

7 Techno-Economic Assessment of Microbe-Assisted Wastewater Treatment Strategies for Energy and Value-Added Product Recovery

Bikash Kumar, Komal Agrawal, Nisha Bhardwaj, Venkatesh Chaturvedi, and Pradeep Verma

Abstract

In the twentieth century, wastewater has emerged as one of the most appalling problems facing mankind. In recent times, numerous steps have been taken to conserve the water bodies, and a variety of wastewater treatment strategies have been developed to treat wastewater in order to make it reusable. The high operational cost associated with these strategies makes the process economically unfeasible. Therefore, looking into the high nutrient content of wastewaters from domestic and industrial establishments, it has been proposed that these treatment plants may be integrated with energy generation (bioenergy) and resource recovery (N, P, K fertilizers and molecular intermediates as value-added products) for making the overall process self-sustainable. Overall, the man-made problem caused due to wastewater can be used as an opportunity for economic benefits through technological advancements. The present chapter evaluates technical and economic aspects of various wastewater treatment strategies with special emphasis on energy and value-added product recovery. It will not only highlight crucial features of each process but also suggest probable areas of improvements keeping in mind the future prospects for establishing self-sustainable wastewater treatment plants.

Keywords

Microbe assisted · Wastewater treatment · Resource recovery · Value-added products · Techno-economic

Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Ajmer, Rajasthan, India e-mail: pradeepverma@curaj.ac.in; vermaprad@yahoo.com

B. Kumar · K. Agrawal · N. Bhardwaj · P. Verma (\boxtimes)

V. Chaturvedi SMW College, MG Kashi Vidyapith, Varanasi, Uttar Pradesh, India

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7.1 Introduction

Air, water, and soil are some of the most important factors for the survival of life on earth. Water is vital for existence of life and also acts as universal solvent. It is a vital component of all living organisms, and without it, life is impossible. Leonardo da Vinci had rightly described water as "the vehicle of nature" ("vetturale di natura"). The entire human civilization has evolved around water like Nile River was lifeline for Egyptian civilization and Indus River for the Indus Valley civilization (Pradeep and Anshup [2009](#page-31-0)). It is truly said that accessibility to clean water is a clear sign of wealth, health, serenity, beauty, and originality. Water, which is free of hazardous chemicals and microorganisms, is considered as pure and also necessary for human health (Pradeep and Anshup [2009](#page-31-0)). However, due to different anthropogenic activities, water is being polluted and unfit for human use, leading to water crisis. Further, climate change causing irregular rainfall has added more woes to this escalating problem. Many countries in Africa and Asia are facing severe water crisis. This water shortage is also accompanied by the depletion of the resources (energy and important chemicals) available to growing human society.

Researchers are working on development of various technologies to utilize the wastewater. The conventional effluent and sewage treatment plants for reclamation of water resources are chemical and energy intensive and also require various posttreatment approaches because of the unwanted by-products formed. These methods require high capital investment, operational and maintenance cost, including larger areas, larger infrastructures, or centralized systems (Capodaglio [2017\)](#page-27-0). In order to tackle these problems, several integrated approaches for simultaneous wastewater treatment and resource recovery had been developed. It is an already established fact that microbes (bacteria, fungi, algae, cyanobacteria) play a crucial role in wastewater treatment or water purification and can be pivotal in resource recovery. The sewage sludge, dairy wastewater, industrial and domestic effluents are some of the sources of wastewater. The huge amount of wastewater is available for application, and there is no lack of raw materials for development of integrated approach. The recovery of different resources from wastewater can minimize the environmental footprint of wastewater treatment (Yan et al. [2018b\)](#page-34-0) and simultaneously result in recovery of resources such as energy, N, P, K fertilizers, different organic compounds, and essential chemicals and nutrients.

The microbe-mediated wastewater treatment can help in generation of electricity and bio-methane; the wastewater can act as substrate for the growth of micro-algae and cyanobacteria for generation of bioethanol and biodiesel. In the last two decades, a concept of microbial nutrient recovery cell (MNRC) was derived which is used by the metallurgy scientist and microbiologist for generation of costly metals and industrially important chemicals, respectively. This book chapter gives an insight into types of wastewater available for resource recovery, different microbe-assisted techniques available, treatment and resource recovery, and cost analysis of the process, thus giving a complete techno-economic perspective of microbe-mediated wastewater treatment strategy for resource recovery. There are different types of wastewater available for microbial assisted treatment for the generation of bioenergy and extraction of value-added products. The wastewater can be broadly classified into three units as described below.

7.1.1 Domestic Wastewater

Rapid economic/industrial development and population growth have led to the increased migration of people to urban areas in search of jobs and financial stability. This has put severe pressure on fulfilling the need of food, water, energy, and other resources. Due to large population, the amount of domestic wastewater released to the environment has greatly increased. Human households generate enormous amounts of wastewater on a daily basis. The reports from several government agencies suggest that an average household generates approximately 300–500 gallons of wastewater daily. The wastewaters are generated from washing, bathing, kitchen, and toilets, that are released to sewage systems. This wastewater is then treated, and then it is either reused or released to rivers, etc. The domestic wastewater consists of mostly kitchen waste, human fecal waste, etc., which are mostly biodegradable (Liu et al. [2018](#page-30-0)). Along with these biodegradable components are some non-degradable plastic waste which increases pollution by choking the sewer and drainage system.

The established wastewater and sewer treatment plants are exposed to increased pressure, and their running cost is very high. Therefore, a large amount of domestic wastewater is discharged to water bodies such as rivers and lakes. This has led to deterioration of groundwater and surface water quality, which triggers several potential health and environmental hazards to both animals and mankind. Thus, in the last few decades, attempts are made to turn this crisis into opportunity as domestic wastewater contains organic waste (from kitchen and toilet) and essential elements such as nitrogen, phosphorus, potassium, etc. Various research groups are working on different microbial treatment strategies for simultaneously treating this waste, generation of electricity, and recovery of different resources such as volatile fatty acids (Li and Li [2017](#page-30-1)), nitrogen, phosphorus, and potassium (Shin and Bae [2018\)](#page-32-0) and several other essential organic chemicals. The microbe-based systems are considered self-sufficient for energy requirement with involvement of less amount of chemical for recycling water and resources. To combat this problem, an efficient, economical, and feasible process needs to be developed. A number of techniques have been tested so far by several scientists and stakeholders; however, the best suitable process is yet to be established. The domestic wastewater is basically rich in carbon (C), nitrogen (N), and phosphorus (P) which can be recovered through different recovery mechanisms and techniques involved. After treatment of wastewater, a huge amount of sewage sludge is generated; these are a potential substrate of different organic matter with high nutritional value (proteins, lipids, and carbohydrates) and essential organic chemicals such as PHA for microbes which could be used as a raw material (Balasubramanian and Tyagi [2016;](#page-26-0) Cole et al. [2016](#page-27-1)) in different resource recovery techniques.

