

Bioremediation of Wastewaters

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Oluwadara O. Alegbeleye

Abstract

Wastewater generation, handling, management, and disposal or recycling is an important issue, globally. Wastewater treatment and reuse is a critical socioeconomic issue, due to several reasons including increased strain on freshwater resources and environmental protection. This overview covers the main types and sources of wastewaters, together with their characteristics. The major alternative uses for (treated) wastewaters are highlighted, exploring the agronomic and socioeconomic intricacies and benefits of wastewater reuse. Over time, there has been extensive research and commercial optimization of suitable wastewater treatment strategies that can ensure reuse or safe environmental discharge. Bioremediation is regarded as a sustainable approach because it is relatively cheap and environmentally friendly. Activated sludge, an important precursor or driver of pollutant removal due to wide abundance and diversity of microorganisms is discussed in this chapter, as well as other technologies such as the use of membrane bioreactors, aerobic granulation technology and hybrid technologies for biological remediation of wastewater-associated pollutants. Known pollutantdegrading microbial groups including bacteria, fungi, and microalgae are discussed and finally, key research gaps are identified.

Keywords

Bioremediation · Activated sludge · Bioreactors · Microalgae · Wastewater

O. O. Alegbeleye (\boxtimes)

Department of Food Science, Faculty of Food Engineering, University of Campinas, São Paulo, Brazil

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20.1 Introduction

The main sources of wastewaters include domestic, agricultural, and industrial activities (such as petroleum exploration, textile production, pharmaceutical activities, as well as other chemical and manufacturing processes) (Changotra et al. 2020). Wastewaters may contain potentially hazardous biological and chemical pollutants including heavy metals, other organic and inorganic chemicals, nitrogen, phosphorous, or other nutrients, microplastics, suspended solids, dispersed oils, salts, clinically relevant pathogens, antibiotic resistance genes, radioactive substances, endocrine disrupters, and so on (Changotra et al. 2019b). Wastewaters are typically characterized based on appearance, temperature, pH, salinity, biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), total phosphorous (TP) levels of total suspended solids (TSS), total dissolved solids (TDS), and non-biodegradable organic compounds (Fazal et al. 2018). Management and disposal of wastewaters constitute a significant technical problem for governments, industry leaders, and other relevant stakeholders since the continuous discharge of raw or poorly treated wastewaters into the natural environment may pose substantial ecological and human health risks (Ferronato and Torretta 2019). Wastewater-associated contaminants may be washed off into surface waters via runoff or seep through soils to contaminate groundwater resources (Alegbeleye and Sant'Ana 2020) and could enter into the human food chain, posing significant human health risks.

20.2 Alternative Uses of Wastewaters

First, a definition of terminologies used in this section is provided, for proper context. Graywater refers to all wastewater that does not contain sewage, i.e. water from a po

source that has been used for laundry and other domestic purposes. Urban wastewater refers to combined effluent that contains sewage. Water that has been adequately treated and is considered suitable for particular specified uses such as domestic purposes, irrigation, etc. is known as reclaimed water. Green water is reclaimed water that has been comparatively highly treated, making it suitable for general purposes, as a non-potable source in parallel with a potable source. Drinking water is very high-quality water certified suitable for human consumption.

Generally, water sources are ranked in the following order in terms of microbiological and chemical quality: rainwater or potable water, deep groundwater, shallow groundwater, surface water (e.g. rivers, lakes, and so on), and raw or poorly treated wastewaters (Alegbeleye et al. 2016; Alegbeleye et al. 2018). Wastewater recycling options depend on the amount and quality of wastewater available, but also on the intended end use (Corominas et al. 2020). For example, in residential or domestic settings, available graywater is usually comparatively limited and treating domestic wastewater generates considerably larger volume of reusable water

(Radingoana et al. 2020). Wastewater treatment however generates significant amounts of sludge (discussed in later sections), which require further management.

Globally, particularly in semi-arid, arid, and other water-stressed regions, freshwater resources are increasingly becoming scarce (Mizved 2013). Wastewater (and graywater) reuse is thus becoming attractive, as it is technically and logistically advantageous for many reasons (Alegbeleye et al. 2018). Fundamentally, the reuse of wastewaters contributes meaningfully to the conservation of natural resources. In certain cases (such as agricultural farming), it serves as a source of nutrients and eases economic and environmental pressures, providing a suitable alternative to environmental disposal of industrial or municipal effluents (Power 2010). Some of the most popular alternative uses of wastewater include: for irrigation of non-food and food crops as well as urban green areas, domestic sanitation, in industrial cooling or other industrial processes, fire systems, recovering arid land, and so on (Englande et al. 2015; Dery et al. 2019). It is important to clarify that the unregulated reuse of graywater is not recommended, because even though graywater does not include sewage, it may contain human pathogens. Many countries, especially advanced countries have stipulated safety standards regulating the recycling and use of (treated) wastewaters. Generally, standards for effluent treatment are based on the required water quality criteria to protect the well-being and beneficial purposes of the end-user or receiving environment. This broadly cuts across the following categories: (1) protection of terrestrial and aquatic ecosystems, (2) agricultural water uses, (3) recreational water uses, (4) groundwater and soils, and (5) human consumption or food production.

While treated wastewaters are usually of slightly poorer quality compared to rainwater, most conventional treatment strategies sufficiently reduce human pathogens and other pollutants to safe levels rendering it colorless, odorless, and suitable for most domestic purposes and agricultural farming (Yadav et al. 2019). Generally, wherever possible, such as in regions with adequate rainfall such as Latin America and sub-Saharan Africa, it is better to use rainwater or potable water for higher-grade purposes such as drinking. The use of raw or poorly treated wastewater for drinking or irrigating crops that will be consumed raw or minimally processed is not recommended (Alegbeleye et al. 2018).

