Ritu Singh · Sanjeev Kumar *Editors*

Green Technologies and Environmental Sustainability

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Dedicated to my family (Ritu Singh)

Dedicated to my late elder brother
Naveen Kumar & late friend Dr. Shailesh
Tripathi (Sanjeev Kumar)

Preface

Decoupling environmental degradation and resource consumption from economic and social development is an enduring challenge and requires a paradigm shift in our approach. Presently, green technologies are playing a significant role in changing the course of the world's economic growth towards sustainability and providing an alternative socio-economic model that will enable our present and future generations to live in a clean, healthy environment and in harmony with nature. The concepts of green technologies, if endorsed and disseminated into the lives of all people, will facilitate the aim of the Millennium Development Goals of keeping the environment intact and improving it for civilization to survive. This book focuses on the goals of green technologies, which are becoming increasingly important for ensuring sustainability. It provides a different perspective of green technology in sectors like energy, agriculture, waste management and transportation. This book also offers recent advancements made towards sustainable development in the field of bioenergy, nanotechnology, green chemistry, bioremediation and degraded land reclamation and helps bridge the gap between the scientific community and policymakers.

Safe, economic and effective disposal of domestic wastewater is one of the most challenging problems today. In response to the growing public concern and enforcement of environmental legislation, the greatest challenge has been in the sanitation sector to minimize domestic wastewater pollution. Chapter 1 presents an overview of the pros and cons, operational design variables and effectiveness of traditional and recently developed constructed wetland systems for the treatment of domestic sewage. The chapter also discusses the role of plants, media materials, microorganisms and oxygen transfer in domestic wastewater purification through constructed wetlands (CWs) along with the research needs for enhancing stability and sustainability of wetland systems. The continuous contamination of water bodies worldwide by the presence of emerging pollutants has raised several issues in the last decades. Although scientific data have made evident the potential threats of emerging pollutants to public and environmental health, there is still limited information on the ecotoxicity, concentration and distribution of these compounds in environment, which makes their ecological regulation, detection and treatment difficult.

Thus, the search for green technologies to detect and treat potential environmental pollutants is critical for ecologic and human health protection. Chapter 2 explores the potential of laccase enzyme as an element of biosensing and bioremediation and identifies the drawbacks that have to be overcome in order to demonstrate their feasibility and implement a large-scale process.

The quest for sustainable and environment-friendly energy sources has become a pressing need. Declining fossil fuel reserves, worldwide demand for energy and undesirable effects of greenhouse gas emissions have led to increased interests in biofuels worldwide. The generations, environmental benefits, compatibility, performance, emission characteristics and global perspectives of biofuels have been highlighted in Chap. 3. The need for developing sustainable criteria and certification of standards for biofuel production and trade has also been addressed. Chapter 4 deliberates on the role of biodiesel in greening the transport sector. Chapter 5 is dedicated to microalgae which play a quintessential role in the energy sector. The conversion of microalgae into liquid fuels provides an inherently renewable, economical, eco-friendly and sustainable alternative to fast-depleting fossil fuels. Moreover, the production of value-added by-products after biofuel extraction is an added advantage of microalgae. The role and performance of natural and synthetic flocculants in harvesting microalgae for the purpose of biofuel production has been conferred in Chap. 6. Chapter 7 deals with solar photovoltaics which is inexhaustible, affordable and the most promising of all the active solar energy technologies. The chapter presents a succinct picture of the solar PV technology along with its classification and application areas. The economic aspects, energy-exergy and status of the maturity of PV technology have also been addressed.

The crop–weed competition is one of the most important factors responsible for potential loss of crops around the world. Therefore, herbicides have become a major tool to tackle weed interference. Glyphosate is one of the widely accepted herbicides which has broad-spectrum activity and effectiveness. Its ubiquitous presence in the environment due to anthropogenic activities and recalcitrance has the potential to affect animal behaviour and interfere with ecological processes. Chapter 8 explores the available information on glyphosate biodegradation over the course of 40 years of study, the different pathways involving the C-Plyase, the genetic and physiological regulatory system that governs these processes and the factors limiting the development of glyphosate bioremediation technologies.

Urbanized areas cover less than 3% of the land, but the majority of the Earth's population and industry is concentrated in these areas. The development of reliable bioindicators on the basis of systematic approach would contribute greatly to rational land use and sustainable functioning of the urban environment. Chapter 9 aims to review the existing approaches to the bioindication of urban areas, i.e. microbial and plant bioindicators, as well as complexity of urban ecosystem, soil and its types, anthropogenic impacts, pollutants, effect on microbial community and other existing problems in this field, and suggests possible ways to solve them. Chapter 10 provides an overview to understand the concept of smart cities. The chapter highlights development theories, sustainability dimensions, indicators and key challenges for smart cities. Chapter 11 discusses the need for an integrated approach towards environmental quality control in developing countries. Conceptual considerations along with challenges for sustainable development, the role of research and development in solving environmental problems, anticipatory actions, environmental quality control and approaches to solutions are presented here. With increased urbanization, solid waste management has emerged as one of the major concerns. Among various categories of municipal solid waste, postconsumer waste, which is no longer recycled and has the possibility of creating aesthetic pollution is the focus of Chap. 12. The chapter presents vermitechnology as the simplest, cost-effective technology for the management of post-consumer wastes.

Nanotechnology is an area which touches almost every aspect of the modern world, ranging from research application, medicine and information technology to consumer goods. At present, smart nano-biomaterials have gained much attention. Nanocellulosic fibres in particular have diverse applications. The sources, types, properties, production cost and future perspectives of nanocellulosic fibres have been covered in Chap. 13. The synthesis of metal nanoparticles in bulk is a challenge to researchers due to their aggregation behaviour. Thus, the stabilization of metal nanoparticles becomes a challenging task. In the last decade, ionic liquids (ILs) were found to be a potent alternative for the stabilization of metal nanoparticles. Chapter 14 aims to contribute to the understanding of the synthesis of metal nanoparticles with tetrazolium ring–based ionic liquid as a solvent and stabilizer. Greener approaches for synthesizing nanoparticles are presently becoming popular due to their eco-friendly nature and cost-effectiveness. The plants which are being used for the synthesis of metal nanoparticles have been documented in Chap. 15.

Green analytical techniques refer to approaches that decrease or completely remove preservatives, reagents, solvents and other substances that are harmful to mankind and the environment. Chapter 16 discusses the basic principles of green analytical techniques which aim to reduce the impact of chemical activities on man and the environment. Emphasis has been placed on green separation techniques, green spectrophotometric techniques, the basics of green analytical techniques and the problems associated with the formulation of ideologies of green analytical chemistry to existing analytical laboratories.

Arsenic is a toxic element whose widespread contamination in highly populated regions of the world has led to environmental and human health concerns. As the extent of the problem is large, there is a need to devise cost-effective measures to tackle this problem. Chapter 17 focuses on different bioremediation strategies for arsenic removal/stabilization such as microbial and phytobial remediation and phyto-, myco- and phyco-remediation. The chapter also discusses prospects of utilizing biological components for restricting arsenic entry into crop plants, specifically rice. Detailed information on the biological methods which are practiced for the treatment of distillery wastewater with major emphasis on microalgae is given in Chap. 18. The merits and demerits of existing processes have been also summarized in this chapter.

Chapter 19 provides a brief insight into the need for ecologically restoring degraded land sites, factors influencing them, the choice or selection of species for undertaking such a work, indicators of ecological restoration that can be applied for monitoring purposes and some of the popular models of ecological restoration in India that have been successfully established and the techniques used in these models. Biochar is one of the modern, inexpensive, rapidly scalable and widely applicable technologies that can be used for climate mitigation and soil health enhancement. The production processes and diverse applications of biochar have been revealed in Chap. 20.

This book will be beneficial as a source of educational material to graduate and postgraduate students, faculty, researchers, chemists, environmentalists, soil scientists, agronomists, policymakers and industrial personnel who are interested in green technologies for sustainable development. It could also be used as a reference book by research scholars, scientists, academicians and readers from diverse backgrounds across various fields such as biotechnology, nanotechnology, chemistry, environmental science, water engineering and energy. We hope that the chapters of this book will provide readers with valuable insights into the major areas of green technologies.

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Contents

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Ritu Singh is an assistant professor in the Department of Environmental Science, School of Earth Sciences, Central University of Rajasthan, Rajasthan, India, and editor of the *Journal of NanoScience, NanoEngineering & Applications* and *Journal of Water Pollution & Purification Research*. She received her doctorate degree in environmental science from Babasaheb Bhimrao Ambedkar University, Lucknow. She has worked as research fellow in CSIR-Indian Institute of Toxicology Research and made significant contributions in the field of nano-remediation. She has published her work in several international journals of repute and has contributed several chapters in international and national books. She is the recipient of the 'DEF-Young Scientist Award' in the field of environmental science. She serves as a life member of various academic bodies. Her research interests include integrated nanotechnologies, wastewater treatment, environmental monitoring and assessment, analytical method development and microextraction techniques.

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Chapter 1
A Review and Perspective of Constructed Wetlands as a Green Technology in Decentralization Practices

Alireza Valipour and Young-Ho Ahn

Abstract Constructed wetlands (CWs) could be an environmentally acceptable option in treating domestic sewage. But, the successful implementation of this technology in decentralization practices is still under debate. Increasing the knowledge regarding the use of CWs in coastal regions where domestic sewage seriously stressed with total dissolved solids (TDS) and cold weather conditions is additionally imperative. A comprehensive review is therefore needed to have a better understanding of this state-of-the-art technology to inspire a sustainable solution for onsite sanitation. This chapter covers the role of plants, media materials, microorganisms, and oxygen transfer in domestic wastewater purification through constructed wetlands (CWs). The pros and cons, operational design variables, and effectiveness of traditional and recently developed CWs, and the necessity of an induced biofilm attachment surface (BAS) in these systems for the treatment of domestic sewage are presented. This chapter also elucidates the ability of CWs to deal with TDS-contaminated wastewater. Adaptive strategies to mitigate the impacts of cold climate on the effectiveness of CWs are also summarized. Future research that needs for enhancing stability and sustainability of wetland systems is highlighted. Overall, by more advanced investigation, the biofilm attachment surface (BAS) CWs can be specified as an ideal treatment process in decentralization. The success of CWs responding to environmental stress can occur by optimum engineering design and operation.

1 Introduction

Safe, economic, and effective disposal of domestic wastewater is one of the most challenging problems in today's civilization. In response to the growing public concern and enforcement of environmental legislation, the greatest challenge has been

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paid in the sanitation sector to minimize domestic wastewater pollution (Valipour et al. 2010). Most cities and towns in developing countries have generally fallen behind in wastewater management (Jhansi and Mishra 2013). In fact, only about 10% of urban sewage in developing countries receives treatment (Snauffer 2007). Decentralized wastewater management in advance to centralized systems can consider being an efficient and long-term solution for meeting public health and water quality goals. Improper zoning, scattering of low population densities, isolated settlements, lack of funding, and requirements of highly skilled personnel can be counted to be major restrictions of using sophisticated centralized treatment systems. It was found that more than 60% of the total budget is responsible for collection system in centralized wastewater management (Massoud et al. 2009). A large number of technologies, such as septic tanks, drain-field systems, lagoons, activated sludge processes, and membrane bioreactors, etc., are available for onsite wastewater treatment, whereas most of them are ineffective, costly, and requires complex operations and maintenance (Wu et al. 2011a). Hence, the implementation of low cost sewage treatment method to ensure environmental preservation can be recognized as an innovative essential component in decentralization practices.

In recent years, constructed wetlands (CWs) have received most attention in the urban wastewater treatment, particularly in decentralized sanitation, for the reason that they are affordable, reliable, simple in design and operation, and offer environmentally sound approach (Wu et al. 2011a). CWs seemed to be started in Germany based on research by Kathe Seidel in the 1960s and by Reinhold Kickuth in the 1970s (Kadlec and Wallace 2009). These ecological engineered systems are known to be effective at removing many pollutants such as organic compounds, suspended solids, pathogens, nutrients, and emergent pollutants. They are designed to take advantage of the same processes occurring in natural wetlands, but within a more controlled environment. Gaining a better understanding of the mechanisms associated with CWs has led to a wide variety of designs and configurations in an effort to achieve a more efficient domestic sewage treatment, for example, single-staged modification (Chale 2012; Kumari and Tripathi 2014), multi-staged in series (Melian et al. 2010), and/or combination with other treatment technologies (Singh et al. 2009). There are growing researches in use of CWs treating domestic wastewater under the specific influent condition seriously stressed by total dissolved solids (TDS) which could be faced in such areas as coastal regions where seawater applies to indoor activities (Valipour et al. 2014b). Many studies have also been done on the adaptation of CWs to the cold climate through the sound operational approach (Ouellet-Plamondon et al. 2006). In view of that, several authors have published review papers relating to the use of CWs in wastewater treatment (Vymazal 2002, 2005, 2013; Babatunde et al. 2008; Mbuligwe et al. 2011; Haynes 2015; Liua et al. 2015; Vymazal and Březinová 2015; Wu et al. 2015). Nevertheless, to date, the reviews of the current knowledge which aimed to onsite sanitation still remain comparatively scarce and incomplete. Still, there is a hesitation about the selection of the appropriate type of CW which is more suitable for domestic wastewater treatment in decentralized sanitation concepts. It is, therefore, imperative to briefly look into the application of CWs for domestic wastewater treatment by giving more attention to decentralization practices. In addition, a valuable overview concerning the potential ability of wetland systems in dealing with TDS-stressed wastewater and operational strategy taken in a cold climate is also an important issue that would be a ready tool in the implementation of CWs for small-sized communities and isolated areas; this is rare in published literature reviews.

This chapter provides a brief review of the essential components and the mechanism of pollutant removal associated with CWs. In a concise manner, it also provides a review of the traditional and advanced models of CWs in treating domestic wastewater. The performance of various types of wetland processes on the contaminant removal (focusing on organic matters, suspended solids, and nutrients) is also summarized. Through this study, it may better define the scope and issues at hand to deal with high TDS wastewater and cold climate condition. This chapter further highlighted potential areas worthy for future sustainable application of CWs. The conceptual framework of this chapter eventually creates a context in which a new ideal could be inspired for decentralization practices.

2 Ecology in CWs

The main compounds employed in the CWs are vegetation, microbial communities, and media material (soil strata or any other material used as the matrix within the constructed wetland). These systems utilize a combination of chemical, physical, and biological processes to remove contaminants from wastewater (Table 1.1).