7.1.2 Industrial Wastewater

Industrialization is considered as one of the major parameters to evaluate the progress of a nation. In the last century, rapid urbanization and industrialization have led to great socio-economic changes in several nations. Till the beginning of the eighteenth century, rate of industrialization was very slow. With the introduction of industrial revolution in the late eighteenth century came the fossil fuel-based engines and an increase in number of chemical and textile industries. These industries use freshwater as one of the important raw materials from washing and cooling (heat absorption), thus leading to the entry of a large number of chemicals leading to water pollution (Han-chang [2002\)](#page-28-0). This wastewater needs to be treated prior to its release to the environment. But the exponential growth of industries is not accompanied by the similar intensity of wastewater treatment setup. This is because the conventional processes are cost intensive and require big infrastructure, and the industrialists in order to make higher profits have neglected to follow the effluent treatment strategies which need to be implemented. Different combination of pollutants is generated by different industries with different chemical and physical properties. On the basis of type of industries, the wastewater is divided into the following categories:

7.1.2.1 Agro-industries

A number of agro-industries, e.g., food processing units, use larger quantity of freshwater, resulting in the huge amount of wastewater after processing of the food items. Some of the important agro-industries are as follows:

(i) *Canning Wastewater*

Canning is used to preserve processed food materials in an airtight sealed jar or can. The canning industries generates huge amount of cane processing water basically rich in phytochemical compounds released during processing of plants parts used as food materials. For example, citrus canning industries involve sequential acidic and alkaline treatment of the citrus membrane, which involves intermediate washing with water that results in huge amount of processing water with very high chemical oxygen demand (COD~10,000 mg/L). It is estimated that for the production of 1 ton of peeled segment used in canning, it will result in generation of 3.6 tons of effluent wastewater with high COD. These effluents contain beneficial phytochemicals such as pectin, flavonoids, and oligosaccharides. These phytochemicals are of great commercial importance such as food, feed, and medicine. Therefore, wastewater treatment strategies may be developed to avoid environmental problems along with the recovery of phytochemicals as valuable organic food compounds (Yan et al. [2018a\)](#page-34-1).

(ii) *Molasses Wastewater*

One of the major by-products of the beet sugar and cane sugar refining industries is molasses wastewater with high chemical oxygen demand (COD) (80,000–130,000 mgL^{-1}), thereby making it one of the most polluted wastewaters released by any

food industry (Onodera et al. [2013](#page-31-1); Ren et al. [2018\)](#page-31-2). However, molasses wastewater has potential to act as medium for microbial growth as it mainly contains various natural sugars, along with nitrogen, salts, and vitamins added during sugar processing from sugarcane and beetroot (Ren et al. [2018;](#page-31-2) Avci et al. [2014](#page-26-1)). The molasses act as an important raw material for production of alcohols, hydrogen, and several amino acids (Ren et al. [2018](#page-31-2); Yan et al. [2012](#page-33-0); Sirianuntapiboon and Prasertsong [2008\)](#page-32-1).

(iii) *Dairy and Livestock Wastewater*

Dairy waste mainly consists of the cheese whey permeates, and livestock industries generate huge amount of wastewater containing cattle fecal waste (cow, buffalo, swine, etc.). Dairy waste has high BOD and COD, along with presence of antibiotics (i.e., tetracycline, sulfonamides, macrolides, and fluoroquinolones) which are used extensively in the dairy industries, thus making treatment or disposal a major obstruction. Dairy waste also consists of huge amount of casein which can be recovered as a value-added product during treatment. Similarly swine and cow fecal materials are rich in phosphorus and nitrogen; the manure which is a major type of agriculture waste is rich in ammonium and phosphorus (Chandra et al. [2018;](#page-27-2) Kim et al. [2008\)](#page-29-0). The direct releases of these fecal matters and livestock waste/ wastewater have several negative environmental consequences such as pollution of freshwater water bodies; hence, it should be properly treated prior to its discharge. Current treatment approaches for livestock waste/wastewater focus on removal of organics and nutrients via biological processes (Wu et al. [2018](#page-33-1); Kim et al. [2008\)](#page-29-0). A popular treatment method, i.e., anaerobic digestion (AD), can effectively reduce organic concentration and recover useful biogas as bioenergy.

(iv) *Brewery Wastewater*

Several grains such as barley, oats, rice, wheat, and millets are used extensively in brewery industries. The freshwater is used in washing and rinsing of these grains, machines and barrels, filters, bottles, etc. The brewery industry wastewater consists of the suspended solids, detergents, and high concentration of COD and BOD due to soluble and insoluble inorganics (Han-chang [2002\)](#page-28-0). The brewery wastewater does not consist of toxic effluents and mostly consists of biodegradable substances; thus, it can be subjected to microbial digestions for effluent treatments (Lu et al. [2019;](#page-30-2) Han-chang [2002\)](#page-28-0).