The unintended (or indirect) use of wastewaters, which occurs when untreated, partially treated or treated wastewater is released into environmental resources such as canals and rivers that supply agricultural water is one of the most significant problems associated with the discharge and recycling of wastewaters (Jeong et al. 2016). This is prevalent in developing countries and poses significant public health hazards as the end user is unaware. The probable human and ecological health hazards include: occupational, i.e. (risks to growers, fishermen, and others working on or within the area), residential (residents or those who otherwise have to frequently be in the contaminated area), and the risk of subsequent animal or human infection via the handling or consumption of contaminated foodstuff or contaminated animal (Narain et al. 2013). As urbanization steadily outstrips urban planning infrastructure/provisions in many parts of the developing world, indirect wastewater use is projected to rise (Satterthwaite et al. 2010; Butsch and Heinkel

Category	Example
Biological	Pathogens Antibiotics, mobile genetic elements (MGEs), class
	1 integrons
Metals (especially heavy metals)	Cadmium, nickel, chromium, arsenic, lead, mercury
Nutrients and salts	Phosphorous, nitrate
Organic chemicals	Hydrocarbons, pesticides, other toxic organic compounds
Inorganic chemicals	Fluoride
	Cyanide
	Hydrogen sulfide
Emerging contaminants	Pharmaceuticals
	Endocrine disrupters
	Other veterinary residues
	Detergents
	Other active pharmaceutical ingredients (API)
Other	Suspended matter/solids, acids, and bases

Table 20.1 Pollutants that may be present in raw or ineffectively treated wastewater (Buechler and Scott 2006; Jechalke et al. 2015; Jaramillo and Restrepo 2017)

2020). Types of pollutants that are typically occurring in wastewaters are shown in Table 20.1.

The reuse of treated wastewater is also fraught in that the safety at the point of use depends not only on the source and efficaciousness of treatment, but also on the storage or holding conditions and distribution systems (Alegbeleye et al. 2018). All of these contiguous factors and systems must thus be optimized to yield the full benefits of wastewater recycling. Human perception and attitudes are also significant as people are mostly averse to consuming treated wastewaters (Murray and Ray 2010; Wester et al. 2015). For example, in Singapore wastewater is treated to drinking water standard using membrane filtration, but the water is rarely used for drinking because most people will not knowingly consume treated wastewater (Ormerod 2017; Tortajada and Nambiar 2019). The primary consideration for wastewater reuse is not the direct or indirect municipal or economic benefits, but public health protection (Janeiro et al. 2020). Therefore, the most critical goal of all wastewater reuse endeavors should be to eliminate or at best, minimize potential health risks.

20.3 Treatment of Wastewaters

Wastewaters are treated to sufficiently improve their chemical and microbiological quality such that they can be safely released into waterways or reused for agricultural farming or certain domestic purposes, without exerting any significant environmental or human health hazards (Kehrein et al. 2020). To protect public health, it is important to approach wastewater collection, treatment, and disposal (or recycling) as constructively as possible.

Dhysicschamisel	(i) Coordination
Physicochemical	(i) Coagulation.
	(ii) Filtration.
	(iii) Sedimentation.
	(iv) Flocculation.
	(v) Chemical disinfection, e.g. chlorination.
Biological	(i) Aerobic, e.g. activated sludge (continuous, fill, and draw), membrane
	batch reactors (crossflow, submerged), sequence batch reactors.
	(ii) Anaerobic, e.g. anaerobic filters, anaerobic sludge reactors,
	anaerobic film reactors
Membrane processes	Nanofiltration, ultrafiltration, microfiltration, electrodialysis reversal,
	reverse osmosis, membrane bioreactors, combinations of membranes in
	series
Advanced oxidation	Hydrogen peroxide, ozonation, perozonation, transition metals and
processes	metal oxides, Fenton reactions, photolysis, photocatalysis,
	electrochemical oxidation, ultrasound irradiation, wet air oxidation
Hybrid technologies	For example, advanced oxidation processes + biological treatment
Other	(i) Phytoremediation.
	(ii) Bioremediation.
	(iii) Biosorption.
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 Table 20.2
 Wastewater treatment options (Scott et al. 2002; Deegan et al. 2011)

Most times, treatment strategies and sequence depend on the source and characteristics of the wastewaters. Wastewaters typically contain various particles of differing sizes categorized as supracolloidal (> 100–100 mm), settleable (> 100 μ m), dissolved (< 0.08 μ m), and colloidal (0.08–1 μ m) (Kinyua et al. 2016). Generally, physicochemical and biological treatment strategies are popular as they are deemed cheaper and more sustainable (Barak et al. 2020). Popular wastewater treatment approaches are shown in Table 20.2.

Physicochemical treatment may represent the sole stage/phase in wastewater treatment or may be incorporated as an auxiliary strategy during treatment to improve biological degradation or secondary treatment (such as polishing). Physicochemical wastewater treatment works fundamentally based on the separation of colloidal particles (Samer 2015). Addition of chemical destabilizers such as flocculants and coagulants can alter the physical state of colloids, enhance their stability and in turn, their ability for particle or floc formation, thereby improve settling properties (Sánchez-Martín et al. 2012; Tetteh and Rathilal 2019). Examples of physicochemical or mechanical wastewater treatment processes include: filtration, clarification, dissolved air flotation, aeration, coagulation, sorption, ion exchange, sedimentation, and chlorination, among others (Table 20.2). These processes remove large particles such as fillers, coating materials, bark particles, and other suspended organic solids from wastewaters (Mohan and Pittman 2007). Passing the water through a filter or screen typically can remove large solid compounds and then the water could be passed through grinders or grit chambers to further disintegrate the residual solid wastes or sift out gravel, sand, and other inorganic materials.

For proper perspective/context, very fine particles (colloids), which are highly stable and do not aggregate and subsequently settle, cannot be efficiently separated by flotation, settling, or filtration as they will pass through any filter. In such cases though, separation via other physicochemical treatments like activated carbon filtration and other advanced oxidation processes is possible (Kehrein et al. 2020). Comparatively large particles can be separated by settling using flotation or gravity, depending on the relative densities of water and solids. Physicochemical treatment of wastewaters is evidently not specifically suited for the elimination of microorganisms (Bello et al. 2008; Skouteris et al. 2020). Urban wastewater treatment plants typically consist of a pretreatment step (screening, oil removal, grit removal), a primary settling treatment, designed to retain suspended solids and reduce turbidity. This is followed by secondary treatment (i.e. biological treatment) using microorganisms to catalyze the oxidation of the biodegradable organic matter, which in many cases is the activated sludge (Bertrand et al. 2015).