In particular, it should be recognized that most wastewater-related diseases are caused by bacteria and viruses rather than worms and protozoa, so the literatures deals primarily with these two groups of organisms (Cronk and Fennessy 2001). Pathogenic bacteria and viruses are removed in aquatic plant systems by sedimentation and filtration, predation, exposure to biocides (natural pesticides) released by plant roots, and die-off from unfavorable environmental condition, including ultraviolet radiation in sunlight and temperatures unfavorable for cell production. The contribution of each of above routes is suggested to be a function of wastewater flow rates, nature of macrophytes, and type of the wetland system (Polprasert 2006; Mburu et al. 2008).

2.1 Marshy Vegetation

Marshy plants play a crucial role in creating a pleasing landscape which can be incorporated into residential developments. They provide a valuable ecological habitat for wildlife. In considering the application of wetlands to treat wastewater, plants have several properties in relation to the treatment process that make them an essential *biotic* component in the CWs. Depending on the plant species and dense coverage, plant effects have a strong influence on the treatment performance, based

Table 1.1 Pollutant removal mechanisms involved in CWs treating domestic wastewater (modified from Watson et al. 1989)

	Contaminant affected						
Mechanism	SP	BOD	N	P	TDS	B&V	Description
Physical							
Sedimentation	P	I	I	I		I	Gravitational settling of solids
Filtration	S	I	I	I		I	Particles filtered mechanically as water passes through media materials and plant biomass
Adsorption	S						Interparticle attractive forces (van der Waals force)
Chemical							
Precipitation				P			Formation of or coprecipitation with insoluble compounds
Adsorption			S	P			Adsorption on media materials and plant surfaces
Biological							
Bacterial metabolism	P	P	P				Removal of biodegradable particulate and soluble organics by suspended, and biofilm bacteria. Bacterial nitrification and denitrification
Plant metabolism			S	S	P	S	Under favorable environmental conditions, these contaminates taken up by plants. Root excretion may be toxic to organisms of enteric origin
Natural die-off						P	Natural decay of organisms in an unfavorable environment
Predation						P	Numerous predator–prey relationships exist in the treatment process, e.g., protozoa, nematode, and zooplankton species act as predators of pathogenic organisms

SP solid particles, *BOD* biochemical oxygen demand, *N* nitrogen, *P* phosphorous, *TDS* total dissolved solids, *B&V* bacteria and viruses, *P* primary effect, *S* secondary effect, *I* incidental effect (effect occurring incidental to removal of another contaminant)

principally on the microbial communities, activities, and their population by providing ideal attachment sites (through roots, stems, and leaves), uptake capability, releasing oxygen, and filtration (Valipour et al. 2014b). However, beside the multi-role of wetland vegetation, contaminant uptake by plants has a minor role. Plant species used for phytoremediation should likely be native, and have high growth rate, high biomass, adapt ecologically to diverse habitats, and ability to accumulate the target pollutants in the aboveground parts. In fact, both pollutant removal efficiency and plant productivity are significant in selecting a right plant for wastewater treatment applications. For the construction of constructed wetlands, four kinds of aquatic macrophytes are typically used (Brix 1993; Taylor et al. 2007):

1. Emergent macrophytes: These are generally grow on water saturated or submersed soil from where the water table is about 0.5 m below the soil surface to where the sediment is covered with approximately 1.5 m of water or more; e.g. *Acorus* sp., *Carex* sp., *Phragmites* sp., *Scirpus* sp., and *Typha* sp.
2. Floating leaved macrophytes: These are rooted in submersed sediments having water depth of approximately 0.5–3.0 m, and possess either floating or slightly aerial leaves, e.g., *Nuphar* sp. and *Nymphaea* sp.
3. Submerged macrophytes: These have their photosynthetic tissue entirely submersed by water (occur at all depths), only grow well in oxygenated water, and are used mainly for polishing secondary treated wastewater, e.g., *Ceratophyllum* sp., *Myriophyllum* sp., and *Rhodophyceae* algae.
4. Freely floating macrophytes: They are not rooted to the substratum, and float freely on or in water column and usually restricted to non-turbulent, protected areas, e.g., *Azolla* sp., *Eichhornia* sp., *Lemna* sp., *Pistia* sp., and *Spirodela* sp.

Among various aquatic macrophytes, *Phragmites* sp. is introduced to stand out as a most prominent aquatic weed in impurities elimination (Valipour et al. 2014a). This plant promotes high below- and above-ground biomass and rigorous root system in the wetland media. The water hyacinth (*Eichhornia crassipes*) also appeared to be a promising candidate for pollutant removal among floating species owing to its massive growth potential and extensive root system (Valipour et al. 2012; Kumari and Tripathi 2014). *Phragmites* sp. and water hyacinth showed, respectively, a relative growth rate (RGR) of 5.44 and 0.98% per day in treating domestic wastewater (Valipour et al. 2009, 2011a). The features of some plant species used commonly in constructed wetlands are presented in Table 1.2.

The physical impacts by aquatic macrophytes in wetland systems include *filtering,* slowing down the flow velocity, improving sedimentation, increasing the detention time, decreasing resuspension, maintaining the hydraulic pathway of water distribution, preventing clogging, and provisioning a surface area for attached microorganisms (Scholz 2011). As suspended solids pass through the plant biomass, they can be trapped, accumulate, and eventually settle under the force of gravity or become metabolized by microorganisms, while particulate matter sinks to the bottom. However, according to Kim et al. (2003), the removal efficiency mostly depends on the retention time in wetland systems. It is reported that most of the removal occurs within the first few meters beyond the inlet zone owing to the quiescent conditions and the shallow depth of liquid in the system (USEPA 1988). Other wastewater contaminants (i.e. BOD (biochemical oxygen demand), nutrients, and pathogens), can also be removed by removing solids from wastewater since their elimination correlated with the removal of suspended solids (Brix 1993).

Nutrients (N & P) and other impurities are taken mainly up by wetland plants through the epidermis and vascular bundles of the roots and further transported upward to the plant (Valipour et al. 2014a, b) (Fig. 1.1). Some uptake also occurs through the immersed stems and leaves from the surrounding water (Brix 2003). On the other hand, a small amount of N (<10%) and P (<5%) has been reported to be removed by macrophyte harvesting compared to their total removal in vegetated

Table 1.2 Properties of some aquatic plants used in CWs

Macrophyte	Type	Preferred temperature (°C)	Optimal pH	Root penetration (cm)	Nutrient uptake (kg/ha/year)	
					Nitrogen	Phosphorous
Bulrush (*Scirpus* sp.)	Emergent	16–27	4–9	75	125~1825	18~438
Cattail (*Typha* sp.)	Emergent	10–30	4–10	75	600~2630	75~403
Papyrus sedge (*Cyperus papyrus*)	Emergent	10–30	6–8.5	60	1100~3650	50~1059
Reed (*Phragmites* sp.)	Emergent	12–33	2–8	60	225~2500	35~120
Rush (*Juncus* sp.)	Emergent	16–26	5–7.5	25	800	110
Duckweed (*Lemna* sp.)	Free-floating	6–33	6.5–7.5	2	350~1200	116~400
Water fern (*Salvinia rotundifolia*)	Free-floating	10–30	6–7.7	–	350~1700	92~450
Water hyacinth (*Eichornia crassipes*)	Free-floating	12–35	6.5–7.5	100	1950~5850	350~1125
Water lettuce (*Pistia stratiotes*)	Free-floating	15–35	6–6.8	80	900~3248	40
Water pennywort (*Hydrocotyle* sp.)	Free-floating	15–35	6.5–7.5	–	2957	730
Coontail (*Ceratophylum demersum*)	Submerged	15–35	6–10	–	100	10
Sago pondweed (*Potamogeton pectinatus*)	Submerged	10–37	6–10	–	500	40

( ): Scientific name

beds (Mander et al. 2003). The plant uptake efficiency differs in relation to the system configurations, loading ranges, pollutant concentrations in wastewater and environmental conditions. The rate of plant uptake also tends to be limited by their net productivity (growth rate) and tissue nutrient concentrations (Table 1.2).

Rhizodeposition, i.e., root exudates (e.g., polysaccharides, sugars, amino acids, vitamins, organic acids, and fatty acids) and other organic substances released by plant roots, takes place in the rhizosphere. The magnitude of this input of organic carbon compounds is still unclear, but reported values are generally 5–25% of the photosynthetically fixed carbon (Bialowiec et al. 2012a, b; Lesage 2006). It is conceivable that rhizodeposition is more important in constructed wetlands where

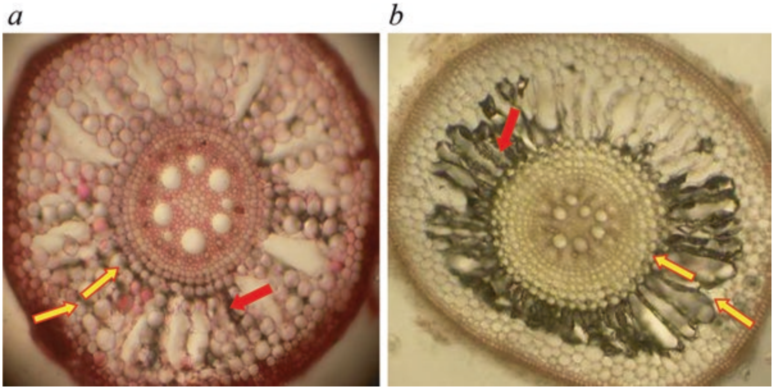

Fig. 1.1 Cross sectioning of (**a**) *Phragmites* sp. and (**b**) water hyacinth root after using in treating domestic sewage. *Red arrow* presence of sewage. *Yellow arrow* translocation of impurities to the vascular bundles from the epidermis, and then upward the plants

carbon load wastewater is extremely low due to the relatively low amount of carbon released by plants compared to the urban wastewater flow (Stefanakis et al. 2014). Rhizodeposition may initiate the mobilization of nutrients, allelopathic effects, and stimulation of microbial growth and activity, generally denitrifies to increase nitrate removal within the root system (Wiessner et al. 2006).

Evapotranspiration plays an additional important role by increasing the hydraulic retention time significantly in wetland treatment systems. The transpiration mechanism (rely on species and environmental condition) is positively related to the impurity absorption, volatile compound emission into the atmosphere, and water purification capability index of plants (Valipour et al. 2015). Plant species differ in transpiration rate due to metabolic and anatomical differences. The height, mass, size, leaf surface area, and growth stage of the vegetation can also affect the rate of transpiration. Transpiration is generally maximal at high temperature, moderate wind, low relative air humidity, and high light intensity (bright sunlight) (Pilon-Smits 2005; Pivetz 2001).

Furthermore, age greatly influences the physiological activity of the plants, particularly its roots. The roots of younger plants can display greater ability to absorb impurities and release oxygen than old plants due to the increased lignification and suberization processes occurring with increasing the age of the plants and tissues (Heers 2006).

2.2 *Microorganisms/Biofilms*

Microorganisms, such as bacteria, fungi, and algae, play a central role in the transformations and mineralization of nutrients and organic compounds in CWs (Dordio et al. 2008). Among the various groups of microorganisms, the presence of bacteria, either in the form of suspension or attached biofilm, is particularly more important since they have versatile metabolic pathways, high metabolic rate, and very short generation times (van der Valk 2012). The rhizosphere has been reported to be associated predominantly with gram-negative bacteria (Valipour et al. 2009, 2011a, 2014b). This can be related to their ability to utilize efficiently the growth substrates available in the rhizosphere and to cope with polluted environments because of the presence of detoxifying enzymes (Valipour et al. 2014b).

Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, as either extracellular (free-living bacteria) or intracellular (symbiotic bacteria), in accordance with the degree of association with the root cells (Ahmad et al. 2008; Bumunang and Babalola 2014). Certain varieties of PGPR strains belong to Bacillus, Enterobacter, Burkholderia, Acinetobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Beijerinckia, Erwinia, Flavobacterium, Rhizobium, and Serratia (Kang et al. 2014). PGPR can be resulted to improve plant nutrition and growth, lessening or preventing the plants from diseases, and responses to external stress factors (Martínez-Viveros et al. 2010; Kang et al. 2014). Free-living bacteria generally refer to the organisms situated on the root surface and in the intercellular spaces of the root cortex, colonizing the plant tissue intercellularly in a competitive environment. Symbiotic bacteria are nitrogen fixation microorganisms that live inside the root cells, produce nodules, and are localized inside the specialized structures (Martínez-Viveros et al. 2010; Bouizgarne 2013; Bumunang and Babalola 2014). Due to the high energy requirement for nitrogen fixation and relatively low metabolic activity, non-symbiotic bacteria provide merely a small amount of the fixed nitrogen that the bacterially associated host plant requires (Ahemad and Kibret 2014; Martínez-Viveros et al. 2010). Many rhizobacteria are capable of lowering the increased plant endogenous ethylene levels (which inhibit plant growth) to reestablish a healthy root system that needs to be faced with environmental stress (Martínez-Viveros et al. 2010; Gontia-Mishra et al. 2014). In addition, arbuscular mycorrhizal of the adapted fungi can sequester toxic substances and promote plant growth by increasing nutrient absorption by the roots to enhance the host plant’s resistance against environmental stresses (Kapoor et al. 2013).

Biodegradation of organic matter is governed by either facultative or obligate aerobic/anaerobic bacteria respiration in the wetland systems, while the faster metabolic rate of the aerobic bacteria makes them more effective for the elimination of the organic pollutants among others (Valipour et al. 2009, 2011a). The bacteria utilize the carbon found in organic matter (organic matter contains up to 50% carbon) as an energy source and convert to carbon dioxide under aerobic conditions or else to methane under anaerobic conditions (Metcalf and Eddy Inc. 2003). Since domestic wastewater discharges into treatment systems is often rich in dissolved organic matter, insufficient supply of oxygen can be a main reason to affect the performance of the aerobic microbial activity. If the oxygen supply is not limited, the aerobic degradation can significantly affect by changes in substrate availability.

Nutrients (N & P) can be assimilated into microbial cellular biomass at the same level as plants (<10% N and <5% P) (Mander et al. 2003), but once a steady stand of biomass is reached within the bed no net nutrient removal will result thereafter. Hereupon, only a small proportion (<20%) of phosphate removal by constructed wetlands can be attributed to nutritional uptake by microflora (Moss 1988). This transformation accepted to have a minor role in the removal of nutrients from a soluble phase, especially when influent wastewater contains a high nutrient concentration. The major biological processes responsible for the elimination of nitrogen in the wetland systems include ammonification, nitrification, and denitrification (Jamieson et al. 2003; Gerke et al. 2001). Yet, its removal efficiency can be attributed to the dissolved oxygen available for nitrification, the denitrification, the strength of organic carbon, and the nitrogen level in the influent sewage. Unlike nitrogen, phosphorus does not have an atmospheric component. Therefore, its removal is notoriously poor in any CW, and can only occur efficiently by incorporating special matrix material with high sorption capacity (Ciria et al. 2005).