7.1.2.2 Paper and Pulp Wastewater

The demand of paper is very high throughout the world, which makes the paper and pulp industry one of the biggest industries. In 2016, as per the record of FAOSTAT, the world production of paper is 410.9 million ton (FAOSTAT). It is a common perspective that the larger the industry, the higher the amount of waste generated that eventually affects the environment. Paper and pulp industry is the largest consumer of freshwater where it uses $5-100 \text{ m}^3$ of water in different steps of pulping for 1 ton of paper produced. The amount of water utilization depends upon the

characteristics of substrates, paper type/quality, and extent of water being reused (Doble and Kumar [2005\)](#page-27-3). The overall process generates huge amount of wastewater during different stages of papermaking, which makes paper and pulp industry the third largest generator of wastewater after metal processing and chemical industries (Ashrafi et al. [2015;](#page-26-2) Savant et al. [2006\)](#page-32-2). The standard industrial technologies and approaches for solutions of problems arising from the industries determine the condition of surrounding environment and the quality of life. Mostly in developing countries, the wastewater generated is more as they are less aware about the water reuse, wastewater treatment plants are poorly regulated, and they lack strict guidelines for water quality measures before its release to environment, whereas in developed countries, they reuse the water more readily along with different technological advancements in treatment process; thus, the amount of wastewater generated has low toxicity (Toczyłowska-Mamińska [2017\)](#page-33-2). However, now the world is getting aware about the toxicity and negative impact of paper and paper mill effluents, and so the governments are tightening the regulations related to wastewater treatment measures. The paper and pulping industry wastewater characteristics at different stages of the paper making depend on the type of process, the type of wood materials, the process technology applied, the internal recirculation of effluent, and the amount of water reused. The paper mill effluents have high COD (1100–2000 mg/L), high total suspended solids (TSS) (300–510 mg), several organic compounds and inorganic compounds such as organic halides (12.5 mg/L), chlorinated compounds (chlorinated hydrocarbons, chlorate, catechols, dioxins, furans, guaiacols, phenols, syringols, and vanillins), volatile organic compounds (VOC), residual lignin, and resin acid which mostly originate from lignins, resins, tannins, and chlorine compounds (Vashi et al. [2018;](#page-33-3) Farooqi and Basheer [2017](#page-27-4); Ashrafi et al. [2015\)](#page-26-2). The paper and pulp wastewater has detrimental impacts on the environment. There are several conventional methods such as physicochemical and biological treatment (aerobic granulation) methods and some hybrid technologies such as MFC-BES and pilot-scale column-type sequencing batch reactor (Farooqi and Basheer [2017\)](#page-27-4). These hybrid methods are based on the concept to develop self-sustainable, energyefficient systems which make the wastewater reusable by removing pollutants and also help in recovery of energy (electricity) and value-added compounds.

7.1.2.3 Textile/Dyeing Industry Wastewater

Industrial revolution started with mechanization of the textile mills, and till date it contributes in large amount to the wastewater generated and released in the environment. Textile processing and dyeing involve use of several acids, alkalis, bleaching agents (peroxide), starch, surfactant, dyes, and metals (Ozturk and Cinperi [2018\)](#page-31-3). As the process involves several washing and rinsing processes, some of these chemicals are washed away during each step, and thus, textile mill effluents consist of these components in less or higher quantity. Due to the presence of these chemicals, textile wastewater has relatively high toxicity, COD, BOD, intensity of color, and salts (Holkar et al. [2016\)](#page-28-1). Various technologies have been developed in order to treat this wastewater for its reclamation and recovery of various industrially important compounds (Sahinkaya et al. [2018\)](#page-32-3).

7.1.2.4 Tannery Industry Wastewater

Leather tannery industries contribute largely to wastewater generation as the processes involved in the tannery are water intensive. The quality of tannery wastewater depends on the different mechanical and chemical processes involved in the leather processing. The water-intensive process involved in these industries basically includes soaking and washing, liming, plumming, and batting followed by drumming and rinsing. The tannery wastewater has high COD (1500–2500 mg/L); high chloride content (5 g/L); highly alkaline, heavy metals such as chromium; and high quantity of settable substances (10–20 g/L), emulsified fat which causes foaming in tannery wastewater (Han-chang [2002](#page-28-0)). The tannery wastewater can be efficiently used for recovery of different biodegradable compounds and metals used during leather processing and generation of bioenergy (biogas).

7.1.2.5 Pharmaceutical Industry Wastewater

Several chemical manufacturing units such as pharmaceuticals, organic dyeing materials, glue, adhesives, soaps, synthetic detergents, insecticides, pesticides, and herbicides generate wastes based on the raw materials used and working process. The large chemical industries simultaneously produce several chemicals and pharmaceutical products; thus, wastewater includes extraction from natural and synthetic compounds, specific poisonous substances, nutrients, and several organic compounds (Stadlmair et al. [2018\)](#page-32-4). Therefore, the BOD/COD is lower than 30% as COD is in the range of 5000–15000 mg/L and BOD is relatively low that results in poor biodegradability, varying range of pH, and bad color (Han-chang [2002\)](#page-28-0). These wastewaters require intensive treatment strategies (Stadlmair et al. [2018;](#page-32-4) Shi et al. [2017\)](#page-32-5) and as they contain a wide range of chemicals so the recovery potential of these wastewaters is really high.

7.1.2.6 Petrochemical Industry Wastewater

Petrochemicals are group of compounds/chemicals derived from petroleum and natural gas. These widespread applications of petrochemicals have led to the contamination of almost every natural resource, say, air, water, and soil. Areas near petroleum refineries have high rate of surface soil and water pollution (Shokrollahzadeh et al. [2008\)](#page-32-6). The animals and plants are adversely affected by products or by-products generated from these refineries. The petrochemical refineries generate huge quantity of the wastewater which has high COD, BOD, oil, grease, metal salts, volatile compounds (Behnami et al. [2018](#page-26-3)), phenols, and mineral oil (Han-chang [2002](#page-28-0)). Several wastewater treatment strategies such as activated sludge treatment and membrane bioreactor (MBR) have been developed for reclamation of the depleting water resources.

It is very clear from the above discussion that water is very essential in almost all human activities such as household, industrial, and agricultural. But the clean water resources are depleting, and climate change has added more worries due to uneven rainfall. Therefore, there is urgent necessity to treat wastewater and extract all possible resources based on circular economy concept or best from waste. In the next part, we will discuss different microbe-based technologies developed for the achievement of the above-mentioned objective of clean water and resource recovery.

7.2 Microbe-Assisted Technologies for Wastewater Treatment: Techno-Economic Evaluation

Several new umbrella concepts such as circular bio-economy and NEWEL (Nutrients-Energy-Water-Environment-Land) (Mo and Zhang [2013\)](#page-30-3) are established. Under these umbrella concepts, innovative research measures have resulted in development of broad spectrum of technologies for utilization of wastewater as resource rather than just another waste generated through anthropogenic activity. The development of these technologies has led to different comparative analysis and technical and economic evaluation in order to identify a self-sustainable technique, which is an economically feasible alternative to the physical and chemical processes. Under this heading, we will discuss the different microbe-assisted process developed for wastewater treatment based on the literature survey, the technical steps involved in developed processes, and economic or market feasibility of the technique.