20.4 Bioremediation of Wastewaters

Bioremediation exploits the metabolic capability of bacteria and other microorganisms to degrade organic compounds and other pollutants into less hazardous compounds, carbon dioxide, water, and minerals (Balseiro-Romero et al. 2019). Aerobic lagoons, anaerobic biological processes, activated sludge systems, and fungal treatment are some of the approaches applied to improve the overall physicochemical properties and neutralize (or at least reduce the toxicity) of chemicals and other hazardous compounds occurring in wastewaters (Pant and Adholeya 2007; Ullah et al. 2020).

Bioremediation technologies can be categorized into two: in situ and ex situ, where in situ refers to the application of bioremediation at the place or point of contamination using natural conditions and ex situ indicates the treatment of wastewater at a separate facility as in the case of centralized and/decentralized wastewater treatment (Kumar Singh et al. 2020). In situ treatment uses natural conditions at the contaminated site, is believed to be relatively cheaper but might be slower. Ex situ treatment approaches are comparatively more expensive as they require waste excavation and transport (Azubuike et al. 2016). In practice, particularly in natural habitats, in situ biodegradation of contaminants is achieved by synergistic interactions among a wide variety or consortia of microbial groups, rather than a single species with known degrading capabilities (Li et al. 2018). In any case, microorganisms with known degrading capabilities may not satisfactorily degrade contaminants in situ (Li et al. 2020a), a trend demonstrated by studies showing that microbial consortia can better degrade pollutants attributable to structural and functional co-operations among diverse members of microbial communities including direct degraders and the so-called non-direct degraders (NDDs) (Hesnawi et al. 2014). Bacterial, fungal, and microalgal species secrete unique enzymes and possess unique degrading genes, which are different in terms of substrate specificity and function over a range of pH and temperature (Roccuzzo et al. 2020). Conversely, however, there is the possibility for interrelationships other than symbiosis such as competition, antagonism, parasitism, neutralism, or commensalism to occur potentially altering the efficacy of co-cultures. In addition, these associations are not static and can in fact evolve over time, which necessitates research on the specific and co-operative roles that the various microbial groups play, specific or co-metabolic degradation steps, and metabolic pathways to permit optimum process control (Roccuzzo et al. 2020). Several strategies including bioventing, biopiling, and bioaugmentation among others are some of the processes that have been developed for the manipulation/modulation and optimization of microbial metabolism of hazardous compounds, colorants, and nutrients occurring in contaminated wastewaters (Ruberto et al. 2009; Alegbeleye et al. 2017a).

Ex situ bioremediation uses bioreactors and there are many different types whose process parameters have been optimized, including aerobic, anaerobic, advanced aerobic, and other wastewater treatment systems (Vikrant et al. 2018; Villegas-Plazas et al. 2019). In one common approach, raw wastewater is suspended in the presence of autochthonous or exogenous microorganisms (in the case of exogenous strains, it is usually those that have been characterized as proficient degraders), thoroughly homogenized and aerated (Samer 2015; Jesus et al. 2019). In another approach, the wastewater can be sprayed over trickling filters or beds of stone covered with microbial biofilms or a cocktail of microbial slime, which act on it to break them down into less toxic byproducts (Ahmed 2007).

The highly controlled and usually conducive conditions in a bioreactor significantly enhance pollutant degradation as well as process rates and efficiency (Alegbeleye et al. 2017a). In most cases, the conditions (such as continuous agitation) in bioreactors improve contaminant bioavailability since it facilitates improved contact between inoculants and pollutants, which enhances contaminant mass transfer phenomena (Bakri et al. 2011). Treatment in bioreactors also offers a dilution effect, which may reduce the impact of pollutant toxicity on degrading microorganisms (Balseiro-Romero et al. 2019). Fundamentally, bioremediation parameters (temperature, pH, and redox conditions) can be more easily manipulated and optimized, but also electron acceptors, solvents, and surfactants can be used to enhance the bioavailability of pollutants (Robles-González et al. 2008; Alegbeleye et al. 2017).

One of the major advantages of ex situ treatment approaches is that it makes the use of exogenous strains with known degradation capacity more feasible while avoiding potential pitfalls such as ecological disruption/stress associated with in situ treatment approaches (Azubuike et al. 2016). Ex situ approaches are also useful because they can be used to assess bioremediation potential, i.e. a pre-validation approach to verify whether or not pilot or field scale remediation may be feasible.

Apart from the management or treatment approach, another critical, yet fundamental factor that influences the type and success of bioremediation is the type of wastewater (Robles-González et al. 2008). The microbial community (diversity and prevalence) depends on the type of wastewater, and this directly affects the success of bioremediation. Increased levels of novel, emerging or recently emerged contaminants such as polychlorinated biphenyls and pharmaceuticals constitutes significant public health challenge particularly because they might not be readily biodegradable. Pharmaceutical wastewaters, for example, might be relatively more challenging to bioremediate seeing as many groups of pharmaceuticals are toxic to microbes; some are antimicrobial agents that can significantly reduce microbial populations in wastewater treatment systems (Iranzo et al. 2018; Changotra et al. 2020).

20.5 Conventional Activated Sludge Methods

Activated sludge is one of the most conventional technologies for the biological treatment of wastewater in wastewater treatment plants (WWTPs), where a suspension of bacterial biomass (i.e. the activated sludge) initiates the biological treatment of pollutants and nutrients (Salama et al. 2019). The process utilizes a dense microbial culture in suspension to aerobically biodegrade organic compounds and form a biological floc for solid separation in the settling units (Tyagi and Lo 2013; Fang et al. 2018; de Rollemberg et al. 2019). The mechanism of treatment is contingent on establishing and maintaining a population of proficient microbial degraders, assuring adequate levels of dissolved oxygen and other environmental/ physicochemical parameters, as well as suitable contaminant-microbe contact (Salama et al. 2019). In some treatment systems, biomass may grow attached to a surface (broadly referred to as biofilms) as, for example, in trickling filters, rotating biological contactors/reactors, granular media biofilters, fixed media submerged biofilters, fluidized bed reactors, among others (Rusten et al. 2006). Some other types of biological suspension systems are moving bed biofilm reactors (MBBR) (discussed in subsequent section 20.4.1), and integrated fixed-film activated sludge systems (which can be regarded as a variation of the MBBR process) (Gernaey and Sin 2011).