Ammonification refers to the process where organic nitrogen (amino groups) is *biologically* converted into ammonia (NH_3) and ammonium (NH_4^+) as an end product. In wetland systems, ammonification is faster in the aerobic zones compared to the zones where the environment switches from facultative anaerobic to obligate anaerobic conditions (Stefanakis et al. 2014; Strock 2008), because of the higher efficiency of heterotrophic decomposition under aerobic condition. Kadlec and Knight (1996) state an optimal temperature of 40–60 °C and a pH of 6.5–8.5 for ammonification. Kinetically, ammonification proceeds more rapidly than nitrification, so that excessive ammonium can be removed by other processes including biological assimilation, adsorption, and volatilization. It was, however, generally known that these processes only make a minor contribution to the elimination of ammonium compared to nitrification–denitrification. If the influent wastewater mainly consists of organic nitrogen, ammonification can accept to be the first step in nitrogen transformation chain.

During the nitrification process in constructed wetlands, ammonium is oxidized to nitrites (NO_2^-) by the genus of Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosovibrio, and Nitrosolobus bacteria, and subsequently to nitrates (NO_3^-) by several genera such as Nitrospira, Nitrospina, Nitrococcus, and Nitrocystis. However, the most famous nitrite oxidizer genus is Nitrobacter, which is closely related genetically within the alpha subdivision of the Proteobacteria (Kadlec and Knight 1996; Ahn 2006; Jóźwiakowski and Wielgosz 2010).

Denitrification is a facultative respiratory pathway in which nitrogen oxides that formed via the nitrification process serve as terminal electron acceptors for respiratory electron transport and organic compounds serve as electron donors. Biological denitrification successively reduces nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and eventually to nitrogen gas (N_2) by the groups of bacteria known as *Bacillus, Enterobacter, Micrococcus, Pseudomonas*, and *Spirillum.* Other species such as *Proteus, Aerobacter*, and *Flavobacterium* can only reduce NO_3^- to NO_2^- (Abou-Elela et al. 2013; Kadlec and Knight 1996). This process possibly can be considered as a key mechanism for total nitrogen (TN) removal in the constructed wetlands if there is a readily available carbon source.

2.3 Media

Media materials (soil, sand, and gravel) strongly affect the movement of water through the bed (hydraulic conductivity) and macrophyte growth. These materials provide a huge surface area for microorganisms to attach additionally to plant biomass (roots, stems, and leaves) and also act either as filtration and/or adsorption medium for pollutants (Taleno 2012).

Media materials selection depends upon the type of the wastewater, and hydraulic regime chosen. Soil strata that consists of mineral particles (e.g., gravel, sand, clay, and silt), and organic matter (e.g., compost and decomposed plant litter) is a common preferred material used in wetland treatment systems (Taleno 2012; Tiner 1999). If the organic matter content of the soil strata is less than 20–35%, it is considered a mineral soil (Tiner 1999; Cooray et al. 2012). Both chemical soil composition and physical parameters such as grain-size distributions, interstitial pore spaces, effective grain sizes, degrees of irregularity, and the coefficient of permeability are the key criteria influencing the treatment performance (Tore et al. 2012).

Ionized ammonia may be removed from wastewater through exchange with soil strata, detritus, humic substances, and organic and inorganic sediments or else fixed within the clay lattice in wetland systems. On the other hand, adsorbed ammonium bounds loosely to the materials and can be released easily in response to water chemistry changes. At equilibrium condition, a fixed amount of ammonium is adsorbed to the exchange sites. Once the ammonium level decreases in the effluent occurring inside treatment unit (e.g., as a result of nitrification), some ammonium will be desorbed to regain an equilibrium with the new concentration. Ammonium ion adsorption capacity increases with increasing initial ammonium concentration in the wetland effluent (Kadlec and Knight 1996; Kadlec 2009).

Phosphorus primarily removes through bed materials rich cations (typically divalent or trivalent) by adsorption and ion exchange reactions. As these associated mechanisms have a finite capacity, the elimination of phosphorus will cease when that capacity is reached (Brix et al. 2001). Soil materials used in constructed wetlands usually do not have a great quantities of ion cations; achieving generally very low phosphorus removal.

Alternative used media materials can be classified into natural materials (e.g., apatite, bauxite, dolomite, zeolite, laterite, limestone, opoka, shale), industrial byproducts (e.g., bauxsol, burnt oil shale, coal fly ash, orchre, red mud, slage) and man-made products (e.g., alunite, filter P, filtralite, lightweight aggregates, norlite, oyster shell, polonite) (Vohla et al. 2011). Most researchers employ natural zeolite exchangers (volcanic tuffs, usually clinoptilolite-rich tuff) as low cost, effective, readily available materials to treat ammonium-containing wastewater (Copcia et al. 2010). Among various industrial byproducts, the highest phosphorus removal capacities stated for some furnace slags up to 420 g P kg^{-1}. The natural and man-made media materials have been reported for maximum removal capacities of about 40 g P kg^{-1} and 12 g P kg^{-1}, respectively (Vohla et al. 2011).

2.4 Oxygen Transfer/Diffusion

The amount of oxygen released from plant roots vary strongly by *species-specific differences,* seasonal variation and different wetland techniques used in wastewater treatment. Aquatic plants transfer atmospheric oxygen to their root system *through an internal gas space, called aerenchyma,* and release a fraction of this oxygen (30–40%) into the rhizosphere for aerobic microbial activity (Wiessner et al. 2006; Li et al. 2011a). The aerenchyma tissue also plays a role in the emission of other gasses, such as carbon dioxide and methane, into the atmosphere through plants (USEPA 2000; Armstrong and Armstrong 2005). Porosity (gas volume/tissue volume) in plant tissues can differ markedly between species. Aerenchyma typically provides a low resistance internal pathway for the movement of oxygen and other gases between shoot (i.e., above-ground or -water plant parts) and root extremities. For that reason, the plants adapt morphologically and anatomically to growing in a water-saturated substrate by further enhance and formation of aerenchyma (Hondulas 1994; Li et al. 2011a). Depending on the degree of adaptation, aerenchyma can account for as much as 60% of the total tissue volume. Obvious processes associated with above-ground or above water gas exchange and gas transport inside the plants (through aerenchyma) are driven by photosynthesis, diffusion, thermoosmosis, convective flow induced by pressure gradient, and humidity pressurization (Wiessner et al. 2006; Allen 1997). However, the types and combination of these transport mechanisms are also specific to each plant species. Besides, dead and broken shoots in some plants, such as *Phragmites* sp. which has the ability to grow in variable water depths, can also allow for the transport of some oxygen to the root zone. This can be attributed to venturi effects of wind flow, stagnation pressures of wind, pressure oscillations induced by turbulent wind flow, and the effect of heat tube, e.g., evaporation and condensation at opposite ends of a tubes (Kadlec and Wallace 2009; Willcox 2013; Allen 1997); perhaps some of these phenomena provide a reliable prediction in wetland operation under cold climates. The thermoosmosis processes occur when the temperature of the internal gas phase in leaves exceeds the temperature in the atmosphere resulting uptake of gas molecules by leaves. The warmer interior of the leaves successively causes an influx of gas molecules, overpressure buildup inside leaves, and gas molecules transport into the root system (Wegner 2010). The pressure gradient is formed by low pressure in oxygen-consuming tissues of the plant caused by different solubilities of the oxygen used and the carbon dioxide formed in this process, and higher pressure in the plant's leaves; initiating air to flow throughout the entire body of the plant (Wiessner et al. 2006). Humidity-induced pressurization is the dominant mode of pressurization under most conditions. It is related to vapor pressure differential between the leaves and atmosphere separated by a porous partition (plant cell membrane). The total pressure will be greater on the more humid side of the partition (Reddy and DeLaune 2008; Steinberg 1996).

3 Traditional Wetland Systems

The wetland systems based on traditionally on a water flow regime can be classified into surface flow (SF) and subsurface flow (SSF) CWs (Kadlec and Wallace 2009; Mitsch and Gosselink 2007). The SSF CWs are further subdivided into horizontal flow and vertical flow paths. The SF CWs often utilize free-floating, free-leaves, emergent, and submerged macrophytes, whereas the SSF CWs are limited to emergent macrophytes. A simple scheme for various types of CWs is shown in Fig. 1.2.

3.1 SF CWs

A constructed wetland designed for surface flow consists of a basin or channels, sometimes with a natural or synthetic liner to prevent seepage. In these systems, water intended to flow horizontally above ground and exposed to the atmosphere. A large aspect ratio (length–width ratio) can ensure plug-flow conditions and optimal treatment performances (Heers 2006; Taleno 2012). SF CWs with free-floating macrophytes (typically ~50–100 cm depth) having three distinct zones, aerobic, facultative and anaerobic, depending on the oxygen transfer through the floating plants (Fig. 1.3a). These floating plant systems function as a horizontal trickling filter, where the submerged roots mainly provide physical support for a thick bacterial biofilm (Valipour et al. 2015). Moreover, the soil or any other suitable medium

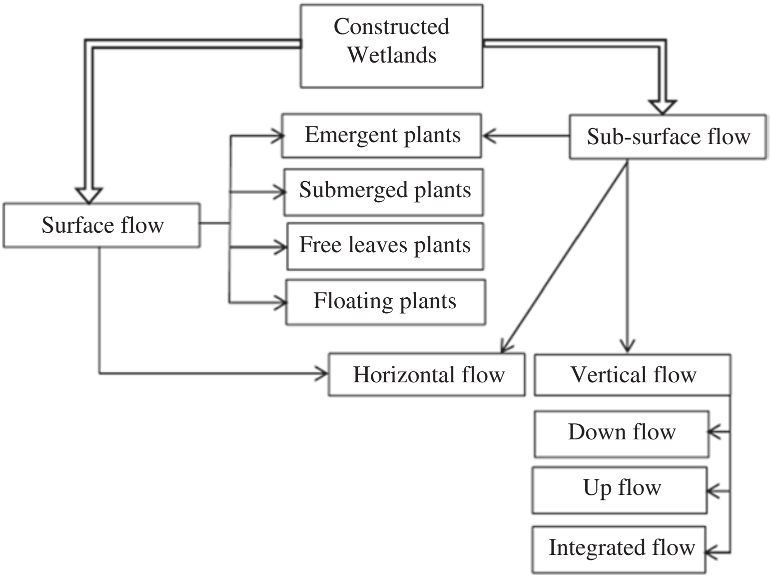

Fig. 1.2 Classification of traditionally designed CWs in domestic wastewater treatment

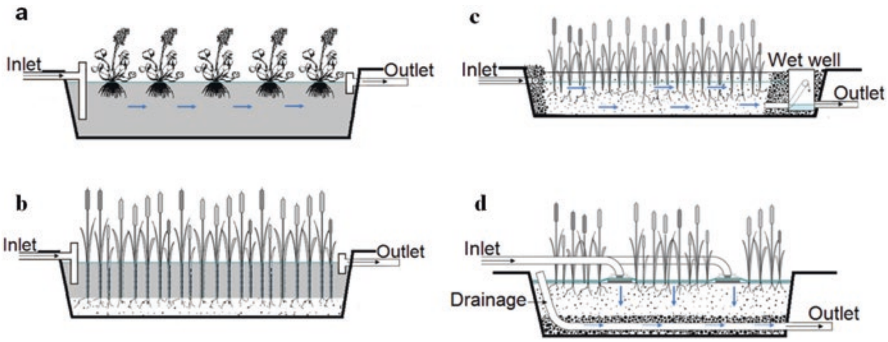

Fig. 1.3 CWs used in wastewater treatment, (**a**) SF CWs with free floating plants, (**b**) SF CWs with emergent plants, (**c**) HSF CWs, (**d**) VSF CWs

(at least 20–30 cm) may serve to support the rooted vegetation (if they are dominant plantation) in the SF CWs (floodwater depth ≤50 cm) (Heers 2006) (Fig. 1.3b). The microbial activities (aerobic/anaerobic) primarily take place in the superior layer of the soil, on the surface of the *immersed* stems and leaves of the plants.

The advantages of SF CWs include the relative ease to construct, simple designs, low maintenance, inexpensive operation, and habitat value. They can also be used for wastewaters with higher levels of suspended solids. On the other hand, these constructed wetlands have lower removal rates of contaminants per unit volume. Therefore, they require more land space and are expensive to construct in terms of capital cost. Odors and insects are a problem due to the free water surface and low treatment efficiency. These complications have limited the use of SF CWs for treating wastewater, especially in decentralization practices.

3.2 SSF CWs

SSF CWs consist of beds that are usually sealed by an impermeable substance to block leakage, and media which assist the growth of emergent plants (Bashyal 2010). The media (in the active zone) are generally composed of rock or crushed gravel of 5–20 mm diameter, sand of 0–4 mm diameter (having a permeability of 10^{-3} to 10^{-4} m/s) and different soils, or in various combinations (UN-HABITAT 2008; Kadlec and Wallace 2009). In most of these systems, the flow path is horizontal (Fig. 1.3c), although some use vertical flow paths (Fig.1.3d). In horizontal subsurface flow (HSF) CWs, the wastewater essentially flows horizontally (5–15 cm below the media surface) through the support media (50–100 cm active zone) and comes into contact with a network of aerobic, anoxic, and anaerobic zones. An average depth of <50 cm, taking into considerations of the precipitation, would cause surface flow (UN-HABITAT 2008; Ríos et al. 2007; Heers 2006; Tee et al. 2012). The inlet and outlet zones are arranged with 20–80 mm media (each 0.5–0.8 m in length) in the HSF CWs (UN-HABITAT 2008). The microbial aerobic respiration more predominates around roots and rhizomes. In the zones that are largely free of oxygen, the system performs the anaerobic microsites to develop (Valipour et al. 2014b; Tee et al. 2012). In vertical subsurface flow (VSF) CWs, the wastewater is typically discharged onto the entire surface via a distribution system and passes vertically into the media (having 50–100 cm active zone). However, it is recommended to use media depth of 70 cm which provides adequate nitrification in addition to the organic pollutants removal (UN-HABITAT 2008; Heers 2006). VSF CWs, depending on whether the wastewater is fed onto the surface or to the bottom of the wetland, includes up-flow (VUF CWs) and down-flow (VDF CWs). The latter is used more generally in wastewater treatment compared to the former system. This can be explained by the fact that they provide good oxygen transfer and are more suited for aerobic conditions resulting in the better elimination of pollutants (Zhao et al. 2011). In view of that, VDF CWs are usually fed intermittently with large batches (3–12 times a day), subsequently surface flooding occurs. Wastewater then gradually percolates down through the bed materials and plant root systems, which allows air to refill the bed (Stefanakis et al. 2014). The effluent in VDF CWs is drained at the bottom of the bed via drainage pipes which are covered with a layer of gravel of 20–60 mm (0.2–0.3 m depth) (Brix and Arias 2005; UN-HABITAT 2008; Kadlec and Wallace 2009). By intermittent dosing, the ventilation pipe connected to the drainage system can contribute to aerobic conditions in the VDF CWs. It is reported that the intermittent dosing system has a potential oxygen transfer of 23–64 g $O_2/m^2/$day whereas the oxygen transfer through plant (common reed species) has a potential oxygen transfer of 2 g $O_2/m^2/$day to the root zone (UN-HABITAT 2008).