7.2.1 Microbial Fuel Cell (MFC) Technology: Bio-electrochemical System (BES)

The novel approach of microbial fuel cell (bio-electrochemical system) can be exploited for wastewater treatment in order to meet the energy and water crisis. The MFC involves microbial conversion of chemical energy stored in biodegradable organic materials by bio-electrochemical catalytic activity. This electrochemical energy involves transfer of electron between cathode and anode, which leads to generation of electricity (Kumar et al. [2018\)](#page-29-1); similarly reaction between electron and proton results in formation of methane, hydrogen, hydrogen peroxide, and several by-product recoveries such as redox chemicals, heavy metals, and different value-added compounds (Jadhav et al. [2017\)](#page-29-2).

The MFC consists of anode (oxidative) and cathode (reductive) fuel cells to produce energy and other value-added products by integration of electrochemical and biochemical processes. Figure [7.1](#page-8-0) represents the schematic design of MFC, where in anodic chamber, electrochemically active microbes catalyze the oxidation of organic electron donors and deliver the electrons to anode, where it is captured as electrical energy. In order to maintain the electro-neutrality, the catalytic conversion also results in generation of protons $(H⁺)$ in anodic chamber, and in order to maintain the electro-neutrality, protons travel through semi-permeable cation exchange membrane to cathodic chamber (Kumar et al. [2018\)](#page-29-1). In bio-electrochemical system under the influence of external potential, protons transferred to cathodic chambers are utilized for generation of value-added chemicals/compounds (Jadhav et al. [2017\)](#page-29-2). Bio-electrochemical system offers a flexible platform for oxidation of pollutants for energy generation and simultaneous reduction-oriented methods for product recovery. Thus, it has provided an integrated solution for wastewater treatment and resource recovery in the form of clean water, bioenergy, and chemicals. The

Power Supply / Electricity Generation

Fig. 7.1 Schematic diagram of MFC/bio-electrochemical system

recovery of different resources reduces the additional energy required in individual synthesis process of each resource. In the technological advancement of MFC, where an external voltage is applied in the MEC, this external voltage acts as the driving force in accelerating the microbial electro-catalysis for production of highvalue chemicals such as methane, hydrogen gas, hydrogen peroxide, and caustic soda at the cathode at very low energy cost (Sharma et al. [2014](#page-32-7); Foley et al. [2010;](#page-27-5) Rabaey and Rozendal [2010;](#page-31-4) Rozendal et al. [2008a\)](#page-31-5). This electricity-driven method is now also applied in the area of bioremediation and exploited for inorganic resource recovery as well. These chemicals have higher market value as compared to the external electricity applied, and several studies associated with life cycle assessment of BES suggested that BES is not only economical but also can help in providing several significant environmental benefits (Foley et al. [2010](#page-27-5)). Table [7.1](#page-9-0) contains details of different value-added products extracted during different wastewater treatments along with the microbes involved in the process.

7.2.2 Economics of the BES system

The techno-economic feasibility of the BES system at larger scale beyond laboratory scale (few milliliters to liters) and pilot scale (30–50 l capacity) needs to be assessed. In the early twentieth century, most of the feasibility studies demonstrated

Table 7.1 (continued) **Table 7.1** (continued)

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in the literature are based on the pilot scales with simpler pollutants such as synthetic wastewater which definitely does not consider the solid waste matters and environmental effect on the pollutants before its entry to the BES system. These studies are limited to a smaller span of operating time. As the BES technology was evolving in past 10–15 years, several attempts have been made to study the technical and economic feasibility in terms of key performance parameters such as electrode material, electrode connections, flow modes, and different range of actual wastewater with different pollutant removal (Hiegemann et al. [2016](#page-28-7); Liang et al. [2013\)](#page-30-8). The scale-up studies by Feng et al. ([2014\)](#page-27-10) and Cotterill et al. [\(2017](#page-27-11)) demonstrated the scale-up with 1000-L stackable horizontal MFC and 175-L microbial electrochemical cell (MEC), respectively. It is evident from the observation made during these studies that the laboratory scale reactor design may not work at large scale as evidenced by the lower pollutant removal and hydrogen yield as compared to laboratory scale. It is a well-established fact that development of any technology on larger scale is driven by capital involvement in operation of the system such as land required for the setup, the cost of electrodes and reactors, etc. However, as per studies available in the literature, BES technology is more studied technology for simultaneous waste treatment and resource recovery. The amount of research going around on the BES technology suggests that BES will have lower operating cost than the traditional aerobic treatments as it involves anaerobic process in the anode section. But the cost of land required, electrode separator, membrane, and reactor material and its construction may lead to enhancement in the capital requirement; however, the concept of resource recovery during the process may balance the enhanced cost and help in development of a sustainable system. As demonstrated by Ge and He [\(2016](#page-28-8)), they suggested that 200-L MFC could be cost competitive to 10000 gpd traditional wastewater treatment system in a decentralized system. The mass transfer balance in BES system is also very important. The mass movement must be limited to its own specific environment; any deviation from that may adversely affect the BES performance. Therefore, the reactors must be designed very carefully to prevent any leakage/overflow in substrate or liquid; the distance between the electrodes and separators must be precisely determined.

7.2.3 Microbial Enzyme-Based Wastewater Treatment

Microorganisms which are capable of generating different catalytic enzymes are used in bioremediation of the wastewater containments. However, direct application of microorganism is a slow process and an energy-intensive process as it requires ambient environment for the growth of microbes (Sharma et al. [2018](#page-32-10); Ghosh et al. [2017\)](#page-28-9). Thus, in the last few decades, focus has been shifted to the microbial enzymes separated from microbes (Thatoi et al. [2014\)](#page-32-11). Enzymes are biologically derived macromolecules which act as catalyst for biochemical degradation of different pollutants (Kalogerakis et al. [2017\)](#page-29-7). The major advantages of application of enzymes are as follows: (a) high selectivity to specific substrate, (b) nontoxic by-product formation by enzymatic biotransformation, (c) high mobility due to small size, and (d) limits the application of high energy and harsh chemical employed in the physicochemical processes (Sharma et al. [2018;](#page-32-10) Maloney et al. [2015](#page-30-9); Gianfreda and Bollag [2002](#page-28-10)).