Activated sludge treatment removes organic carbon compounds, nutrients such as nitrogen and phosphorous as well as pathogens. In some cases, further treatment (thermal or anaerobic) may be applied to efficiently denature pathogens (Wang et al. 2014). Biological decontamination of pollutants may be brought about by a combination of processes including volatilization, surface binding, and microbial decomposition (Fang et al. 2018). Crucial factors such as environmental variables (including pH, temperature, and oxygen), nutrients (usually suitable, biodegradable waste from the bulk of the "nutrients"), microbial diversity, and abundance influence the overall efficiency of activated sludge systems (Zhai et al. 2020; de Rollemberg et al. 2019). Microbial decomposition may not always proceed at a desirable rate, or effectively eliminate, or reduce hazardous compounds due to unfavorable environmental or physicochemical conditions and sub-optimal levels of proficient microbial degraders (Zhao et al. 2017a; Rastogi et al. 2020). These drawbacks can be countered by strategies such as prior acclimatization (or adaptation) of the microbes to contaminants, i.e. biostimulation (Alegbeleye et al. 2017b; Nikolopoulou et al. 2013; Yang et al. 2019). Oxygen conditions and aeration may be improved by diffused or mechanical aeration, such as in bioreactors (e.g. introducing air via agitation, in the form of bubbles, or through diffusers), or the use of models to determine, forecast,

and monitor suitable environmental factors for the elimination of respective compounds in activated sludge systems (Brdjanovic et al. 2015).

Activated sludge systems are designed depending on factors such as the ratio of the microorganisms (or activated sludge) to the substrate (waste) and other characteristics of the waste. In most systems, healthy, active microbial groups/ population that will feed on new, incoming batch of organic compounds is maintained by replenishing the microbe concentration (sludge) that has drained through the tank and settled out in a secondary sedimentation tank and disposing part of the settled material. For domestic sewage, adsorption of most organic compounds by the sludge floc occurs within 15–45 min, although most conventional plants are designed to provide up to 90 min contact time to ensure adequate adsorption by the sludge floc.

The efficaciousness of activated sludge systems for pathogen inactivation or removal varies tremendously. For instance, there is some indication that despite high removal efficiency and rates, parasites can survive the activated sludge process and that the treatment does not completely inactivate them. Parasites are mostly inactivated during secondary sedimentation, where some studies have reported enhanced protozoa settling during secondary sedimentation, although this seems to vary depending on the type of protozoa. Cryptosporidium removal seems to be comparatively poorer and slower, and both primary and secondary sedimentation may be necessary for the removal of helminth eggs. Researchers have detected the presence of helminth eggs of Ascaris spp., Trichuris trichiura, Hookworm, Taenia saginata, Hymenolepis spp. and protozoan oocysts of Giardia spp., Cryptosporidium, and Entamoeba spp. in activated sludge effluents (Ben Ayed et al. 2009). Helminth eggs are the infective stage of a variety of intestinal worms and although not all helminths are the same, all known helminth eggs are enclosed in a strong protective membrane that consists of an internal lipoidal shell, an intermediate quitinose layer, and a proteic external shell (Robles et al. 2020). This feature makes them highly resistant to most conventional treatment protocols (Robles et al. 2020). Reported removal percentage may, however, depend on the sampling pattern (i.e. if the effluent samples were collected after aeration and sludge separation, or following activated sludge treatment (after secondary sedimentation).

Though activated sludge effectively improves sanitation and minimizes overall environmental health impacts, drawbacks such as associated complex process design, large land footprint, bad odor emissions, and management of treatment byproducts have over time created the need for advancements (Ferronato and Torretta 2019; Cichowicz and Stelęgowski 2019; Guo et al. 2019). In addition, activated sludge processes generate significant amounts of sewage sludge, which may themselves be heavily contaminated (Cichowicz and Stelęgowski 2019; Al-Gheethi et al. 2018). For example, Iranzo et al. (2018) reported sufficient degradation of pharmaceuticals in wastewaters, but elevated levels of the pharmaceuticals were detected in resultant sewage sludge. Sewage sludge can be managed, but imposes additional financial and logistical costs. Sewage sludge management practices vary depending on the type and properties and includes agricultural land application, land reclamation, land filling, anaerobic digestion, energy recovery through incineration, and so on (Kehrein et al. 2020). In recent times, sewage sludge is to a large extent, not regarded as waste per se, but as a source of organic matter, energy, and nutrients (Lalander et al. 2016; Kehrein et al. 2020). There are several strategies for decontaminating sludge such as composting and anaerobic digestion, while composting eliminates most pathogens, as well as a wide range of environmental pollutants, it does not eradicate all pharmaceuticals.

Although several physicochemical and biological approaches such as the use of biofilters, bioscrubbers, and biotrickling filters have been developed over time to minimize the nuisance of bad odors (Barbusinski et al. 2017), odor emissions from activated sludge processes persist as a public health problem. Part of the problem is that certain challenges such as accumulation of toxic metabolites in the treatment systems, moisture control, short-circuiting of gas, media plugging, and so on have been identified as significant drawbacks in some of the intervention techniques developed (Fan et al. 2020; Wu et al. 2018). Contemporary approaches such as aqueous activated sludge biotechnologies have been proposed for end-of-pipe odor removal or prevention of odor emissions. Although they still need to be more robustly characterized and optimized, they are promising because they offer the benefit of simultaneously treating odor and decontaminating wastewater and because of associated low cost (since they can use existential WWTPs facilities).