SSF CWs is thought to have several advantages over SF CWs. The soil matrix provides a large surface area available for attached microbial biofilms. As a result, the treatment responses are faster, and lower space is needed compared to SF CWs. The subsurface position of the water and the accumulated plant debris on the surface of the bed may offer a greater thermal protection in cold climates in SSF CWs. Nevertheless, these systems can still be restricted by choking, clogging, odors and vectors, slow mass transfer, poor root penetration into the multilayer soil column, high area requirements and capital investment. In fact, when choking and clogging occurs most likely because of both biofilm growth and suspended solids accumulation, the void spaces inside bed matrices are blocked, and the infiltration rate of wastewater is considerably reduced. Subsequently, the supply of oxygen into the matrices diminishes, and the treatment ability of the reed beds decreases rapidly (Sun et al. 2007). Hence, applying these systems in practice may also not prove to be practicable in decentralization.

4 Process Modification

A wide range of expended designs, configurations, and combinations with other technologies was trialed to prevail over the limitations of CWs and establish them as an effective tool for purifying municipal wastewater effluent (Fig. 1.4).

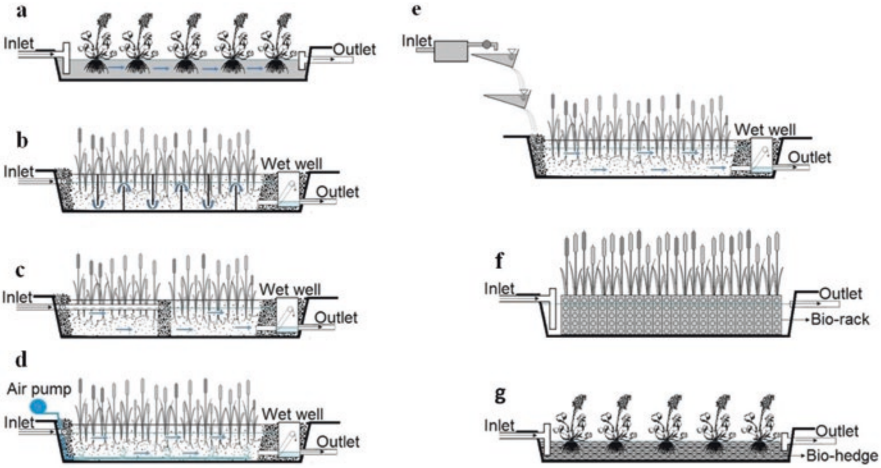

Fig. 1.4 Examples of advanced wetland systems: (**a**) shallow pond, (**b**) baffled follow, (**c**) step-feed, (**d**) artificial aeration, (**e**) multilevel drop aeration, (**f**) bio-rack, and (**g**) bio-hedge

4.1 Single Wetland Systems

4.1.1 Shallow Pond Water Hyacinth System (SPWHS)

The excess pond depth in traditional SF CWs (with free floating macrophytes) reduces oxygen transfer efficiency though roots and sustaining high anaerobic microbial growth. Higher pond depth can rise to anaerobic zones resulting in slow organic degradation rate and foul odor emission. In that order, the shallow pond system using water hyacinth was attempted to enhance the performance and to minimize the constraints associated with SF CWs (Fig. 1.4a). This system has the maximum water depth of 145 mm with the fully matured plant root submerged (90–120 mm) to avoid the anaerobic zone. This condition ensures the optimal interactions between the wastewater effluent and microbial biomass in the phytoremediation treatment practice. The vegetation (water hyacinth) had a coverage area of 192–193 plants/m^2, indicating a highly dense plant population (Valipour et al. 2011a).

The SPWHS offers the advantages of better oxygen diffusion efficiency through the roots and the accumulation of a larger aerobic bacterial population. This process leads to a higher biological degradation rates, resulting in lower space requirement and capital investment per volume unit than other traditional CWs. The nuisance odors prevented, and insect-related problem minimized. Nevertheless, SPWHS may be still thought to associate with the drawbacks of a high land area and capital investment. The risk of insect vectors is yet inevitable due to the free water surface. Hence, the use of shallow pond water hyacinth technique could be limited by potential users for wastewater treatment in decentralization practices.

4.1.2 Baffled CWs

Insufficient oxygenation of the rhizosphere in HSF CWs leads to incomplete oxidation of organic compounds and nitrification, and consequently denitrification (Chen 2012; Tee et al. 2012). The VUF CWs are also reported to be more favorable for anaerobic condition which can hamper the treatment capacity of the system (Zhang et al. 2013). However, they are more efficient than HSF CWs, simply because the water-root contact can be optimized in the system through an upward flow that thereby promotes plant uptake (Hamdan and Mara 2014). In contrast, the VDF CWs is stated to have a better oxygen transport ability which allows comparably higher pollutant removal efficiency, whereas denitrification is limited in such systems (Tee et al. 2012; Zhang et al. 2013). In that order, HSF CWs incorporating up and down flows sequentially along the treatment path is developed to enhance the removal of pollutants (Fig. 1.4b) (Tee et al. 2012, 2015). The design is able to nurture sequential aerobic, anoxic, and anaerobic conditions within the same wetland bed. This was achieved by inserting vertical baffles along the width of the wetland thus forcing the wastewater to flow up and down instead of horizontally as it travelled from the inlet to the outlet. The baffles help to protect the system from short circuiting, i.e., water finding a quick route from the entrance to the outtake.

The performance of the baffled CWs over traditional wetland systems can provide quantitative advantage given relatively smaller footprints and lower capital cost. This could be attributed to the longer flow path provided by the up-flow and down-flow conditions sequentially in the wetland resulting in more contact of the wastewater with the rhizomes and micro-aerobic zones (Tee et al. 2012). Nevertheless, the disadvantages, such as choking, clogging, large land area, capital investment, and so forth, would still exist in the baffled wetland unit (Xing 2012). Therefore, the baffled wetland system may face with difficulties in decentralization practices.

4.1.3 Step-Feeding CWs

For performance enhancement of organic matter and nitrogen in the CWs, step-feeding strategy is used by the gradational inflow of the wastewater into the bed (Fig. 1.4c) (Fan et al. 2013a; Stefanakis et al. 2011). In this practice, the wastewater typically discharges at multiple input points along the length of the cell. In the SSF CWs, it relies more on inlet and outlet structures for uniform flow distribution, especially for small length-to-width (L/W) ratio. In fact, inlet structure is expected to affect the performance of the CWs through selected flow distribution along the wetlands length (Stefanakis et al. 2011). In full-scale CWs, the main purpose of the step-feeding strategy lies in the effective utilization of wetland area through uniform loading distribution (i.e., BOD and TSS) (Hu et al. 2012). The step-feeding for full-scale CWs with longer L:W ratio and reduced width, resulting in lower needs for piping, simplifying inlet construction, and as a result, reducing construction costs. In cases of high strength wastewaters (characterized by high BOD and TSS), the small length to width ratio (L:W < 1) could be confronted with step-feeding adoption. In addition, the design/operation parameter might be great importance to avoid rapid clogging of subsurface flow systems and increase the lifespan of the material used in the bed (Stefanakis et al. 2011).

Step-feeding configuration offers some significant advantages and unique capabilities when compared to traditional CWs. This strategy ensured uniform distribution of influent wastewater through the reactor bed, prevents overloading of the influent, enhances the treatment performance, and consequently, reduces land area requirement and capital investment (Stefanakis et al. 2011; USEPA 1988). On the other hand, the drawbacks associated with traditional CWs could be still unavoidable with step-feed systems. In step-feed SF CWs, there is still the risk of odors and insect vectors. Also, there is a possibility of choking, clogging, and foul odors in the step-feed SSF CWs. These types of CWs may still require a large land area and capital investment. Moreover, Fan et al. (2013a) stated that the classic route of denitrification in CWs with step-feeding strategy is generally not completed, and the TN removal remains at a low level. However, the additional carbon source supply in the new influent may believe to improve the denitrification. Therefore, the application of step-feed CWs could be limited in the decimalization practices.

4.1.4 Artificial Aeration CWs

Dissolved oxygen is often a limiting factor during removal of organic and inorganic pollutants including nitrogen in CWs (Kumari and Tripathi 2014). Wetland oxygen is rapidly depleted by microbial nitrification and decomposition of organic matter, resulting in a large anaerobic zone (Tao et al. 2010). Therefore, in order to improve the oxygen availability and to enhance the removal of organic and inorganic pollutants, the use of artificial means for oxygen supply in CWs has been proposed (Fig. 1.4d). The ideal is to use an air pump (a blower or injector) to provide compressed air to the bed (Stefanakis et al. 2014; Tao et al. 2010). It is reported that the aeration of wetland with compressed air requires about half of the power of an equally performing and sizing activated sludge system for nitrogen removal (Wu et al. 2011b). The use of supplemental aeration can facilitate aerobic biodegradation of organic materials, nitrification, and consequently denitrification (Kumari and Tripathi 2014; Tao et al. 2010). So far, the artificial aeration in most CWs studies is performed in continuous mode (i.e., 24 h/day). This arrangement results in the faster depletion of influent carbon source and the lack of effective anoxic zone which both of them inhibit the subsequent denitrification step in CWs, and also, the high operation cost remains doubtful. Intermittent artificial aeration, instant of continues oxygen supply, can well develop alternate aerobic/anoxic redox conditions in wetland systems, allowing the occurrence of the coupled nitrification/denitrification processes. In general, intermittent aeration could be much energy-benefit than continuous mode (Fan et al. 2013a; Tao et al. 2010; Kumari and Tripathi 2014). In SF CWs, aeration can cause a hydrodynamic mixing effect and more uniform distribution of dissolved oxygen at a considerable distance from the air diffusers. For that reason, aeration is typically provided via high-volume devices. On the other hand, in SSF CWs, the hydrodynamic mixing is limited since the distance of an air bubble does not exceed 30 cm. This can result to the effectiveness of aeration and uniform distribution of small air quantities merely across the bottom of the bed (Stefanakis et al. 2014).

Besides, as a performance improvement of wetland, the artificial aeration increases the aerobic microbial activities, and enhances activates of the plant rhizomes and root systems (Tao et al. 2010). The performance of the system could diminish land requirement, and correspondingly, capital cost. The higher dissolved oxygen content can prevent the nuisance odors in the wetland units. Aeration also reduces the settling of suspended solids and flushes them out of the systems. This may lower the choking and clogging dilemma in SSF CWs. Accordingly, the insect vector problem can be minimized in the SF CWs. The bottom aeration in VDF CWs can also result in the combined action of the vertical downward drainage of the wastewater with the up-flow movement of air bubbles leading in a very good water mixture within the bed as described by Stefanakis et al. (2014). Nevertheless, the greater microbial activity could lead to a change of both microbial community structure and diversity, and higher microbial biomass yield. In view of that, the possible long-term effect of artificial aeration on SSF CWs, such as choking and clogging difficulties, is lacking and should investigate the case further (Chazarenc et al. 2009). For that reason, limiting the aeration efficiency and treatment performance as well as odor difficulties may occur in long-term operation of SSF CWs. The risk of insect vector is probably still apparent in the SF CWs. The land requirement and capital investment could be found considerable in wetland units. Furthermore, artificial aeration anyhow requires energy input and additional cost. Hence, it can be argued that artificial aeration makes the system less environmentally sustainable for decimalization practices.

4.1.5 Multilevel (Two-Layer) Drop Aeration CWs

Considering the limitations such as the low pollutant removal efficiency and oxygen transfer capability in traditional wetland treatment systems, the process of vertical flow CWs feed with drop-aerated influent has been configured (Fig. 1.4e) (Zou et al. 2012). In this practice, the additional oxygen is supplied into the wastewater by using multilevel, two-layer drop aeration devices that are installed at above CW. The cross-section of the inclined two-layer drop aeration units is triangular and parallel to each other with a distance of 0.3 m vertically. The wastewater flowed to the top of the multilevel, two-layer drop aeration unit and then being dropped into the next one by gravity. The amount of oxygen transferred into wastewater strongly depends on the designed flow rate and drop height of the aeration devices. The maximum oxygen transfer efficiency is possible to achieve at low flow rate as well as drop height up to 1.2 m (by using four units at a distance of 0.3 m vertically from each other); it slows at above this drop height (by increasing the units). The multilevel, two-layer drop aeration device supplies 2–6 mg/L higher dissolved oxygen (DO) per meter of the height into wastewater compared to the direct drop aeration process.

This type of CWs has numerous advantages of high hydraulic loading rate, high pollutant removal efficiency, low land requirement, and capital investment. There is no additional cost of energy consumption due to supplementary aeration. The nuisance odor can be prevented within wetland unit. Nevertheless, this system may still associate with some limitations; however, no any operational difficulties were reported during the study (27 months) by Zou et al. (2012). The low temperature would freeze the influent dropping device in cold climates. Nuisance odors and insect problems may occur because of the exposure of wastewater to the atmosphere in the influent dropping device. The chocking and clogging may also a problem due to soil strata in CWs. Accordingly, the odor nuisance could be faced. To apply for SF CWs, there is still question about the risk of insect vectors and public exposure due to open water surface. These systems may still be associated with high footprints and capital cost. Therefore, the application of the multilevel, two-layer drop aeration CWs could face with difficulties in decentralization practices.