Water Research Commission Project report (No:1170/1/04) by Rhodes University BioSURE Process® identified the active role of group of hydrolase enzymes such as endoglucanases, glucosidases, lipases, phosphatases, proteases, and sulfatases (Watson et al. [2004;](#page-33-9) Whiteley et al. [2002a](#page-33-10), [b,](#page-33-11) [2003](#page-33-12)). They demonstrated the presence of these hydrolase enzymes in biosulfidogenic reactors used for the industrial wastewater treatment. They also demonstrated that these enzymes could be used for treatment of the wastewater from acid mine drainage, abattoirs, textile dyeing, and tanneries (Agrawal et al. [2018](#page-26-7); Mutambanengwe and Oyekola [2008\)](#page-31-12). As per literature survey, it is much evident that very less work has been carried out on direct involvement of enzyme-mediated resource recovery. But the enzymes are highly selective and result in breakdown of several toxic organic and inorganic compounds into different nontoxic residues. These residues can be separated before their release from the treatment plants. The enzyme-mediated microbial fuel cell/biological fuel cell (Kumar et al. [2018](#page-29-1)) is one such another approach for the involvement of enzymes in electricity generation. Therefore, enzyme is not directly involved in resource recovery but can be used as a biocatalyst of electricity generation and resource recovery.

7.3 Methodology Used for the Enhancement of Enzyme Performance in Wastewater Treatment

7.3.1 Immobilization

The application of enzyme-mediated transformation and valorization of raw materials dates back 50 years. The enzymes were used in different batch reactors, which has major drawbacks such as high operating costs (enzyme production cost is more), loss of catalytic activity due to inactivation, etc. During the early twentieth century, the idea of enzyme mobilization has evolved as an interesting alternative to overcome above-mentioned limitations. During the immobilization, biocatalysts are ensured in a localized space which helps in the prevention of loss of the enzyme, enhances the shelf life of the enzyme, and above all increases reusability of the enzyme. Different immobilization techniques have been proposed such as adsorption or covalent binding of enzyme on solid support like nanoparticles, inclusion in a capsule or magnetic beads, embedding in matrix, fiber, or resins, etc. Bayramouglu and Arica ([2008\)](#page-26-8) demonstrated that covalently immobilized horseradish peroxidase on magnetic beads showed higher phenol conversion, high activity, and stability as compared to its free enzyme. Thus, it can be suggested that HRP can be successfully used in a large-scale continuous enzymatic degradation of phenolic pollutants. HRP has wide substrate specificity from an azo dye Remazol Blue (Bhunia et al. [2001](#page-26-9)) to chlorinated dibenzodioxins and dibenzofurans (Köller et al. [2000\)](#page-29-8). Peroxidasebased bioreactors can be designed to treat wastewater by immobilization into such

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medium to allow efficient interactions with substrate/pollutants for its degradation. Several nano-based imbedding materials suggested are carbon nanotubes (Campbell et al. [2013,](#page-26-10) [2014b;](#page-26-11) Dinu et al. [2010](#page-27-12)), graphene oxide (Zhang et al. [2010\)](#page-34-2), graphene oxide sheets (Campbell et al. [2014b\)](#page-26-11), metal-oxide particles (Campbell et al. [2014a\)](#page-26-12), nanotubular aluminosilicate (Zhai et al. [2013\)](#page-34-3), and nanodiamonds (Krueger and Lang [2012\)](#page-29-9) on which the peroxidases are embedded through encapsulation into a gel (De Lathouder et al. [2008](#page-27-13)) or membrane (Zhang et al. [2012a](#page-34-4); Shen et al. [2011\)](#page-32-12). The material should be chosen such that it has minimum interaction with nanosupport or encapsulator in order to preserve the specificity and catalytic behavior of enzyme. However, improvement in stability, selectivity, and efficiency of nanobased immobilized enzyme has provided ample opportunity for its application in wastewater treatment (Zhang et al. [2012b](#page-34-5)). Similarly, Jamie et al. [\(2016](#page-29-10)) demonstrated covalent immobilization of lipase enzyme from *Candida rugosa* that was embedded in modified multiwalled carbon nanotubes (MWCNTs). These lipasebased MWCNTs were used in oily wastewater treatment which showed enhanced resistance of enzyme to severe conditions under industrial applications. The CNTimmobilized enzyme showed 93 times higher catalytic activity as compared to those immobilized on other support material. It also helped in retention of about 98% of biological activity. Laccase from *Phoma* sp. UHH 5-1-03 was cross-linked by electron beam irradiation to polyvinylidene fluoride membrane. The immobilized laccase addressed high removal efficiency of >85% for the acetaminophen and mefenamic acid from a municipal wastewater containing pharmaceutically active compounds (PhACs; applied as a mixture of acetaminophen, bezafibrate, indometacin, ketoprofen, mefenamic acid, and naproxen). The immobilized laccase also displayed higher wastewater stability as compared to non-immobilized laccase (Jahangiri et al. [2018](#page-29-11)).

7.3.2 Biocatalytic Membrane Reactor/Enzymatic Membrane Reactor (BMR/EMR)

Enzyme-based membrane systems were introduced in order to overcome the limitations of enzyme immobilization technique, i.e., decrease in enzyme activity due to steric hindrance effects that arise due to distortion of enzyme structure during immobilization process and the interfacial limitations. The enzymatic/biocatalytic membrane reactor (EMR/BMR) is a bioreactor in which a biochemical transformation takes place in the presence of enzyme and a selective membrane is used to separate the enzymes and end product generated (Vladisavljević [2015;](#page-33-13) Rios et al. [2004\)](#page-31-13). Different types of configurations of membrane catalytic bioreactors are based on relative positions of the catalyst such as the following: (a) the enzyme is separated with the help of a membrane; (b) the enzyme is incorporated within the membrane wall as a filter; (c) the enzyme is encapsulated in the core-shell microcapsule; and (d) a matrix is prepared where the enzyme is encapsulated (Vladisavljević [2015](#page-33-13)) (Fig. [7.2](#page-17-0)).

