

- **Silica nanoparticles**: Enzymes are immobilized onto silica nanoparticles via covalent linkage using silanol groups present on their surface. Silica nanoparticles impart excellent enzyme stability and are less toxic due to fabrication. During enzyme immobilization, silanol groups present on the surface of silica nanoparticles are functionalized with 3-aminopropyltrimethoxysilane to generate -NH$_2$ followed by addition of glutaraldehyde (act as a cross-linker), which provides aldehyde groups to enzymes as well as aminolyted silica nanoparticles containing -NH$_2$ and forms imine bonds in both cases. The size of silica nanoparticles can easily be controlled based on synthesis protocols; they are biocompatible and provide a
very large surface area with an abundance of sites for surface functionalization. These features are responsible for excellent immobilization efficiency and better catalysis by immobilized enzymes. In addition, immobilized enzymes are less susceptible to damage by adverse physical and chemical conditions like pH, temperature, organic solvents, ionic strength, etc. Further, a minimal amount of aggregation has been observed in the case of immobilized enzymes kept in the reaction mixture for longer duration. Water-treating enzymes immobilized onto silica nanoparticles are lipase, peroxidase, α-amylase, and keratinase; while sensing and monitoring enzymes immobilized onto silica nanoparticles are catalase and glucose oxidase [122–125].

### 3.6 Combo-technology: bridging the gap between market and bench work

Water treatment using nanoparticles with attached enzymes (combo-technology) has been found to be more efficient and less toxic. Lab experimentation has found this technology to be very useful in effective removal of recalcitrant pollutants and combining the two is complementary. However, the combo-technology has not yet arrived on the market due to various challenges. The foremost of these are scale-up optimization, unstable physical states due to aggregation, and determination of an accurate number of cycles of reusability. Nanoparticles have gained more attention in enzyme immobilization, as they present a very high surface-to-volume ratio, leading to very high immobilization efficiency. The major problem that has been frequently observed is that immobilized enzyme on nanoparticles has a higher tendency of aggregating during storage. Microparticles with larger size, though not having higher immobilizing efficiency, at least have a tendency to aggregate due to less surface energy [126]. The optimal conditions under which aggregation of immobilized enzyme onto nanoparticles can be minimized have not yet been determined. Aggregation leads to heterogeneity and lowers the efficiency of water treatment. It has been found that removal of immobilized enzyme from the reaction medium after catalysis can help in minimizing aggregation. This creates a nuisance, however, as separation of immobilized enzyme (onto nanoparticles) is not an easy job due to the very small size. The concept is then to look for those nanoparticles that have less surface energy and can be separated due to added properties like magnetism. Enzymes immobilized onto magnetic nanoparticles can easily be separated from the reaction medium with the application of a magnetic field. Further, magnetic nanoparticles have less tendency to aggregate when the enzyme covers their surface by immobilization.

However, magnetic nanoparticles cannot be used for immobilization of all types of enzymes. Chitosan nanoparticles have been found to be very useful in this respect; however, they themselves have no characteristics for taking part in any water treatment. Therefore, hybrid magnetic chitosan nanoparticles have been made containing a magnetic coating (such as Fe₃O₄-chitosan nanoparticles) by using methods like micro-emulsion polymerization, or in situ polymerization. They have a very small tendency
of aggregation and present very high immobilization efficiency. Further, they provide excellent enzyme stability with higher number of reusability cycles [127]. Magnetic chitosan nanoparticles are resistant to corrosion and offer higher flexibility with respect to functional groups. It has been found that they are a better sensor for monitoring the level of recalcitrant pollutants in treated water [128]. Similar types of nanoparticles are being explored that can fulfill all the requirements mentioned previously.

Water treatment using combined technologies of nanotechnology and enzyme technology has revolutionized both fields, with more advantages than their individual counterparts. They have exceeded the performance of conventional methods for removal of complex and recalcitrant pollutants in a less expensive manner, but there is still a long way to go before these products are accepted in the commercial market. Toxicological studies must be done despite claims of the nontoxicity of enzyme-immobilized nanoparticles with experimental evidence. The phenomenon of nanoparticle aggregation has to be properly taken care of, as it is one of the major reasons for biological system toxicity. Nanoparticle aggregation has been a major reason for disruption of the aquatic food chain, thus playing an important role in the collapsing aquatic ecosystem. Therefore, eco-friendly water treatment would be more effective, sustainable, and acceptable by every section of society.

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