4.1.6 Tidal Flow (TF) CWs

They are significantly developed to enhance the treatment capacity in traditional CWs. The operation of these systems is based on the batch principal by multiple periodical flood and drain cycles per day (Austin 2006; Hu et al. 2014). In these systems, the treatment performance could be attributed to many factors; including flood-to-drain time ratio, oxygen transfer efficiency, and media material characteristics. When reactors are flooded, the wastewater comes into contact with the bacteria in attached biofilms, and ammonium ion adsorbs to negatively charged media materials/roots surfaces. During the drain portion of the cycle, air is positively drawn from the atmosphere into media pore spaces of the bed, so the aeration of the system is significantly improved, resulting in a rapid nitrification of adsorbed ammonium. Accordingly, oxidized ammonium ions are dissolved in the remaining water on the soil particle and root surface. Because nitrification occurs in drained phases, there is little apparent competition for oxygen within biofilms to limit nitrification. In the next flood phase, nitrate ions desorb to bulk effluent (a high organic carbon environment) and are reduced to nitrogen gas by denitrifying bacteria with organic carbon as electron donor (denitrification). Therefore, the selection of media materials with high cation exchange capacity is significantly important to support this process (Austin and Nivala 2009). Moreover, diffused air in the wetland beds allow for enhanced organic particulates decomposition (Sun et al. 2007). The redox status in the bed can be controlled by the duration of the flood and drain periods. In that order, increasing the drain (unsaturated) time would enhance nitrification performance whereas may inhibit denitrification and vice versa (Hu et al. 2014). Flows can be a combination of horizontal and vertical during filling, but mostly vertical downward during draining (Kadlec and Wallace 2009).

In addition to effluent performance, the tidal flow wetland can be found to provide a benefit of smaller footprints, and lower capital investment (Austin and Nivala 2009). These types of wetlands are hydraulically efficient because they resist formation of preferential flow paths. They can build up as deep as 1.2 m without losing hydraulic efficiency. As a result, deeper systems occupy less land area, thereby broadening the number of potential wetland treatment sites (Austin 2006). They also may have fewer odor problems due to high oxygen transfer capacity of the wetland bed. On the other hand, in cold climates wetland beds may face with problem of freeze solid (Austin and Nivala 2009). Over a period of time, the system may cause choking and clogging problems (Sun et al. 2007). This can result in limiting available voids in the bed for atmospheric air diffusion and treatment efficiency, and consequently, odors complication can be faced. The high land area requirement and capital investment may still be considerable. Thus, the tidal flow type CWs could be restricted in decentralization practices.

4.1.7 Biofilm Attachment Surface (BAS) CWs

These systems are incorporated with the advantages of phytoremediation and engineered attached microbial growth processes to overcome the drawbacks associated with traditional CWs (Valipour et al. 2009, 2015). The wastewater is distributed uniformly and flows horizontally throughout the support matrix that used to provide an abundant attachment site for microbial growth (6–7 logs cfu/cm^2). Microorganisms associated with biofilms attached to the support matrix, stems, roots, and water column are responsible for most of the essential transformations and decomposition of contaminants in the wastewater. As initially developed, this type of wetlands includes Bio-rack (Fig. 1.4f) and Bio-hedge (Fig. 1.4g) treatment systems. The Bio-rack system (0.5 m effective depth) is free of soil strata-based root zone system and in lieu, a support matrix (Bio-rack) is provided to enrich the microflora. The Bio-rack assembled by the number of vertical PVC pipes having an effective height of a 500 mm, and 50–60 mm outer diameter (depending on type of vegetation). The pipes contained numerous surface perforations (20 mm diameter) for liquid transportation (Fig. 1.5).

The Bio-hedge CW is an advance form of shallow pond water hyacinth system in which plastic mesh type structures (Bio-hedges) was provided to achieve higher biomass concentrations within wetland unit (having 0.15 m effective depth). Bio-hedges with a length and effective height equal to the treatment unit were hanging vertically in 5.5 cm distances from one another, parallel to the direction of flow of wastewater.

The BAS wetland systems have proven effective in challenging applications of CWs in domestic wastewater treatment. These exceptional processes are less expensive to build and operate, simpler to design, and easier to maintain than any other wetland systems. It has significantly lower total lifetime costs, space requirement, and capital costs because of more intense biological and treatment activity as compared to other CWs. The specific arrangement of the BAS wetland techniques provides efficient oxygen diffusion and a distinguished surface area for a superior microbial population. In these systems, aerobic microorganisms dominate due to high oxygen transfer efficiency through roots. The BAS wetlands can be used to treat domestic wastewater with high concentration of suspended solids. On contrary to SSF CWs,

Fig. 1.5 Ideal root system growth of *Phragmites* sp. in the Bio-rack at steady-state condition

these practices do not have restrictions such as choking and clogging as a result of soil media. Odors and insects also are not a problem as a result of high oxygen transfer efficiency and short retention time. Overall, BAS CWs (especially Bio-rack wetland) can be strongly recommended for decentralization practices since there is a lack of specific technical constraints associated with any other CWs.

4.2 CWs Combined with Other Technologies

In application of CWs in domestic wastewater treatment, the pretreatment stage recommended to avoid clogging problems of the porous media (particularly where soil strata is used), lower the organic load rate, and reduce the land area requirement and capital investment (Jamshidi et al. 2014; Singh et al. 2009). The pretreatment methods can include screening, settling basin, stabilization pond, and anaerobic treatment units. The treated effluent of stabilization pond and anaerobic systems could be totally nutrients rich, and containing high concentration of unacceptable pathogen (Singh et al. 2009; dos Santos et al. 2013; Nasr et al. 2009; Kim et al. 2003). They are, however, typically known as efficient, economical and sustainable options in the decentralization (Nasr et al. 2009; Gikas and Tsihrintzis 2014). They require post-treatment for removing the remaining organic matters, nutrients, and pathogens. Therefore, the application of CWs as a post-treatment unit has been introduced as promising solution in wastewater treatment (Singh et al. 2009; Nasr et al. 2009; dos Santos et al. 2013; Kim et al. 2003). Yet, the behavior of constraints is the same still there is in types of CWs characterized by SSF (i.e., the bed with soil layers) and SF operation as a post-treatment unit. For that reason, these integrated treatment processes may be associated with complications in decentralization practices. In contrast, the incorporation of the BAS wetland systems could be recommended.

4.3 Hybrid Systems

Hybrid constructed wetlands are in operation in many countries around the world (Tee et al. 2012). The traditional CWs alone cannot achieve high removal efficiency of nitrogen because of their inability to provide both aerobic and anaerobic conditions simultaneously. They typically require low hydraulic loading rates, and large surface areas. As such, they are normally combined to complement each other, and are ideal to achieve higher treatment efficiency, especially for nitrogen removal (termed hybrid system) (Ávila et al. 2014; Tee et al. 2012; Tuncsiper 2009). The VDF CWs have a very high nitrification capacity, whereas HSF and VUF CWs show very efficient denitrification (Tuncsiper 2009; Zhao et al. 2011; Zhang et al. 2013). In that order, the hybrids from VDF and HSF CWs arranged in a staged manner are most frequently used to achieve, in addition to BOD_5 and SS removal, nitrification and denitrification (Abidi et al. 2009). However, HSF followed by VDF CWs were also used to enhance treatment efficiency. HSF-VDF CWs were designed by a large HSF bed and a small VDF bed. In this system, for nitrate removal, it is necessary to recirculate the effluent back to the front end of the system where denitrification takes place by using the raw feed as a source of carbon needed for denitrification (Vymazal and Kropfelova 2011). The potential combination of VDF followed by VUF CWs may likewise attempt for achieving higher treatment effect, particularly toward nitrogen removal (Zhao et al. 2011; Zhang et al. 2013). Besides VDF-HSF, HSF-VDF, and VDF-VUF systems, other combinations of various types of constructed wetlands including FS CWs and modified processes (consisting of two or more stages) can also be used (Vymazal and Kropfelova 2011; Ávila et al. 2014; Zhao et al. 2011; Zhang et al. 2013).

On the other hand, despite the performance of the hybrid systems, the limitations may still present in the need for large land area and capital investment (either by using traditional or modified processes), and typically, a recycling system to enable the wastewater to undergo treatment under oxidation and reduction conditions repeatedly (Tee et al. 2012). The most introduced modified systems (depend on the type of modification) often faced difficulties transitioning from traditional wetland treatment methods. There is a possibly of choking and clogging problems in the SSF stages where the beds filled with soil strata. The risk of odors and insects is unavoidable in the stages that build up based on kind of SF CWs. Therefore, the hybrid systems may also be restricted as model for decentralization.

5 Process Performance

A review of the literature has shown that the most treatment wetland studies deal primarily with organic, suspended solids, and nutrient pollutants removal. The performance of CWs depends on many factors, including the environmental conditions, degree of vegetative completeness within a wetland unit, types of plant, operational strategy taken, bacterial population, and oxygen concentration. In that order, emergent *Phragmites* sp. and free-floating water hyacinth has been found to have a great potential for use in phytoremediation of wastewater among other species. The potential ability of these plants can be explained by their higher growth rate and extensive root systems responsible for greater microbial mass contribution, the desirable rate of the oxygen transfer efficiency, nutrient cycling, filter bed stabilization, and water quality improvement throughout the treatment unit. A mixed culture of plant species might not play an important role during wastewater treatment compared to monoculture systems. Agreeing the hydraulic retention time (HRT) is a noteworthy direct predictor in a performance evaluation of CWs. An excessively short HRT will result in low treatment efficiency, whereas an excessively long HRT will not be economically feasible and might cause clogging of the filter material.

5.1 Organics and Suspended Solids Removal

Regardless of the traditional SF CWs, which is operated in batch mode (see Table 1.3), high levels of organic matter (≥50% COD, ≥70% BOD_5) are found in all types of CWs (Tables 1.3, 1.4, 1.5, and 1.6); despite this, the systems differ in terms of the operating conditions applied. Similarly, the removal of suspended solids is also high in all types of CWs (≥67% TSS).

As shown in Table 1.3, high organic removal efficiencies can be adopted for traditional HSF CWs using *Phragmites* sp. at a minimum HRT of 1 day. On the other hand, there is concern regarding the feasibility of traditional CWs as a cost-effective method because they typically require a long HRT to achieve efficient pollutant removal.

Among the cited modified processes applied in treating domestic wastewater (Table 1.4), the Bio-rack system planted with *Phragmites* sp. offers an excellent opportunity for the high efficiency of organic removal at a shorter HRT (0.42 day). As an obvious effect of vegetation, the Bio-rack system in the presence of *Typha* sp. can provide an effective organic removal efficiency comparable to that planted with *Phragmites* sp. at a longer HRT of 0.71 day HRT; but it can still operate at higher loading rates than other cited modified CWs. In fact, extensive root system of *Phragmites* sp. occupies thoroughly over the depth of the treatment unit, and correspondingly, enhances aerobic microbial mass contribution as well as organic and inorganic removal efficiencies. Despite this, the Bio-hedge system (at low HRT of 0.6 day) also appears to be an effective process in organic pollutant removal compared to other modified wetlands. The shallow pond water hyacinth process is less efficient than Bio-rack and Bio-hedge wetland systems (by increasing 20–50% HRT), but more effective in removing organic pollutants than other modified processes. In contrast, the step-feed HSF CWs have been operated for long retention times (6 and 14 days). Furthermore, a comparison of batch-type SF CWs with continuous aeration (at 1.5 days detention time) with the traditional operation (presented

Table 1.3 Examples of design/operational parameters and treatment efficiency of traditional CWs

CW type	WT	Wastewater characteristics							HRT (day)	Flow rate (m^3/day)	HLR (m^3/ m^2/day)	Vegetation	Reduction (%)					Study period (Months)	Country	References
		Temp. (°C)	pH	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	NH_4–N (mg/L)	TP (mg/L)					COD	BOD_5	TSS	NH_4–N	TP			
SF[a]	R	26	7.7	309	155	–	–	–	1.5	–	–	*Eichhornia sp.*	48	50	–	–	–	3	India	1
SF[a]	R	26	7.7	309	155	–	–	–	1.5	–	–	*Salvinia sp.*	37	38	–	–	–		India	1
SF[a]	R	26	7.7	309	155	–	–	–	1.5	–	–	*Eichhornia sp., Salvinia sp.*	48	51	–	–	–		India	1
FSF	R	24–27	7.4	363	212	144	30	–	1.8	0.03	0.27	*Eichhornia sp.*	76	87	67	69	–	22	India	2
HSF	R	28–30	7.2	445	235	152	33	–	1	0.026	0.23	*Phragmites sp.*	80	90	69	73	–		India	2
HSF	R	19–42[b]	6.5	477	167	159	–	20	2	0.0125	0.014	*Phragmites sp.*	69	95	90	–	96	6	India	3
HSF	R	–	7.69		105	33	16.6	5.03	1.5	0.198	0.34	*Typha* sp.	–	80	87	59	53	3	Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	1.5	0.198	0.34	*Canna* sp.	–	77	84	56	58		Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	1.5	0.198	0.34	*Typha* sp., *Canna* sp.	–	79	86	63	64		Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	3	0.099	0.17	*Typha* sp.		86	90	69	77		Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	3	0.099	0.17	*Canna* sp.		84	91	68	79		Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	3	0.099	0.17	*Typha* sp., *Canna* sp.		87	87	72	82		Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	6	0.0495	0.086	*Typha* sp.		90	92	86	86		Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	6	0.0495	0.086	*Canna* sp.		92	93	84	86		Thailand	4
HSF	R	–	7.69		105	33	16.6	5.03	6	0.0495	0.086	*Typha* sp., *Canna* sp.		91	90	88	90		Thailand	4
HSF	R	5–22[b]	–	536	340	272	36	23	4.7	2	0.05	*Typha* sp.	79	97	92	20	36	24	Spain	5
HSF	R	5–22[b]	–	744	485	359	43	29	4.7	2	0.05	*Typha* sp.	81	97	92	36	12		Spain	5

SF surface flow, *HSF* horizontal subsurface flow, WT wastewater type, including raw sewage (R)

References: 1 (Kumari and Tripathi 2014); 2 (Valipour et al. 2014a); 3 (Baskar et al. 2009); 4 (Sirianuntapiboon and Jitvimolnimit 2007); 5 (Ciria et al. 2005)

[a] Operated under batch mode

[b] Air temperature

Table 1.4 Examples of design/operational parameters and treatment efficiency of modified CWs