Fig. 7.2 Types of membranes used in EMR. (**A**). Enzyme not embedded in membrane. (**B**) Enzyme embedded in the membrane. (**C**) Enzyme is encapsulated within a core-shell capsule. (**D**) Enzyme is encapsulated in a matrix-type capsule (Adapted from: Vladisavljević [2015\)](#page-33-13)

7.3.2.1 Types of Enzyme-Based Membrane Bioreactors and Their Application in Wastewater Treatment

- (i) *Immobilized Enzyme Membrane Reactor:*
- In this approach, enzymes are immobilized onto a membrane by covalent attachment, electrostatic deposition, gel formation, and physical and chemical mediated adsorption. Wastewater is transferred through the membrane where it interacts with the enzyme, and the products/treated water diffuses from the reaction side to the other side of the membrane, and there they are recovered as a permeate. A batch ultrafiltration cell-based bioreactor having flat polyacrylonitrile membrane with crude enzyme from *Pseudomonas* sp. having catechol 2,3-dioxygenase activity has been used in coke wastewater having phenolic effluent. The phenolic degradation of 40–80% was achieved with this system (Vladisavljević [2015](#page-33-13); Bohdziewicz [1997](#page-26-13); Bodzek et al. [1994](#page-26-14)). A capillary hollow fiber membrane bioreactor having polyphenol oxidase (EC 1.14.18.1) obtained from *Agaricus bisporus* immobilized in a polysulfone membrane was capable of removing up to 25% of phenolics in 8 h from coal-gas conversion plant effluents (Edwards et al. [1999\)](#page-27-14). Lante et al. [\(2000](#page-29-12)) demonstrated application of laccase from *Pyricularia oryzae* imbedded in a polyethersulfone membrane of a SPIRA-CEL spiral wound module for treatment of synthetic wastewater with eighteen different phenolic substrates. The laccase immobilization resulted in good operational stability and shows potential physico-chemical properties for decreasing phenol substance concentration in a synthetic phenolic wastewater. Jolivalt et al. ([2000\)](#page-29-13) demonstrated immobilization of laccase obtained from a white rot fungus *Trametes versicolor* on a modified polyvinylidene difluoride (PVDF) microfiltration membrane of a frame plate reactor. This system resulted in efficient removal of phenylurea herbicide (N',N'- (dimethyl)-N-(2-hydroxyphenyl)urea) from wastewater.
- (ii) *Extractive Membrane Bioreactor*
- The extractive *membrane bioreactor* consists of a separate membrane system and a biological component (enzyme/microbes). The wastewater first enters into the

Fig. 7.3 Sketch of an extractive membrane bioreactor (Based on Livingston [1994;](#page-30-10) Vladisavljević [2015\)](#page-33-13)

membrane where the pollutants which are hostile to the enzyme or microbes are separated out. The pollutants separated through the membrane are recycled into the bio-medium where the biodegradation occurs. In the recycling unit, the nutrient, pH, and temperature are regulated for proper activity of the biological system (Livingston [1994\)](#page-30-10). If enzyme-producing microbes are directly used, then nutrients, oxygen, and pH are regulated. Livingston [\(1994](#page-30-10)) has also given an insight into the technology where he explained about an integrated system in which a series of membranes may be used for different pollutants and the biological priority pollutants can be subjected to biodegradation. Kojima et al. [\(1995](#page-29-14)) demonstrated an application of hollow fiber bioreactor fitted with polyethersulfone membrane; the bio-medium is supplied with glucose oxidase enzyme obtained from the *Aspergillus niger* that helped in the removal of organic components (glucose) from the synthetic wastewater (Fig. [7.3\)](#page-18-0).

- (iii) *Membrane Separation Reactor (MSR)*
- It is a stirred tank reactor fitted with membrane module. The membrane is used to bound a dissolved or dispersed catalyst (may be enzyme molecules or cells) in the batch reactor vessel. The untreated permeate leaves the reactor through the permeable membrane, whereas the excess solvent and product are withdrawn regularly in feed and bleed fashion. MSR are used by Gallifuoco et al. [\(2001](#page-27-15)) for de-polymerization of polygalacturonic acid using pectolyase obtained from *Aspergillus japonicus*; the membrane material used was polyethersulfone. Similar reactor was used by Lopez et al. [\(2002](#page-30-11)) in dye decolorization by using the manganese peroxidase enzyme obtained from *Bjerkandera* sp. Soybean peroxidase obtained from ground soya bean hulls was used in reactors for degradation of phenols in an MSR; however, better results were obtained when soybean

hulls were used directly that contributed to degradation of the phenols as well as adsorption of excess dye (Flock et al. [1999](#page-27-16)).

(iv) *Anoxic-Oxic Membrane Bioreactor (A/O MBR)*

An anoxic-oxic membrane bioreactor is designed which consists of the modified activated sludge process, anoxic system coupled with contact stabilization, and membrane system (Komala et al. [2011\)](#page-29-15). This system helps in overcoming the fouling of membrane system. Fouling is loss of membrane performance which occurs due to deposition of the suspended particle on its pore (Wang et al. [2017\)](#page-33-14). Fouling causes increase in pressure and decrease in the flux (Komala et al. [2011\)](#page-29-15). Different methods such as aeration and backwashing with water or permeate pump are applied to reduce fouling. Among them, aeration is one of the best suited methods as it increases the flow circulation by inducing shear stress on the membrane. Aeration also helps in providing oxygen to the biomass, maintaining the activated sludge suspension apart from reducing fouling by scouring the membrane (Le-Clech et al. [2006\)](#page-29-16). This AO-MBR system was employed for the dye biodegradation of Remazol Black V (azo dye) with a co-substrate tempe industrial wastewater as carbon source (Komala et al. [2011](#page-29-15)). A long-term performance of the system was tested for dye degradation. The effect of aeration on fouling of membrane was also evaluated, and it was reported that in long-term operation, for stable flux and membrane recovery, a filtration-backwash time of 61 minutes with aeration intensity of 0.7–1 bar was optimum. Xu et al. [\(2014](#page-33-15)) demonstrated that Fenton-Anoxic-Oxic/MBR system can be successfully employed for reclamation of water from the pharmaceutical waste, i.e., avermectin fermentation wastewater. The toxicity of the effluents was reduced significantly along with pollutant removal and improved biodegradability. They also demonstrated that HCl + NaClO system with aeration is used for removing fouling, increasing the flux of MBR and acceptable trans-membrane pressure (TMP). Xiang et al. [\(2003](#page-33-16)) demonstrated a pilot-scale (10m3 /d) plant of AP-MBR for treating the dyeing wastewater of woolen mill (without wasting sludge) for 125 days operation. The water obtained from the treatment plant is reusable in plant.