20.5.1 Membrane Bioreactors

Membrane bioreactors combine the features of conventional activated sludge processes with membrane separation (microfiltration or ultrafiltration in the range $0.05-0.4 \mu m$) to remediate pollutants (Barak et al. 2020). The retention of biomass within the reactor promotes the growth and action of slow-growing autotrophic bacteria, which in many cases, translates to enhanced nutrient and pollutant removal. In fact, when nutrient removal is a priority, MBRs offer a competitive advantage (Yeo et al. 2015). There are a variety of commercially available membranes for use in membrane bioreactors and the most important characteristics that determine the choice include pore size, source material, and structure (Subtil et al. 2014). Based on structural characterization, membranes are categorized as anisotropic (i.e. consists of a thin layer of membrane supported by a dense layer of porous understructure) and isotropic (homogeneous composition). The hollow fiber membrane bioreactor is the most commonly used type of membrane bioreactor (Gede Wenten et al. 2020). There are two possible cell immobilization modes in membrane bioreactors: cells are either immobilized within the membrane or on the membrane in the form of biofilms, or cells are separated from the bioreaction medium by the membrane and maintained in a separate compartment (Nemati and Webb 2011). Regardless of how the cells are immobilized, one critical advantage of MBRs is that the membrane protects the cells from existent bubble bursting and shear forces, which are detrimental to plant and mammalian cells (Nemati and Webb 2011). Generally, because MBRs prevent direct exposure of microbial cells to toxic compounds, it may be considered the preferred choice in the treatment of certain types of waste streams that contain hazardous agents (Sun et al. 2019). There is also some research indication that MBRs may have comparatively better potential (20–50%) for removing micropollutants and other emerging contaminants (Kumar Singh et al. 2020), partly attributable to lengthier solids retention time, which permits more complete oxidation of pollutants.

With membrane pore sizes of less than 0.1 μ m, MBR can mount a barrier to some chlorine resistant pathogens, an indication that it can eliminate a wider range of pathogenic bacteria and viruses (Giorno et al. 2011). Membrane reactors do not require sedimentation and media filtration for suspended solids or mixed liquor separation from treated effluent and the secondary clarifier can be eliminated. This is because aeration, clarification, and filtration are merged into a single unit, which makes the process simpler and enables the use of smaller bioreactors, saving space, and exerting low visual impact. Other advantages include reduced CO₂ footprint, high aeration rates, increased separation efficiency, decreased sludge production, and superior effluent quality compared to conventional activated sludge treatment approaches (Barak et al. 2020). The rate and efficiency of bioremediation in MBRs is hinged on controlling and monitoring operational parameters such as influent pH, organic loads, nitrogen and phosphate levels, and aeration within the bioreactors to ensure optimal conditions for microbial species (Awolusi et al. 2015). Careful selection of microbial species and the carrier material for use is also necessary to ensure high exposure area and provisions of sufficient reaction sites to avoid problems such as limited diffusion or enhanced toxicity (Roccuzzo et al. 2020). The use of MBRs offers simultaneous product separation and bioconversion, usually in form of a concentrated stream and in the last decade, their use for municipal and industrial wastewater treatment has increased. Cost-effective design and operation that has easily built on available technologies makes MBRs more attractive. For example, activated sludge models (ASMs), an important advancement in the modeling and operation of conventional activated sludge processes, though developed for use in activated sludge systems have been easily transferred and applied to MBR processes. Despite these merits however, certain disadvantages such as the need to periodically replace membranes and the need to control membrane fouling have been identified.

20.5.2 Moving Bed Biofilm Reactors (MBBR)

Moving bed bioreactor is a hybrid, advanced wastewater treatment approach that merges the features of attached growth processes/media and suspended growth in a single aerobic tank, utilizing the whole tank volume for biomass growth, which increases biomass quantity within treatment tanks (Ødegaard 2006; Marques et al. 2008). In addition to suspended biomass, attached biomass is usually grown on some specially designed biocarriers that have a high surface area, which is applied for enhanced pollutant removal. It does not require any sludge recycle since the biomass grow on carriers that move freely in the water volume of the reactors (Rusten et al. 2006). These carriers are held within the reactor volume by a sieve system fitted to the outlet of the reactor.

The media is in many cases, fluidized through aeration or by mechanical mixing. The premise is to achieve a continuous operating bioreactor with high surface for biomass growth and minimal head losses. This process has proven efficient for pollutant removal and its comparative (compared to traditional suspended growth systems) minimal footprint makes it particularly attractive (Kumar Singh et al. 2020). In addition, it is easy to retrofit existential aerated treatment processes to an MBBR process by integrating the effluent screens and plastic media into the system (Rusten et al. 2006).

20.5.3 Aerobic Granulation Technology

Aerobic granules are formed when consortia of microorganisms self-immobilize or self-aggregate in the absence of a support carrier (Liu et al. 2003). Aerobic granulation technology is a development on activated sludge technology targeted at improving among other drawbacks, sludge-water separation issues during wastewater bioremediation (Nancharaiah and Sarvajith 2019). It is a promising wastewater treatment approach because the various occurring microbial species can play different specific roles in the treatment of wastewater-associated contaminants (Li et al. 2014). Aerobic granules are regular in shape and have a dense, compact structure, which enhances settling capacity, multi-microbial functions, higher biomass retention, as well as enhanced tolerance to toxicity and shock loading (Li et al. 2020c; Maszenan et al. 2011). So many studies have explored granulation mechanisms, the extracellular polymeric substance matrix (EPS) (i.e. sticky polymers secreted by bacteria consisting of lipids, phospholipids, polysaccharides, proteins, and humic acids, which trigger cell adhesion and formation of aerobic granules), as well as other factors that contribute to the physical and chemical structure of the granules. The factors responsible for the long-term stability of AGS and other related factors that influence the rate and efficiency of wastewater remediation have also been abundantly explored (Franca et al. 2018; Alshabib and Onaizi 2019; El-sayed 2020; Li et al. 2020b; Ogura et al. 2020; Pei et al. 2020; Phong Vo et al. 2020). Granulation is according to some research (Barr et al. 2010) rooted in the formation of biofilms, i.e. aggregation of microbes (similar to that in AS), facilitated by polymeric entanglement, cations, granules shaping, and then densification and possible disintegration. The consensus, however, seems to be that four important stages: (i) intercellular interactions, (ii) microbial attachment and formation of aggregates, (iii) EPS facilitated attachment, and (iv) shaping of granules in anaerobic granulation (Lv et al. 2014) are involved in the formation of dense aggregates (AGS). The approach for AGS cultivation from activated sludge flocs, which involves operating the sequencing batch reactor with anaerobic, aerobic, and short settling phases in the cycle creates optimum growth conditions for slow-growing microbes such as glycogen accumulating organisms (GAOs), polyphosphate accumulating organisms (PAOs), and nitrifying bacteria as dense aggregates (Bengtsson et al. 2018; Wilén et al. 2018). Results of most studies suggest that the predominant microbial groups in AGS are bacteria: (such as *Dechloromonas* spp.,