		Wastewater Characteristics											Reduction (%)							
CW type	WT	Temp. (°C)	pH	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	NH_4–N (mg/L)	TP (mg/L)	HRT (day)	Flow rate (m^3/day)	HLR (m^3/m^2/day)	Vegetation	COD	BOD_5	TSS	NH_4–N	TP	Study period (Months)	Country	References
Aerated SF[a, b]	R	26	7.7	309	155	–	–	–	1.5	–	–	*Eichhornia sp.*	82	83	–	–	–	3	India	1
Aerated SF[a, b]	R	26	7.7	309	155	–	–	–	1.5	–	–	*Salvinia sp.*	77	77	–	–	–		India	1
Aerated SF[a, b]	R	26	7.7	309	155	–	–	–	1.5	–	–	*Eichhornia sp., Salvinia sp.*	83	85	–	–	–		India	1
SPWHS	R	26–28	7.2	429	222	152	32		0.88	0.023	0.16	*Eichhornia sp.*	81	91	70	74	–	18	India	2
Bio-hedge	R	26–30	7.4	418	215	165	33	–	0.6	0.036	0.25	*Eichhornia sp.*	79	86	73	72	–	12	India	2
Bio-rack	R	27–29[f]	7.3	421	218	154	33	–	0.71	0.071	0.72	*Typha sp.*	78	88	69	73	–	5	Iran	2
Bio-rack	R	25–26	7.3	340	207	171	31	–	0.42	0.127	1.14	*Phragmites sp.*	75	87	73	70	–	19	India	2
Step-feed HSF[c]	S	16	6.8	491	373	–	31	10	14	–	–	*Phragmites sp.*	84	83	–	39	60	8	Greece	3
Step-feed HSF[d]	S	19	6.8	443	379	–	23	14	6	–	–	*Phragmites sp.*	87	89	–	69	78		Greece	3
Aerated VDF[a, b]	S	14–15[f]	–	352	–	–	46	–	3	–	–	*Phragmites sp.*	97	–	–	99		4	China	4
Aerated VDF[a, e]	S	14–15[f]	–	352	–	–	46	–	3	–	–	*Phragmites sp.*	96	–	–	97			China	4

(continued)

Table 1.4 (continued)

CW type	WT	Wastewater Characteristics							HRT (day)	Flow rate (m^3/day)	HLR (m^3/m^2/day)	Vegetation	Reduction (%)					Study period (Months)	Country	References
		Temp. (°C)	pH	COD (mg/L)	BOD$_5$ (mg/L)	TSS (mg/L)	NH$_4$–N (mg/L)	TP (mg/L)					COD	BOD$_5$	TSS	NH$_4$–N	TP			
Aerated VDF$^{a, e}$	S	-	7.5	113			40	3.9	3			*Phragmites sp.*	91			99	65	5	China	4
Aerated VDF$^{a, e}$	S	–	7.4	217	–	–	40	3.9	3	–	–	*Phragmites sp.*	95	–	–	99	79		China	4
Aerated VDF$^{a, e}$	S	–	7.4	429	–	–	40	3.9	3	–	–	*Phragmites sp.*	96	–	–	99	92		China	4
Aerated VDF$^{a, e}$	S	–	7.4	836			40	3.9	3	–	–	*Phragmites sp.*	97	–	–	96	99		China	4
TF	S	20–25^{f}	7.7	590	167	297	42	9.6	(6.75:0.5)	0.003	0.44	*Phragmites sp.*	49	71	67	43	84	5	Ireland	5
TF	S	20–25^{f}	7.7	436	181	107	46	5.1	(5.75:1.5)	0.003	0.44	*Phragmites sp.*	65	83	81	70	85		Ireland	5
TF	S	20–25^{f}	7.5	552	244	168	51	10.8	(4.75:2.5)	0.003	0.44	*Phragmites sp.*	83	94	92	96	95		Ireland	5
TF	S	20–25^{f}	7.7	207	61	69	55	8.2	(4.75:2.5)	0.003	0.44	*Phragmites sp.*	62	79	86	94	88		Ireland	5
TF	S	20–25^{f}	7.6	224	105	65	52	8.9	(4.75:2.5)	0.003	0.44	*Phragmites sp.*	70	87	85	96	88		Ireland	5
TF	S	20–25^{f}	7.6	464	182	157	50	8.6	(4.75:2.5)	0.003	0.44	*Phragmites sp.*	82	92	93	95	91		Ireland	5
TF	S	12–25^{f}	6.4	–	193	–	37	–	(3:3)	0.022	0.9	–	–	84	–	82	–	–	China	6
TF	S	12–25^{f}	6.5	–	193	–	75	–	(3:3)	0.022	0.9	–	–	82	–	74	–		China	6
TF	S	12–25^{f}	6.6	–	366	–	75	–	(3:3)	0.022	0.9	–	–	86	–	67	–		China	6
TF	S	12–25^{f}	6.2	–	366	–	34	–	(3:3)	0.022	0.9	–	–	91	–	33	–		China	6

SF surface flow, *SPWHS* shallow pond water hyacinth system, *HSF* horizontal subsurface flow, *VDF* vertical down flow, *TF* tidal flow, WT means wastewater type, *R* including raw sewage, *S* synthetic sewage

Operated under [a]batch mode, [b]continuous aeration, [e]intermittent aeration

Inflow distributed at three points by percentages of [c]33:33:33, and [d]60:25:15

References: 1 (Kumari and Tripathi 2014); 2 (Valipour et al. 2009, 2011a, 2014b, 2015); 3 (Stefanakis et al. 2011); 4 (Fan et al. 2013a, b); 5 (Hu et al. 2014); 6 (Wu et al. 2011b)

[f]Air temperature; ( ) flood-to-drain time ratio (h: h)

Table 1.5 Examples of design/operational parameters and treatment efficiency of post-treatment CWs

CW type	WT	Wastewater Characteristics							HRT (day)	Flow rate (m^3/day)	HLR (m^3/m^2/day)	Vegetation	Reduction (%)					Study period (Months)	Country	References
		Temp. (°C)	pH	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	NH_4–N (mg/L)	TP (mg/L)					COD	BOD_5	TSS	NH_4–N	TP			
HSF	PE	–	–	266	219	–	–	2.43	1.64	0.79	0.2	*Typha* sp.	74	76	–	–	85	24	Indonesia	1
HSF	PE	–	7.2	123	–	54	–	8.9	1	0.44	0.22	*Canna* sp.	59	–	93	–	12	–	Thailand	2
HSF	PE	–	7.23	136	–	65	–	9.2	2	0.22	0.11	*Canna* sp.	73	–	95	–	23		Thailand	2
HSF	PE	–	7.26	135	–	47	–	9.8	4	0.11	0.055	*Canna* sp.	83	–	96	–	35		Thailand	2
HSF	PE	–	7.2	123	–	54	–	8.9	1	0.44	0.22	*Heliconia* sp.	58	–	92	–	6		Thailand	2
HSF	PE	–	7.23	136	–	65	–	9.2	2	0.22	0.11	*Heliconia* sp.	72	–	95	–	7		Thailand	2
HSF	PE	–	7.26	135	–	47	–	9.8	4	0.11	0.055	*Heliconia* sp.	79	–	97	–	13		Thailand	2
Baffled HSF	PE	23–34	–	156	–	–	33	–	2	0.25	0.25	*Typha sp.*	59	–	–	74	–	12	Malaysia	3
Baffled HSF	PE	23–34	–	150	–	–	33	–	3	0.16	0.16	*Typha sp.*	67	–	–	84	–		Malaysia	3
Baffled HSF	PE	23–34	–	160	–	–	37	–	5	0.1	0.1	*Typha sp.*	79	–	–	99	–		Malaysia	3
HSF	AnE	–	–	–	116	62	–	13.4	3.5	1	0.063	*Phragmites sp.*	–	82	77	–	47	–	Korea	4
HSF	AnE	–	–	197	120	79	52	4.5	3	0.146	0.073	*Phragmites sp.*	71	83	94	89	33	–	Saudi Arabia	5
HSF	AnE	11–30	–	355	–	–	34	12	8	0.6	0.03	*Phragmites sp.*	70	–	–	21	52	12	Italy	6
VDF	AnE	11–30	–	355	–	–	34	12	2.7	1.2	0.21	*Phragmites sp.*	92	–	–	94	27		Italy	6
Drop-aerated VDF	AnE	12–16[a]	7.7	164	69	41	–	–	3.6	0.144	0.192	–	80	91	84	–	–	–	China	7
Bio-rack	AnE	25–29[a]	7.1	420	150	245	–	–	0.4	0.126	1.31	*Phragmites sp.*	77	84	75			12	Iran	8
Bio-rack	AnE	25–29[a]	7.15	290	110	237	–	–	0.52	0.096	1	*Typha* sp.	66	76	70				Iran	8
HSF	SPE	21[a]	7.5	276	112	–	–	16	6	0.28	0.1	*Typha* sp.	64	72	–	–	31	5	Brazil	9

HSF horizontal subsurface flow, *VDF* vertical down flow

WT means wastewater type, including primary effluent (PE), anaerobic effluent (AnE), and stabilization pond effluent (SPE)

References: 1 (Hendrawan et al. 2013); 2 (Konnerup et al. 2009); 3 (Tee et al. 2012); 4 (Ham et al. 2007); 5 (El-Khateeb and El-Bahrawy 2013); 6, (Mietto and Borin 2013); 7, (Zou et al. 2012); 8, (Jamshidi et al. 2014); 9, (dos Santos et al. 2013).

[a] Air temperature

Table 1.6 Examples of design/operational parameters and treatment efficiency of hybrid CWs

		Wastewater Characteristics											Overall reduction (%)							
CW type	WT	Temp. (°C)	pH	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	NH_4–N (mg/L)	TP (mg/L)	HRT (day)	Flow rate (m^3/day)	HLR (m^3/m^2/day)	Vegetation	COD	BOD_5	TSS	NH_4–N	TP	Study period (Months)	Country	References
VDF-HSF	R	17–29	–	462	310	80	124	–	3	0.07	0.33–0.1	*Phragmites* sp., *Scirpus* sp.	83	87	95	85	–	8	Spain	1
VDF-HSF	R	17–29	–	274	162	72	122		6	0.036	0.17–0.05	*Phragmites* sp., *Scirpus* sp.	74	85	95	91	–		Spain	1
VDF-VUF	S	–	7.21	289	–	–	–	3	1.2	0.5	0.5–0.5	*Typha* sp.—*A. donax*	61	–	–	–	51	6	China	2
VDF-VUF	S	–	7.21	289	–	–	–	3	1.2	0.5	0.5–0.5	*Canna* sp.—*Pontederia* sp.	64	–	–	–	50		China	2
HSF-VDF	AnE	10–27	7.5	115	41	26	15	5.1	3	27	0.17–0.15	*Phragmites* sp.	94	95	85		94	17	Italy	3
VDF-HSF-SF	AnE	15–25	7.8	189	133	25	20	–	4	0.2	0.06–0.1–0.1	*Phragmites* sp.	74	90	84	96	–	11	Spain	4
VDF-HSF-SF	AnE	18–24	7.5	294	204	98	43	6.8	–	14	0.04–0.06–0.06	*Phragmites* sp.- *Phragmites* sp. - 6 species	83	97	94	95	22	14	Spain	4
VDF-HSF-SF	AnE	13–15	7.8	549	–	27	31	–	4	0.2	0.06–0.1–0.1	*Phragmites* sp.	91	–	93	97	–	–	Spain	4
VDF-HSF-SF	AnE	15–16	7.8	828	–	53	47	–	2	0.4	0.13–0.2–0.2	*Phragmites* sp.	89	–	96	72	–		Spain	4
VDF-HSF-SF	AnE	18–20	7.5	868	–	60	43		1.5	0.55	0.18–0.28–0.28	*Phragmites* sp.	91	–	95	86	–		Spain	4
THCW	AnE	−4.2–39[a]	7.11	320	–	124	46	6	2.7	43	0.32	5 species	85	–	89	83	64	8	China	5

HSF horizontal subsurface flow, *VDF* vertical down flow, *VUF* vertical up flow, *SF* surface flow, *THCW* towery hybrid CW, *WT* wastewater type, including raw sewage (R), synthetic sewage (S), anaerobic effluent (AnE), and stabilization pond effluent (SPE)

References: 1 (Melian et al. 2010); 2 (Chang et al. 2012); 3 (Masi and Martinuzzi 2007); 4 (Ávila et al. 2013a, b, 2014); 5 (Ye and Li 2009)

[a] Air temperature

in Table 1.3) showed that artificial aeration can compensate for the lack of plant-mediated oxygen supply, and enhance the organic removal efficiency in CWs. On the other hand, a comparison of continuously aerated SF with continuously aerated VSF CWs showed that the type of wetland system and HRT can play a key role in these systems. Moreover, based on the evidence of aerated batch-fed VDF CWs (at 3 days detention time), the systems that operated under intermediate aeration showed comparable removal efficiencies of organic pollutants to that operated under continuous aeration. The experimental results from tidal flow (TF) CWs showed that the system produced the highest organic pollutant removal efficiency with relatively short flood and long drain periods, highlighting the importance of oxygen transfer into the bed matrix.

Among the cited CWs presented in Table 1.5, the Bio-rack system planted with *Phragmites* sp. also appears to be a suitable alternative that can be used to remove organic pollutants from pre-treated domestic wastewater (at a HRT as low as 0.4 day). The Bio-rack system planted with *Typha* sp. was found to have a slightly longer retention time (0.52 day) to achieve an almost comparable organic effluent quality. The HRT of 0.52 day is still shorter than the other cited CWs in this category. The influent wastewater discharged into the Bio-rack wetland (with *Phragmites* sp.) contains somewhat higher concentrations of organic pollutants than that planted with *Typha* sp. The higher influent pollutant concentrations may cause a longer retention time in the CWs. But, by contrast, there would still be the added benefit of dealing with constraints associated either with traditional or other modified CWs when the Bio-rack system (planted with *Typha* sp.) is used. The traditional VDF CW shows better organic removal treatment performance than traditional HSF CWs because they provide an ideal environment for aerobic bacterial respiration. Baffled HSF and drop-aerated VDF CWs have been found to have long HRTs (respectably above 2 and 3 days) to produce the desired organic pollutant removal efficiency, while the pollutant influent concentration was comparatively low in both systems. In fact, the low influent pollutant concentrations and the longer HRT in the cited CWs can be due to the possible long-term effects, such as choking and clogging difficulties. However, wetland systems with long HRT also had adverse effects on effluent quality.