7.3.2.2 Economics of the Enzyme-Based Technologies

The enzyme-based reactors are dependent on biological catalyst which has certain advantages as well as disadvantages. The advantages are as follows: the processes used in the treatment of many pollutants are very specific in nature, so selective degradation can be done. However, cost of production, stability, and activity at specific temperature and pH require infrastructure or sophisticated setup and selection of the special membrane material so that stability is not hampered much by steric hindrance. Fouling is one of the major problems in the MBR, so A/O MBR is introduced. Several other concerns limiting the application of the MBR on large scale are membrane flux, membrane life (adversely affected by fouling), and the high price of membrane price. Liu et al. ([2010\)](#page-30-12) had performed a case study of an A/O MBRbased sewage treatment in Qingdao Liuting International Airport on the technoeconomic evaluation of the operation and maintenance. He suggested that the

relationship between the costs of MBR system is negative logarithm of membrane flux and membrane life while the relationship between membrane price and costs of running MBR system is in linear relationship. However, this is a highly efficient technique for the wastewater treatment, and resource can be recovered as byproducts of degraded organic pollutants. Different materials scientist and stakeholders are interested in developing this technology on larger scale and wider scale of pollutants. With the improvement of membrane technology, reduction of the membrane prices and overall running cost may be decreased.

7.4 Direct Application of Microbes

7.4.1 High Rate Algal Pond (HRAP) Systems

The introduction of high rate algal ponds (HRAPs) for treatment of wastewater was discovered around 50 years ago; they are widely used for growing microalgae using wastewater as substrate for their growth and in turn treat the wastewater, and the algal biomass generated is used for recovery of value-added products and bioenergy (Craggs et al. [2014;](#page-27-17) Oswald and Golueke [1960](#page-31-14)) (Fig. [7.4](#page-20-0)).

HRAP consists of a shallow open pond (depth of 0.2-1.0 m) divided into a channel by central wall fitted with a paddle wheel for proper circulation of the water throughout the channel, where average water velocity ranges from 0.15 to 0.30 m/s (Sutherland et al. [2015](#page-32-13); Craggs et al. [2014\)](#page-27-17). This shallow depth attributed with high nitrogen (N) and phosphorus (P) content of wastewater along with the turbulent eddies resulting in vertical mixing by paddle wheel helps in algal growth by enhancing exposure of cells to sunlight, preventing sedimentation, and enhancing the diffusion of nutrient across the cell boundary layer (Sutherland et al. [2015;](#page-32-13) Hadiyanto et al. [2013;](#page-28-11) Park et al. [2011](#page-31-15)). The appearance of dead zones is common in long channel of large-scale operations (Grobbelaar [2012\)](#page-28-12). Carbon dioxide is added in the HRAP under carbon-limited situation to enhance algal growth (Craggs et al. [2012\)](#page-27-18). The anaerobic ponds or gravity settlers help in removing and digesting the

Fig. 7.4 Advanced pond system comprising of covered anaerobic pond, HRAP with CO₂ supply, algal settling pond, maturation tank, and rock filter (Adapted from Craggs et al. [2014\)](#page-27-17)

wastewater solids (Craggs et al. [2013\)](#page-27-19). HRAP systems require low energy $(\sim 1 \text{ kWh})$ m3 of water treated) (Arashiro et al. [2018](#page-26-15); Garfí et al. [2017;](#page-28-13) Passos et al. [2017\)](#page-31-16), are less expensive, and require less maintenance as compared to conventional techniques such as activated sludge system (Arashiro et al. [2018;](#page-26-15) Garfí et al. [2017;](#page-28-13) Molinos-Senante et al. [2014;](#page-30-13) Craggs et al. [2014;](#page-27-17)). HRAP is used for simultaneous wastewater treatment and the resource recovery; this process helps in generation of economically viable feedstock which can be subjected to different treatment strategies for generation of biofuels such as biodiesel by trans-etherification of lipid fraction (Rodolfi et al. [2009\)](#page-31-17), bio-methane by anaerobic digestion of the whole biomass (Yen and Brune [2007](#page-34-6)), bio-oil by pyrolysis of dry biomass (Miao and Wu [2004](#page-30-14)) or hydrothermal liquefaction of wet biomass (Biller and Ross [2011\)](#page-26-16), and bio-ethanol by fermentation of the polysaccharide/carbohydrate part (Harun et al. [2010](#page-28-14)). The HRAP also helps in recovery of NPK fertilizers; the protein extracted from the algal biomass is rich in different essential amino acids and phyto-hormones (Coppens et al. [2016;](#page-27-20) Garcia-gonzalez and Sommerfeld [2016;](#page-28-15) Uysal et al. [2015;](#page-33-17) Jäger et al. [2010\)](#page-29-17) which can be extracted as value-added product and help in making the entire process economically sustainable.

7.4.2 Anammox Process

Autotrophic anaerobic ammonium oxidation (*anammox*) bacteria have unique capability to metabolize ammonium and nitrate or nitrite to produce nitrogen gas. Anammox process was first observed by Mulder et al. [\(1995](#page-30-15)) in denitrifying fluidized bed reactor designed to treat effluent of a methanogenic reactor, and they patented the process. Most of the genera of anammox bacteria have been discovered in wastewater treatment plants, and some are also identified from laboratory-scale bioreactors. The six different genera of the anammox bacteria are *Anammoxoglobus*, *Anammoximicrobium*, *Brocadia*, *Jettenia*, *Kuenenia*, and *Scalindua*, and they all belong to order *Planctomycetales* (Li et al. [2018](#page-30-16)). The discovery of anammox process has led to understanding that half of the total nitrogen turnover in marine envi-ronment was mainly mediated by these bacteria (Li et al. [2018](#page-30-16); Kuenen [2008\)](#page-29-18). Thus, it was believed that anammox process has great potential in removal of ammonium from waste gas or wastewater (Kuenen [2008](#page-29-18)).

Different type of wastewater systems utilizes anammox process for wastewater treatment and resource recovery. Since the discovery of anammox system was observed in the wastewater treatment system, it has been proven that wastewater with highly contaminated nitrogen and low organic content can be treated with the help of anammox bacteria. Several already established wastewater techniques have employed the application of anammox for recovery of nitrogen. Table [7.2](#page-22-0) describes different treatment strategies involving anammox-based treatment and its applications in nutrient recovery.