Thauera sp., *Nitrospira* sp., zoogloea, among many others). There is, however, some research indication that protozoal and fungal filaments play vital roles in the initial stages of granule formulation, contributing to the development of a core for bacterial colonization (Beun et al. 1999; Weber et al. 2007).

Aerobic granulation technology has in the last several years evolved into a robust biotechnological approach that has been used commercially for full-scale industrial and municipal wastewater treatment in different parts of the world such as the UK, Netherlands, Sweden, Brazil, and South Africa (Li et al. 2020c). Several studies have described remediation of persistent hazardous pollutants including phthalates, chloroanilines, pharmaceuticals, nitroaromatics, azo dyes, phenols, organophosphorous compounds, metal chelating agents, and explosives, in AGS reactors (Sarvajith et al. 2018; Zhao et al. 2015; Ramos et al. 2015). Compared to conventional wastewater treatment strategies, aerobic granulation technology is more effective in terms of energy requirements and land use (Sarma and Tay 2017). The process design is also comparatively simpler because aerobic, anaerobic, and anoxic microenvironments are occurring within microbial granules, eliminating the need for separate aerobic and anoxic compartments for efficient biological nitrogen removal (BNR). As a consequence, it is possible to achieve both biological treatment and biomass separation from treated wastewater in a single treatment tank. Also, secondary clarifiers, key for the AS process is not required because of the good settling velocities of granules (Weber et al. 2007). Some other benefits include lower sludge production attributed to peculiar metabolism of the various involved microbial groups and resourceful utilization of excess sludge (Nancharaiah and Sarvajith 2019). Despite its advantages however, certain drawbacks such as the requirement of complex sequencing batch operation modes and the need for post-treatment to satisfy environmental standards have been identified (Liébana et al. 2018). The technique of a popular commercial brand/application (Nereda®) involves a short fill/draw timer over the cycle time ratio (e.g. 15%) of sequencing batch reactors which imposes a stringent flow requirement on its pumping systems, meaning that it can only handle small treatment demands (Zou et al. 2018).

Considering that most large-scale WWTPs are currently running under continuous-flow operation, instead of upgrading WWTPs to SBR systems like Nereda, which is logistically and financially costly, it is more practical to incorporate aerobic granulation technology into existing continuous-flow operations (Kent et al. 2018). Requirements for cultivating microorganisms in continuous-flow reactors differ significantly from that in SBRs. There are typically low substrate concentrations in CFRs due to constant substrate consumption by microorganisms. Cultivation and utilization of aerobic granulation in CFRs are thus unstable and challenging (Kent et al. 2018; Li et al. 2020c). To mitigate some of these limitations, studies have explored strategies such as the application of selective pressure into CFRs by modifying the configuration to stimulate the growth of slow-growing bacteria (Devlin and Oleszkiewicz 2018; Zou et al. 2018; Li et al. 2020c). Incorporation/implementation of AGS technology into WWTPs for full-scale, routine use requires that certain operational parameters be optimized. Bioreactor conditions such as anaerobic feeding, feast farming regime, and short settling

periods select for slow-growing bacteria that have unique metabolic traits and favor granulation (Li et al. 2020c). The maintenance of granular stability is contingent on optimization of anaerobic feeding and sludge removal strategies. In addition, it is important to further explore molecular aspects of granulation. Over time, studies have developed approaches for improved cultivation of aerobic granules. Recently, Li et al. (2020c) developed a reactor to cultivate aerobic granules under continuous-flow and identified key features and operation conditions for sludge granulation and nitrogen removal during municipal wastewater treatment. In addition to reactor configuration, the study found that dynamic feeding pattern enhanced nitrogen removal performance and nutrient removal in AGS.

20.5.4 Hybrid Technologies

Biological treatment approaches, though widely acknowledged as cheap and effective can be disadvantageous in that they are slow, they generate large amounts of sludge and in some cases, unpleasant odor (Gogate et al. 2020; Brillas 2020). In addition, direct biological treatment technologies are for the most part, suitable for the degradation of biodegradable organic pollutants only (Wang et al. 2014; Changotra et al. 2020). Many times, however, wastewater may contain a significant amount of non-biodegradable, recalcitrant compounds and there is good research evidence indicating that biological treatment may be more effective and faster when combined with other techniques such as physical, chemical, or other biological approaches (Changotra et al. 2019a; Paździor et al. 2019; Rahimi et al. 2020; Bhanot et al. 2020). Examples of pretreatment, auxiliary, or sequential approaches include those based on hydrodynamic cavitation, H_2O_2 , Fenton, ozone, and other chemical oxidation approaches (Gogate et al. 2020). Of the several possible combination of technologies, advanced oxidation processes (AOPs) and biological treatment is according to several recent studies particularly promising (Ganzenko et al. 2014; Thanekar et al. 2020; Popat et al. 2019; Paździor et al. 2019). Some advanced oxidation processes such as Fenton processes offer the dual advantage of coagulation and oxidation, while more contemporary processes such as electro-Fenton aid the rapid and effective degradation of recalcitrant pollutants (Nidheesh et al. 2018). Some laboratory and field scale studies have designed dual or multi-phased chemical/biological treatment schemes for the treatment of wastewaters. For example, Changotra et al. (2019b) applied phenton (dark, solar driven photo and electro) as pretreatment for biological treatment of pharmaceutical wastewaters. These processes significantly reduced the organic load of wastewater, enhanced the BOD/COD ratio, improved biocompatibility for subsequent biological degradation, and the overall biodegradability. The study indicated that of the three applied pretreatment technologies, photofenton was the most efficient and was not toxic to the microorganisms in the biological treatment setup. A recent study by Gogate et al. (2020) corroborated these findings reporting that the application of an ultrasonic pretreatment significantly reduced biodegradation time (36 h compared to 60 h of biological oxidation without pretreatment). The pretreatment also significantly enhanced the biodegradability index. Another recent study by Ceretta et al. (2020) coupled biological and photocatalytic treatment for decontaminating textile wastewaters. The study applied bacterial treatment first and then subsequently used photocatalytic process (ZnO/polypyrrole) and reported improved decolorization efficiency and bioremediation rates.