As shown in Table 1.6, the use of hybrid systems does not eliminate the need for pretreatment. For the treatment of domestic wastewater (100–500 mg COD/L), the hybrid systems were operated optimally in HRT with a range of 3–6 days. Accordingly, a higher organic pollutant removal rate can be obtained at a higher organic loading rate compared to those operated under a low organic loading rate. Nevertheless, caution should be taken to avoid clogging of the filter beds when they operated under high organic loading rates. Zhao et al. (2011) reported that the average organic pollutant removal efficiencies ranged from 73 to 93% by varying the influent COD/N ratios (2.5, 5, and 10) in two-stage hybrid systems (Q=0.04 m^3/day, HLR=0.07–0.07 m^3/m^2/day) (Fig. 1.6). On the other hand, the highest organic matter removal occurred when the COD/N ratios were 5–10 for VDF-VUF CW.

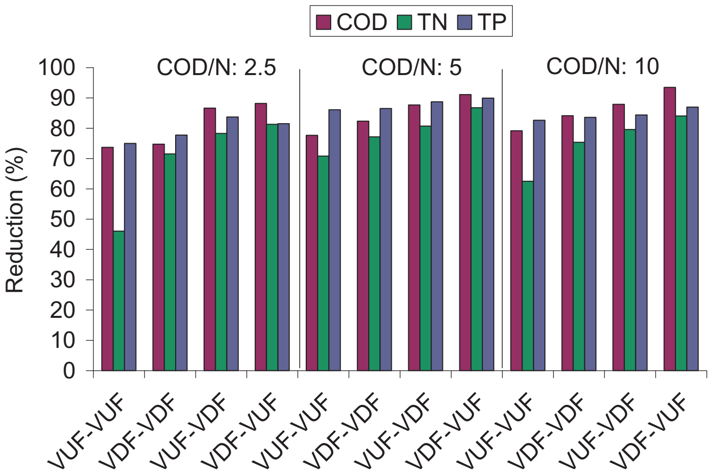

Fig. 1.6 Pollutant removal efficiencies of four types of two-stage hybrid CWs fed with synthetic domestic wastewater (study period: 9 months, vegetation: *A. calamus*, influent temp.: 8–41 °C) (Zhao et al. 2011)

5.2 Nitrogen Removal

As shown in Tables 1.3, 1.4, 1.5, and 1.6, the removal efficiency of ammonium nitrogen (NH4–N) varied (20–99%), which can be due to the input organic and nitrogen concentration, oxygen transfer efficiency of the systems, aerobic microbial population within the wetland beds, process condition (i.e., hydraulic retention time), and a certain adsorption capacity of the materials in the wetland bed. As shown in Tables 1.3 and 1.4, the nitrogen removal rate (nitrification) could be limited by the high organic matter concentrations (≥300 mg BOD5/L) presented in the influents in a single unit CWs, while the organic removal efficiency remains constant. This might be because the available oxygen in wastewater with a high BOD and nitrogen content is utilized quickly by heterotrophic bacteria for the metabolism of organic carbon (Ciria et al. 2005). As a result, clogging of the bed can be observed due to the excessive heterotrophic biofilm growth. Based on Table 1.4, the low nitrogen removal efficiencies may also be due to the high nitrogen levels presented in the influent (≥70 mg NH4–N/L).

Nevertheless, in step-feed CWs (Table 1.4), the nitrogen removal efficiency may be altered depending on the step-feeding scheme. The gradual wastewater inflow at three points across the wetland length with sequence percentages of 60%, 25%, and 15% of the total influent volume is more effective in enhancing the level of nitrogen elimination in the step-feed HSF CWs. Fan et al. (2013a) stated that the classic route of denitrification in CWs with step-feeding strategy is generally not completed, and the TN removal remains at a low level. However, the additional carbon source supply in the new influent may believe to improve the denitrification. In the cited aerated batch-fed VDF CWs (3 days HRT) (Table 1.4), the improved simultaneous removal of organic matter and nitrogen for high-strength nitrogenous organic wastewater can also be realized by incorporating supplementary aeration. Therefore, the intermittent aeration could be an appropriate method for enhancing the TN removal efficiency than continuous aeration due to the nitrification and denitrification conditions that occur simultaneously. Fan et al. (2013a, b) reported intermittently aerated VSF CWs with a removal efficiency of 96–99% NH4–N and 26–94% TN depending on the influent COD/N ratio (from 2.5 to 20). Based on the tidal CWs cited (Table 1.4), a total bed rest time of 2.5 h in an approximately 8 h cycle (i.e., short flood and long drain periods) was adequate for complete nitrification (≥94%). Under this condition, the system achieved effective denitrification when the influent COD/N ratio was above 7 (≥85% TN reduction) (Hu et al. 2014). By considering the results of the cited CWs used as a post-treatment (Table 1.5), it can be verified that the traditional type VDF CW provides higher nitrification activity compared to that of traditional HSF CWs. In hybrid systems (Table 1.6), such as the cited VDF-HSF, nitrification could be limited slightly when they are operated under a high organic loading rate. On the other hand, the high nitrogen concentration in wastewater might not have an effect on the ammonia nitrogen removal performance in hybrid systems (as VDF-HSF). Tower and three-stage (VDF-HSF-SF) hybrid systems can achieve a TN > 80% (Ávila et al. 2013b; Ye and Li 2009). Tuncsiper (2009) reported the highest nitrogen removal efficiency (≥80%) in a two-stage hybrid system (HSF-VSF) by 100% recycling and HLR of 0.03m^3/m^2/day (Table 1.7). Therefore, the removal efficiency has been increased by increasing the recycling ratio and decreasing the HLR. Zhao et al. (2011) reported average removal efficiencies of 46–87% for TN by varying the influent COD/N ratios (2.5, 5, and 10) in two-stage hybrid systems, and VDF-VUF CW showed the highest TN reduction at a COD/N ratio of 5 (Fig. 1.6). As a result, the two hybrid systems of VUF-VUF and VDF-VDF have the lowest TN removal efficiency because of their inability to achieve the aerobic/anaerobic conditions for the nitrification/denitrification.

5.3 Phosphorous Removal

As reported in previous studies (Tables 1.3, 1.4, 1.5, and 1.6), the phosphorus removal efficiency in CWs varies considerably (6–99%) depending on the wetland design, environmental condition, and loading rate. The elevated phosphorus elimination in the cited CWs can be due directly to the plant uptake process, more stable temperature during wetland operation and long contact time within the wetland units. Similarly, the high removal of phosphorus could be due to ligand exchange reactions within the bed (Vohla et al. 2011). In a proposed design system, increasing the hydraulic retention time can ameliorate the treatment performance. The removal of phosphorus is normally low and typically amounts of only 40–60% are observed during the treatment of domestic sewage (Vymazal 2004). In particular, Zhao et al. (2011) reported an average reduction of 75–90% TP by varying the influent COD/N ratios (2.5, 5, and 10) in two-stage hybrid systems (Fig. 1.6). Therefore, VDF-VUF CW with COD/N ratio of 5 was verified for the highest TP reduction.

Table 1.7 Mean nitrogen removal at different recycling ratios and HLR in a two-stage hybrid CW fed with pre-treated domestic sewage (Tuncsiper 2009)

RecirculationRatio	Nitrogen components	HLR: 0.1 m^3/m^2/day		HLR: 0.8 m^3/m^2/day		HLR: 0.06 m^3/m^2/day		HLR: 0.03 m^3/m^2/day	
		HSF (%)	VSF` (%)	HSF (%)	VSF (%)	HSF (%)	VSF (%)	HSF (%)	VSF (%)
0	TN	35	60	45	63	48	50	63	83
	NH_4–N	37	60	45	65	47	50	62	87
50	TN	40	60	50	63	57	52	73	93
	NH_4–N	37	63	50	68	53	53	73	97
100	TN	53	62	57	50	63	60	80	95
	NH_4–N	50	65	53	52	60	63	80	98

[a] Vegetation: *Iris* sp.—*Phragmites* sp., influent temp.: ~20 °C, study period: 15 months

6 Environmental Stress Condition

6.1 High TDS Concentration and Desalination

The domestic wastewater in coastal areas may contaminate with high concentrations of total dissolved solids ranging from 400 to 3000 mg TDS/L (Valipour et al. 2014b). The tolerance to high TDS stress in aquatic macrophytes is a coordinated action in the treatment performance. Caution must be taken to plant selection when dealing with high TDS-contaminated wastewater since they should be stress-tolerant plant in order to have a resilient and effective wetland system. Under high TDS stress wastewater, plants stand by using several physiological mechanisms include *sequestration of TDS in vacuoles of the cells*, *TDS exclusion from the transpiration stream,* excreting excess TDS through TDS glands, and preventing TDS uptake into the roots (Tuteja 2007). TDS affects plants in different ways, such as osmotic effects, specific-ion toxicity, and/or nutritional disorders. The extent by which one mechanism affects the plant over the others depends upon many factors, including the species, genotype, plant age, ionic strength, and composition of the solution (Läuchli and Grattan 2007). Halophytic plants have long been suggested in treating wastewater contaminated with high TDS; however, the use of halophytes to reduce TDS (desalination) is a novel strategy. To act as an accumulator of TDS, the plant has to be able to tolerate a wide gradient of TDS, grow in wetland, and accumulates enough ions within its tissues to significantly reduce the TDS of wastewater (Shelef et al. 2013). In general, the systematic data regarding phytoremediation of TDS-contaminated wastewater are scarce.

The investigation on using the Bio-rack and shallow pond system in treating TDS-contaminated wastewater revealed that the *Phragmites* sp., *Typha* sp., and water hyacinth can tolerate TDS up to 9000, 2500, and 2000 mg/L, respectively (Valipour et al. 2010, 2011b, 2014b). Beyond these concentrations, the plants were highly damaged and the COD level in treated effluent reached to above 100 mg/L. At these detention limits, the removal efficiencies were found to be close to 80% in COD and 20% in TDS (Table 1.8). The results suggested that aquatic macrophytes (such as *Phragmaties* sp., *Typha* sp., water hyacinth) play a role in desalination by the accumulation of TDS in their tissues since the reduction of TDS was observed in these biological processes. *Phragmaties* sp. may more appropriately refer to as a TDS tolerance plant due to osmotic adjustment with attribution of high K^+ content in shoot tissues. Additionally, the same desalination phenomenon has also been reported to occur in halophyte *Bassia indica* by accumulating 10% Na of its dry weight (Shelef et al. 2013).

6.2 Cold Climate Operation

The best prospects for successful wetland treatment should be in the warmer regions. However, the application of this system under cold climate condition has also been reported. From the North America Treatment System Database, 176 wetland

Table 1.8 Performance of shallow pond and Bio-rack CWs treating domestic sewage seriously stressed with TDS at the optimal tolerance limit of plant species (Valipour et al. 2010, 2011b, 2014b)

CW type	HRT (day)	Flow rate (m^3/day)	HLR (m^3/m^2/day)	Inlet (mg/L)		Reduction (%)		Study period	Country
				COD	TDS	COD	TDS		
Bio-rack system (*Phragmiters* sp.)	0.42	0.127	1.14	456	8760	80	14	3	India
Bio-rack system (*Typha* sp.)	0.71	0.071	0.72	388	2512	76	21	3	Iran
Shallow pond system (Water hyacinth)	0.88	0.023	0.16	442	1992	81	19	3	India

treatment sites in Canada and the USA have been listed, of which about 60% of these are located in cold climate regions. Almost all of the cold climate wetlands are SF, and 90% of them treating domestic wastewater (Mæhlum 1999).

In cold season, the microbial metabolism and their bioactivity are rather low; whereas, with increasing temperature, the plant biomasses and activities of microorganisms increase at high speed, which resulted in higher removal rate of pollutant. For a multi-stage pond-wetland system in Dongying city, China, in cold season, the removal efficiency of COD, BOD_5, and NH_4–N were about 85, 40, and 20%, respectively, while in warm season 92, 73, and 71% of these pollutants were removed, respectively (Peng et al. 2005). A long hydraulic retention time could enable microorganisms to grow and reduce organic matters and nitrogen at low temperature, but the capital investment in the system increases.

In actual fact, the CW technologies have to be adapted to subfreezing environments, which would enhance the treatment performance. The plant species that provide structure year-round perform better than those species that die below the waterline after the onset of cold temperature. For this reason, fast-growing emergent species (such as *Phragmites*, *Typha*, and *Scirpus*), that have high lignin contents and are adaptable to variable water depths, are the most ideal for CWs during the cold seasons. Furthermore, in cold climate condition or in response to short-term temperature decreases, a greater oxygen leakage from the root of some plants can sometimes appear due to plant dormancy and reduced internal oxygen consumption. This may offset possible oxygen limitation and improve aerobic microbial respiration in the root zone throughout the cold condition operation (Stein and Hook 2005). However, it is usually assumed oxygen transfer to the rhizosphere would be greater during periods of active plant growth where microbial activities are also maximal. The selection of appropriate plants may have added benefits in protecting the water surface from freezing, trap falling and drifting snow, enhance oxygen transfer, and reduce the heat-loss effects of wind. Besides, some type of insulation strategies can also apply as a response of wetland system to cold climate.

In CWs (dominated by emergent plants), ice and snow can be used as an insulating layer. As an example, these types of insulations have been carried out in northern

China (Li et al. 2011b). In this practice, an ice-air blanket is often purposefully generated by raising effluent levels to allow it to freeze and subsequently lowering it, leaving an air gap between the ice layer and the effluent (Horváth 2012). A pilot scale CW planted with *Phragmites* sp. (in Tianjin, northern China) under ice layers when the average air temperature lower than −4 °C has been shown an effluent quality better than secondary treatment level, e.g., $BOD_5<20$ mg/L, $SS<20$ mg/L, $TN<15$ mg/L, $TP<0.6$ mg/L (Yin and Shen 1995). Ice began to form on a water surface when the water temperature reaches as low as 3 °C because of density differences and convective losses. If the wastewater entering wetland has a temperature >10 °C, aeration can be used to modify surface water temperature near zero. The presence of ice layer can be a benefit for providing insulation and declining the cooling of the underlying water, but the water flow will be reduced as the ice layer thickens. As a result, the constriction of flow beneath the ice layer leads to subsequent flooding, freezing, and hydraulic failure (Mæhlum 1999). Thus, an increased depth up to 1 m is also recommended for SF (water depth) and SSF (effective depth) CWs (Heers 2006). A Canadian FWS CW operated successfully over 4 years by raising the water level at freezing time (Mæhlum 1999; Heers 2006). On the other hand, in SSF CWs, the dead vegetation or mulch (such as poplar bark, wood chips, and reed-sedge peat) can be used as appropriate heat insulation materials for preventing freezing and resulting in hydraulic failure throughout the cold season (Wallace et al. 2000).