Table 7.2 Different wastewater treatment strategies using anammox bacterial system **Table 7.2** Different wastewater treatment strategies using anammox bacterial system

7.4.3 Photosynthetic Bacteria

The photosynthetic bacterium uses light as source of energy and different organic materials as carbon substrate and proton donor in autotrophic and heterotrophic growth. The different photosynthetic bacteria are grouped under different family like groups of microbes such as *Chromatiaceae*, *Chlorobiaceae*, *Ectothiorhodospiraceae*, *Heliobacteriaceae*, and *Rhodospirillaceae* (Li et al. [2011;](#page-30-17) Dong and Cai [2001](#page-27-21)). These photosynthetic bacteria play a vital role in nutrient cycle and different biological processes, i.e., carbon sequestration, dehydrogenation, denitrification, sulfide oxidation, etc. (Han et al. [2008](#page-28-17)). Photosynthetic bacteria have shown great prospective in area of simultaneous wastewater treatment and resource recovery. PSB helps in COD and ammonia nitrogen removal up to 85–99% (Meng et al. [2018;](#page-30-18) Yang et al. [2017](#page-34-10); Saejung and Thammaratana [2016\)](#page-31-18). PSB biomass after cleaning wastewater can be used as by-products for feeding fish and livestock as feed or feed additive as they are rich in single cell protein, thus helping in reducing production cost of fish (Li et al. [2011\)](#page-30-17). SCP have several health benefits for livestock and fish such as the following: it promotes growth, it enhances resistance against diseases, etc. PSB biomass can be used as source for extraction of value-added products such as coenzyme Q10 (CoQ10) and carotenoids (Meng et al. [2018;](#page-30-18) Hao et al. [2017](#page-28-18); Jeong et al. [2008](#page-29-20)). Therefore, the PSB has evolved as an attractive tool for treating nontoxic wastewater for simultaneous wastewater treatment and resource recovery. It has an added advantage over algal based technology as it can be used for wastewater with high COD (Meng et al. [2018](#page-30-18)). As compared to other conventional technology, PSB has several advantages during its application process such as the following: it can work with high organic loading, the space requirement for bacterial growth is less, thus leading to low investment involved as the nutrients are mostly available in wastewater, and it requires less power consumption (Meng et al. [2018;](#page-30-18) Li et al. [2011\)](#page-30-17). PSB can also be used in biological hydrogen production (Meng et al. [2018](#page-30-18)). Different wastewater types have been reported to be treated by the action of PSB tabulated in Table [7.3](#page-24-0). Photosynthetic microbes are used in different bioreactors such as membrane sequencing batch reactor (Kaewsuk et al. [2010](#page-29-21)) (MSBR), photo-bioreactor, and photo anaerobic membrane bioreactor (Hülsen et al. [2016\)](#page-28-19).

7.5 Economics of the Direct Involvement of Microorganisms in Treatment of Wastewater

Microorganism-based biological treatment has been preferred over chemical-based traditional methods. Most of the reactors designed for wastewater treatment nowadays involve the microbes or the bio-molecules generated by the microbes. Some of the systems such as algal based system and photosynthetic-based system involve microbes directly. The applications of microbes in MFC have been explained in a separate section. Considering the economics of the HRAP and PSB systems, Harun et al. ([2011\)](#page-28-20) demonstrated the techno-economic evaluation of the microalga

Table 7.3 Different wastewater treatment strategies using photosynthetic bacterial system **Table 7.3** Different wastewater treatment strategies using photosynthetic bacterial system wastewater (Mingxing and Yanling 2010) are also reported to be treated in the presence of phototrophic bacteria wastewater (Mingxing and Yanling [2010](#page-30-19)) are also reported to be treated in the presence of phototrophic bacteria

photo-bioreactor in biogas and biodiesel generation. They demonstrated that the biodiesel production was integrated with bio-methane production. The methane generated was able to surrogate for the energy required during different steps of biodiesel production from algal biomass such as micro-algal cultivation, extraction, dewatering, and trans-esterification. They theoretically calculated that the energy requirement for the overall process is reduced by 33% and the carbon emission is also reduced by approximately 75%. As evident that microalgae growth can be nurtured in wastewater where the wastewater will provide the essential nutrients such as carbon, nitrogen, etc., in turn the algal biomass results in pollutant treatment. Meng et al. [\(2018](#page-30-18)) suggested that HRAP design require one-fifth of the cost required by any other lagoon system for cleaning wastewater to acceptable water quality. The land requirement is smaller for construction of HRAP as compared to other lagoon system. The best advantage of the microbe-based system is that the microbe produces different metabolites, bio-molecules during the process which are of great economical value. Therefore, strategies may be designed/implemented to recover these components apart from wastewater units for making the process carbon neutral and cost neutral and even generating revenues/profits in some cases.

7.6 Conclusion and Future Perspective

This chapter has elucidated in detail the role of microbes and how different microbeassisted products/systems can be used in wastewater treatment directly or in combination. Currently, the bio-electrochemical system is considered as one of the leading technologies due its potential for direct electricity generation apart from wastewater treatment with simultaneous recovery of economically important chemical intermediates. The scaling up of the system is a major hindrance and limited by loss in electricity yield, cost of electrodes, issues associated with the continuous running of the process, and sludge formation and its separation. The application of photosynthetic microbes has also shown great potential but is limited due to cost involved in designing larger specialized photo bioreactors and the requirement of large land areas for HRAP. The application of microbial enzyme in different bioreactors for wastewater treatment has shown great potential; however, cost of enzyme production, stability, and reusability are a major concern. Although various techniques such as immobilization and membrane-based technology have enhanced the reusability of enzyme, these techniques are faced with decreased specificity and reactivity due to steric hindrance caused by immobilizing material. Thus, scientists have designed several nanoparticle-based immobilization systems, but the overall process at larger scale is still costly.

It is evident that the microbial based technologies are in the naïve stage and need lots of scientific, economic, as well as social impact studies before their application as a large-scale technology. The incorporation of microbes with different treatment strategies has opened a new venture for scientist from different streams such as materialist, physicist, microbiologist, chemist, economist, and sociologist in order to take the technology from the laboratory to land, overcoming the above-described limitations associated with different developed techniques.

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