Some researchers have proposed integrating aerobic granular technology with membrane bioreactors for wastewater remediation (Liébana et al. 2018). This combination would yield a hybrid system known as aerobic granular sludge membrane bioreactors (AGMBRs), where the aerobic granules constitute the biomass and the water is treated via filtration (Liébana et al. 2018). Already, research has shown that utilizing granular sludge in MBRs reduced fouling likely because granular sludge has a more compact structure, higher density, as well as larger particle size compared to floccular sludge (Liébana et al. 2018). Also, the use of membranes for the separation of aerobic granules from the treated water (depending on membrane pore size) would yield high-quality effluents. In a full-scale WWTP, AGMBRs would be advantageous since it would guarantee high-quality effluent, with associated advantages including low-permeability loss, minimal space requirements, and less fouling. Some challenges such as maintaining granular stability in AGMBRs is a limitation for the technology (Li et al. 2005; Vijayalayan et al. 2014; Liébana et al. 2018).

Although hybrid processes where systems or treatment protocols are integrated and methods applied sequentially or consecutively seem promising, certain drawbacks persist. For example, AOPs are quite expensive and are inefficient in terms of energy use and sludge production. Overall, integrated treatment approaches seem promising, and it is important to optimize operational parameters that can achieve maximum degradation of organic and inorganic components of wastewaters without exerting any tangible human or ecological health impacts. It is crucial to determine the most suitable treatment sequence(s) and design standard methods for the characterization and management of byproducts.

20.6 Microbial Groups Used for Bioremediation

The rate, efficiency, and overall success of wastewater bioremediation depend to a great extent on the microbial communities in the system (Barak et al. 2020). The potential of using microorganisms: bacteria, fungi, yeasts, and algae to remediate wastewaters has been abundantly explored (Dellamatrice et al. 2017; Roccuzzo et al. 2020); and it has been determined that some microorganisms have the capacity to metabolize/mineralize toxic compounds into CO_2 , methane, and other simpler compounds, while others contribute to decolorizing wastewaters (Forgacs et al. 2004; Spolaore et al. 2006). Several studies have attempted to elucidate the major microbial groups that catalyze the different processes in wastewater bioremediation streams or reactors (Costa and Duarte 2005; Maintinguer et al. 2013). This is critical, as it forms an important basis for improving overall process efficiency. Bioreactors and other biological wastewater treatment systems typically contain a tremendously

complex and diverse microbial community (Valentín-Vargas et al. 2012; Show et al. 2020; Zhang et al. 2020). The advent, advancement, and increased accessibility of molecular identification and characterization methodologies have greatly enabled our understanding of these microbial degraders (Czaplicki and Gunsch 2016; Malla et al. 2018). The microbial groups required to catalyze the bioremediation pathways, processes, and dynamics depend on whether or not the bioremediation process is aerobic or anaerobic (Juwarkar et al. 2010; Azubuike et al. 2016; Alegbeleye et al. 2017a). Where oxygen is the electron acceptor (aerobic bioremediation), aerobic microorganisms including members of the bacterial phyla Proteobacteria, Firmicutes (which include nitrogen-fixing and denitrifying bacteria), and Bacteroides are some of the most predominant bacterial groups associated with degrading or stabilizing wastewater-associated pollutants (Maintinguer et al. 2013; Zhao et al. 2017b). Some popular decomposing bacterial Genera include Pseudomonas. Bacillus. Rhodococcus, Sphingomonas, Burkholderia, and Mycobacterium (Alegbeleve et al. 2017a; Li et al. 2020a). Members of the pathogenic species Enterobacteriaceae and Enterococcus have been found predominant in some surveys, but their use for bioremediation is not recommended due to their potential health relevance (Robinson et al. 2010; Alegbeleye et al. 2017a; Drzewiecka 2016). Anaerobic bioremediation, however, is triggered and progresses through reducing electron acceptors and specific heterotrophic microorganisms. Notable anaerobic metabolic processes that contribute to biodegradation include fermentation, nitrates respiration (including denitrification), and methanogenesis. Examples of studies that have demonstrated the potential for bacterial species to improve the chemical and microbiological quality of industrial, agricultural, and domestic wastewaters abound in the literature. Paisio et al. (2014) reported that Acinetobacter sp. and Rhodococcus sp. degraded up to 1000 mg/L of tannery and chemical industry associated 2-methoxyphenol, as well as 4-chlorophenol, 2,4-dichlorophenol, and pentachlorophenol efficiently. In addition, after 7 days of treatment, BOD and COD levels had been significantly reduced. Similarly, Hesnawi et al. (2014) used Pseudomonas aeruginosa and Bacillus subtilis for the bioremediation of municipal wastewaters.

Fungi can efficiently degrade pollutants occurring in wastewaters via biosorption/ bioaccumulation, adsorption, or other intra- and extracellular enzymatic mechanisms (Roccuzzo et al. 2020; Sharma et al. 2020). Their use is advantageous because they purify wastewater satisfactorily, yielding good quality effluent, they are renewable, and they produce commercially valuable biomass that can serve as animal feed, biofuel, and fertilizer (Roccuzzo et al. 2020). Examples of proficient fungal degraders are *Aspergillus ochraceus, Scedosporium apiospermum, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor, A. terreus, A. cylindrospora Penicillium purpurogenum,* among many others (Martínez-Gallardo et al. 2020; Sharma et al. 2020).

Although several proficient and potential pollutant-degrading microorganisms have been identified and characterized, a significant percentage of possible degraders remain unexplored. Despite advances in microbial detection, identification, and characterization, several biotechnologically relevant microbial groups remain unidentified and unexploited. Certain studies have indicated that NDDs (i.e. degradation augmenting or assistant strains) can play significant roles in in situ biodegradation and their roles or potential in replenishing contaminated sites should be further explored (Li et al. 2020a). A number of contemporary techniques are used to determine metabolic responses and identify functionality of predominant/active microorganisms in their natural environments. An example is the DNA-stable-isotope-probing (DNA-SIP) technique, a cultivation-independent approach that can be used to identify microorganisms involved in the in situ degradation of contaminants including direct and non-direct degraders in complex microbial communities (Li et al. 2020a). While these approaches are not yet scaled up for real time environmental applications, there is some recent research indicating that the so-called non-direct degraders may play critical roles in bioremediation of compounds such as biphenyl in wastewaters.

20.6.1 Microalgae

Microalgae are a group of eukaryotic or prokaryotic photosynthetic microorganisms that grow in marine and freshwater systems and even wastewater (Khan et al. 2018). Although their photosynthetic mechanism is similar to that of higher plants, microalgae's systematics is based on the type and combinations of photosynthetic pigments occurring in the different species (Moejes et al. 2017; Khan et al. 2018). Most species are, however, capable of capturing solar energy up to 10–15 times better than terrestrial plants (Mondal et al. 2017). They have a simple cellular structure, a large surface to volume body ratio, which enables nutrient uptake and comparatively high growth rates, an indication of efficient CO_2 fixation and high biomass productivity (Singh and Ahluwalia 2013). Additionally, few species can tolerate extreme environmental conditions such as high salinity.

Studies have demonstrated the potential for microalgae to phytoremediate industrial wastes such as dyes, metals, nutrients, and other toxicants in industrial wastewaters (Singh et al. 2016; Fazal et al. 2018). There is also some indication that the cultured microalgae may serve as feedstock for biodiesel production, a potentially sustainable strategy for energy generation, although that is a different subject matter and is not within the scope of this chapter. Microalgae can be cultivated in the wastewaters, which may utilize salts, nutrients, metals, and dyes (depending on the kind of wastewater) as carbon sources for growth and proliferation (Renuka et al. 2015). Some pollutants such as dyes may also adsorb onto the surface structure of microalgae, which has a large surface area and strong binding affinity for some contaminants such as azo dyes and metals, thus acting as a biosorbent (Pathak et al. 2015). Utilization of wastes as a carbon source and biosorption/bioaccumulation may according to some studies occur simultaneously, potentially accelerating the rate and efficiency of bioremediation. Examples of microalgae species capable of pollutant or nutrient biodegradation include Chlorella vulgaris, Chlorella pyrenoidosa, and Oscillatoria tenuis (Forgacs et al. 2004). For example, Chlorella alga isolated by Cheriaa et al. (2009) degraded textile dyes indigo, remazol brilliant orange, crystal violet, and direct blue. Studies have reported

that microalgae–microalgae and/or bacteria–microalgae mixed populations are more efficient than individual microbial strains for neutralizing nutrients and biodegradation of pollutants (Xiong et al. 2018; Roccuzzo et al. 2020). Microalgal–bacterial symbiotic consortia are potentially more sustainable since microalgae can via photosynthesis, provide oxygen for aerobic bacteria while utilizing CO₂ released from bacterial respiration. This improves aeration in the system and can potentially reduce high electricity inputs for aerotion (Roccuzzo et al. 2020).

20.7 Conclusions

As agricultural and industrial activities continue to increase, it can be expected that wastewaters will continue to be generated. The handling, management, and disposal or recycling of wastewaters is an important issue, globally due to the potential public health impacts of poor management. As highlighted in this chapter and several other studies and summaries, wastewater-associated pollutants including human pathogens, endocrine disrupters, and potential carcinogens may enter into terrestrial and aquatic ecosystems, human food chains, and reach humans. Effective and readily accessible treatment technologies are required for the decontamination of wastes prior to reuse or disposal to minimize potential human health and ecological impacts. Bioremediation is widely regarded as a sustainable treatment strategy, as it is a relatively safe and effective treatment approach that degrades toxic wastes into less hazardous compounds. Some of the mechanisms, dynamics and microbial groups and processes, relevant technologies including conventional activated sludge, membrane bioreactors, and aerobic granulation technology have been summarized in this chapter. Despite significant strides, some gaps, which can further improve optimized strategies and contribute meaningfully to protection of public health have been highlighted.

20.8 Future Perspectives

Although wastewater treatment has advanced significantly over time, particularly in response to increasingly more stringent water conservation and public health requirements, there are still some research gaps.

Optimization of critical parameters such as most suitable microbial species, culturing and harvesting requirements/approaches, dynamics, suitable reactors, and other process parameters is necessary. Microorganisms and their potential for biodegradation vary substantially. Some microbes have more versatile biodegradative potential and are thus, more promising biotechnological tools for degradation of wastes. Probably more critical is that of the microbial groups with known biodegradative potential, some groups are more suitable for some types of wastewaters. Wastewaters vary in type and characteristics and some may offer unbalanced nutrient profiles for certain groups of microorganisms. Some pollutants may also be inhibitors to the growth and proliferation of some groups of microorganisms. Adaptability to physicochemical and environmental changes also varies among different types of microbes. It is important to characterize process inhibitors; for example, microbes present in sludge may be unable to degrade the organic compounds in wastewater. Considering that the generation and accumulation of toxic byproducts have been shown to contribute to bacteria die-off and halt or slow down the remediation process, it is important that the generation of metabolites as bioremediation progresses be better characterized and modulated, if need be.

The behavior of pollutants (e.g. their susceptibility to treatment, their persistence potential, their potential for environmental partitioning, their ability to biomagnify along the food chain, their potential non-human hosts, human infection mechanisms, host response, other environmental behavior) has to be properly understood for better design of bioremediation systems/paradigms. Also, future research should evaluate and compare remediation efficiencies of the various remediation approaches in more realistic settings.

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