The amount of mulch material needed to protect wetland bed from freezing damage is highly dependent on the timing and amount of snowfall since snow cover itself is a significant insulator (Wallace and Nivala 2005). It is recommended to use possibly well-decomposed organic materials to avoid degrading treatment efficiency. To be effective, insulation must be uniform in coverage, which requires that it be designed as an integral part of wetland system (Wallace et al. 2000). The wetland surface can also be covered with a porous media having low thermal conductivity, such as expanded clay aggregates, which should be kept unsaturated during the cold season (Mæhlum 1999; Horváth 2012).

In a SSF CW covered with harvested vegetation, the average removal efficiencies were obtained to be 31% for TP, 27% for NH_4–N, and 10% for TN during winter (−3 to 6 °C) which were 16, 10 and 5% higher than that of the control wetland, respectively (Shen et al. 2007). An integrated household VSF CWs (using *Salix* sp.) in rural villages in northern China has been pointed out that a 0.4 m insulating biomass layer (sawdust) maintained a bed temperature above 6 °C in the face of freezing temperatures (even at a very low temperature of −8 °C) during winter. Average removal efficiencies were 95% BOD_5, 96% TSS, 85% NH_4–N, and 88% TP during the winter period. There was a negligible increase in the average removal efficiency for BOD_5, TSS, and NH_4–N out of winter (1.3%, 1.1%, and 5.4%, respectively); while an increase of 0.6% was achieved for TP removal in the winter period (Wu et al. 2011a).

Moreover, artificial aeration can be used to overcome the effect of oxygen limitation in CWs caused by the winter dormancy and mortality of vegetation and the need to use an insulating mulch layer in the bed. As a result, organic material and nitrogen pollutant removal are enhanced during cold season. Oxygen solubility is higher in colder water, but gas exchange in HSF CWs may be reduced by the additional insulation layer and the fact that plants are dormant (Horváth 2012; Ouellet-Plamondon et al. 2006). In SF CWs, the artificial aeration additionally can also create an ice-free zone during cold season (Wallace 2011). Another alternative option which has already been used in northern USA and Canada is to store the discharged wastewater in a tank during cold season and reflow it again through the wetland unit during spring time (Pries et al. 1996). The advantage of this practice is the use of a design for warm weather condition, whereas the disadvantage is the cost of the storage lagoons. It has been reported that a seasonal storage for the winter waste load might be necessary in locations where extended periods of air temperature <−10 °C are experienced. A number of CWs in South Dakota and northwest Canada operated this way (Mæhlum 1999).

7 Future Sustainability of Constructed Wetlands

It has been widely recognized that CWs offer an effective and a reliable treatment to domestic wastewater after years of study and implementation. The current review indicated that the advances in the design and operation of CWs could be desirable to improve the treatment performance. In view of that, among others, BAS CWs allow for a more flexibility in the design and operation for dealing with domestic wastewater. The excellent efficiency level in CWs treating domestic sewage under environmental stress condition can be accomplished by an appreciate adoption strategy. But future research and development work is still needed for successful and sustainable application of CWs, especially in decentralization practices. In summary:

1. However, the knowledge of traditional CWs has been going to develop; but critical studies to overcome constraints (such as problems of clogging, odors, insect vectors, high land area, and capital investment) according to the types of advanced treatment systems (Section 4) are still required for sustainable wastewater quality improvement. Out of this need, several localization frameworks in advance CWs should also be developed to provide a sustainable model for decentralization practices.
2. In BAS CWs, plastic type materials are primarily used for attached biofilm growth. The supporting media can be regarded as a critical step to provide the obligatory surface area for biomass concentration within wetland unit. The greater surface area to volume ratio of expended bed reactor can enhance treatment performance. There is a need to research on application of a more sustainable material with higher surface area in BAS CWs.
3. Hybrid systems are mainly a combination of different types of traditional CWs. Various types of modified wetlands could be combined into hybrid systems to achieve higher organic matters and nitrogen removal efficiencies.

4. Future research should investigate the possible incorporation of media materials with high sorption capacity (such as industrial byproduct, agricultural wastes, etc.) in CWs which can be beneficial to pollutant removal processes from domestic sewage. However, the possible clogging problems should be considered in their applications.
5. The type of plant species used in wetland system can affect treatment efficiency and is critical for sustainable pollutant removal from domestic sewage. This is important to have a comparative assessment on the use of different aquatic weeds in each modified wetland process under the same environmental condition. Further analysis is also needed for identifying potential plant species for climate adaptation strategies, particularly for cold season.
6. The lack of detailed information concerning the tolerance of plants to high TDS concentration is an important issue requesting for more researches. It is challenging to predict under what circumstances the plant contribution to the wetland units will be more remarkable in phytoremediation of TDS-contaminated wastewater. Intensive evaluation to assess the potential of different plant species for TDS accumulation (in desalination practices) is also needed.
7. During cold season, a wide variety of materials such as bark, pine straw, wood chips, etc., would be used as mulch to prevent the wetland bed from freezing damage. The mulch materials can strongly affect the system performance and the establishment rate of introduced plants. As a result, the plant may experience a large drought stresses on seedlings and have extremely poor seed germination. The species can only establish by rhizome spread from mature plants. Therefore, the research needs to verify an appropriate mulch layer over the main media. In addition, future research may be necessary to extend beyond a focus on plant species that can tolerate the mulch layer.
8. Microbial activity levels can be changed temporally in response to root development and plant age, system type, high TDS, and seasonal patterns. Therefore, research needs to identify microbial population and their ecological activity associated within wetland units in details. This may help to develop the design and better management of CWs.
9. The research on the predominant microbial species and macrophytes having a specific gene for nitrogen removal may help to optimize nitrogen removal in CWs.
10. Plant biomass production is an integral part of the wetland system. It is possible that nutrients and other pollutants which trapped in plant tissues released back into water as the plants die and decay during the cold winter. Therefore, it is important to find low cost methods for harvesting, disposal, and/or utilization of excess biomass generated by wetland treatment processes. The research and focus on developing strategies for plant resource recovery and recycling are essential.

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Chapter 2
Laccases: A Blue Enzyme for Greener Alternative Technologies in the Detection and Treatment of Emerging Pollutants

Melissa Rodríguez-Delgado and Nancy Ornelas-Soto

Abstract The continuous contamination of worldwide water bodies, by the presence of emerging pollutants, has raised great importance over the last decades. This group of pollutants comprises a large variety of chemicals, comprehending household and personal care products, human and veterinary drugs, as well as industrial compounds. Although, scientific data have made evident the potential threats of the emerging pollutants to public and environmental health, there is still limited information available concerning the ecotoxicity, concentration, and distribution of these compounds, which makes their ecological regulation, detection, and treatment very difficult. Thus, the search for green technologies to detect and treat potential environmental pollutants is critical for ecological and human health protection. In this context, laccases have gained scientific interest due to their broad substrate range, including recalcitrant environmental pollutants, and their ability to use only oxygen as a co-substrate. This work explores the potential of laccase enzyme as element of biosensing and bioremediation, and identifies the drawbacks that have to be overcome in order to demonstrate their feasibility and implement a large-scale process.

1 Introduction

To date, there is a critical environmental problem of contamination in water resources with persistent, bioactive, and bioaccumulative substances, which cause potential health and ecological effects (Petrovic 2003). Some of these chemicals are daily life articles, such as household and personal care products, human and veterinary drugs, which are extensively used and constantly released into the aquatic ecosystems by human activities and direct discharges from wastewater treatment plants (WWTPs) (Caliman and Gavrilescu 2009). These groups of substances are known

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as emerging pollutants and their detection and removal is crucial due to their high persistence and toxicity, even at concentrations as low as ng/L (Bolong et al. 2009; Gavrilescu et al. 2014). It is well-known that most of the emerging pollutants that pass through conventional WWTPs are not completely removed (Caliman and Gavrilescu 2009). Thus, the development of biosensors resulted as an effort in the search for analytical tools capable to detect these kinds of contaminants at low concentrations with a high specificity (Rodriguez-Mozaz et al. 2006). However, once the pollutants have been identified, a decontamination process has to be performed. In this context, bioremediation of water resources by the use of biocatalysts, such as enzymes, has been suggested in recent years. Enzymes are biomolecules that have the ability to mediate reactions; severe conditions are not required for their action and normally the by-products formed during the catalysis are benign, which implies a great opportunity for the use of enzymes in bioremediation (Senthivelan et al. 2016). Currently, several enzymes have been used in different biotechnological and industrial applications; however, laccases enzymes have received special attention due to its ability to oxidize a wide range of substrates, accompanying the reduction of oxygen to water as a by-product of reaction (Rodríguez Couto and Toca Herrera 2006). Laccases are able to oxidize, polymerize, or transform diverse recalcitrant substances into less toxic molecules; therefore, these enzymes could be suitable biocatalysts for water bioremediation (Majeau et al. 2010). However, several drawbacks have to be surpassed in order to implement the use of laccases for pollution alleviation, mainly, because of the elevated expenses that a large-scale enzyme production involves (Majeau et al. 2010).

2 Laccase

2.1 Laccase Source

As oxidoreductase enzymes, laccases possess the ability to oxidize diverse phenolic compounds with the concomitant reduction of oxygen (Yaropolov et al. 1994; Morozova et al. 2007a; Madhavi and Lele 2009). These biocatalysts have been widely found as extracellular and intracellular enzymes in several organisms, encompassing microorganisms (*Bacillus*), plants (genus *Rhus*) (Omura 1961), fungi (such as genera *Trametes*, *Cerrena*, *Ganoderma*, *Pycnoporus*, and *Coriolopsis*) (Chaubey and Malhotra 2002; Morozova et al. 2007b; Madhavi and Lele 2009), and insects (*M. sexta*) (Bailey et al. 2004). In insect species, laccases are responsible for the sclerotization process; whereas in *Bacillus* species, they play a role related to pigmentation, pathogenesis, and the assembly of UV-resistant spores (Kramer et al. 2001; Claus 2003). In plants, laccases are found in the xylem and, together with peroxidases, are involved in lignification (Omura 1961); moreover, fungal laccases play a role in delignification (Singh Arora and Kumar Sharma 2010) and humification processes (Morozova et al. 2007b).

2.2 Biocatalytic Mechanism and Applications

In an enzyme, the active site is the spot where the recognition, binding, and oxidation of the substrate take place. The active site of laccases comprises four copper nucleuses, each of them are grouped into three different classes of atoms according to both their distribution in the enzyme (T1, T2, and T3 sites) and their spectroscopic nature (Piontek et al. 2002).

The T1 and T2 sites only possess one copper atom; the two remaining atoms are found in the T3 site. Each type of copper has unique characteristics: type 1 is responsible for the emblematic bluish color of the enzyme, is a hydrophobic cavity where the substrate is oxidized during the catalytic mechanism of the laccase (Durán et al. 2002; Madhavi and Lele 2009); type 2 is colorless since no absorption in the visible region is observed; meanwhile, type 3 exhibits a weak peak at 330 nm (Ba et al. 2013). The reduced form of T1 (resulted from substrate oxidation) donates an electron, which is sent to the T2/T3 trinuclear cluster (formed from T2 and T3 copper atoms), which is where water formation occurs (reduction of oxygen) (Durán et al. 2002; Madhavi and Lele 2009) (Fig. 2.1).

The isoelectric point of laccases is around 4.0, showing their optimal performance at acidic conditions, which has been related to the growth conditions where the microorganisms produced these enzymes (Madhavi and Lele 2009). However, it has been recently reported that laccases maintain high stability (above 60% of its activity) at alkaline conditions (up to pH 8) (Ramírez-Cavazos et al. 2014b). On the other hand, the thermostability, high redox potential, and the ability to oxidize a wide range of substrate (including recalcitrant pollutants) are some other properties that make laccase of particular interest to researchers (Giroud and Minteer 2013; Ramírez-Cavazos et al. 2014b). Nowadays, the use of this enzyme is common in some industries (mostly paper, food, and textile industries) (Morozova et al. 2007b). However, the current trend is the use of laccase as biocatalysts in the bioremediation of polluted waters by emerging pollutants (Almansa et al. 2004; Junghanns et al. 2005); in the generation of energy by bioelectrocatalysis in enzymatic fuel cells (Meredith and Minteer 2012; Giroud and Minteer 2013; Holmberg et al. 2015); and in the development of biosensors using this enzyme as bioreceptor, for food

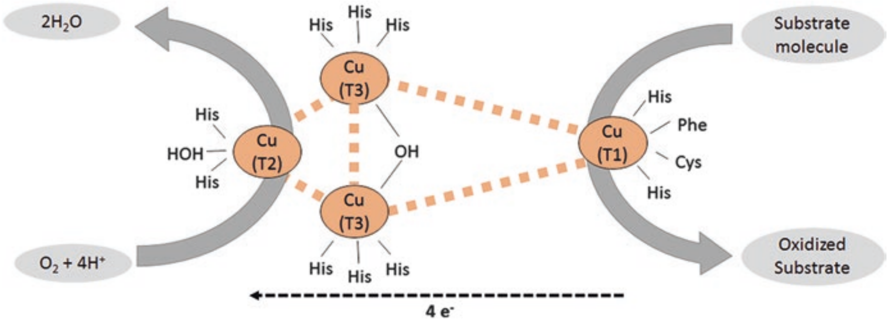

Fig. 2.1 Model of the laccase active site and catalytic cycle

(Ghindilis and Yaropolov 1992; Gamella et al. 2006; Di Fusco et al. 2010), environmental (Torrecilla et al. 2007; Tang et al. 2008), and medical applications (Quan and Shin 2004; Ferreira et al. 2009).

2.3 Laccase Production in Agro-Industrial Residues

Despite the high potential of laccase for biotechnological and industrial purposes, it is important to highlight that a large amount of enzyme is needed for a large-scale process. According to Osma et al. (2011), the culture medium represents the highest cost to the total expenses of laccase production; meanwhile, infrastructure costs are the lowest. Thus, in order to overcome this issue, several studies have been focused on (1) the development of stable genetic modifications of microorganism in order to achieve the gene expression for laccase; (2) optimization and reduction of the costs of culture media using agricultural wastes as cheap growth substrates (also helping to alleviate the environmental pollution); (3) search for new strains of microorganism capable to produce laccase (Bhattacharya et al. 2011; Yang et al. 2012; Theerachat et al. 2012; Nicolini et al. 2013; Ramírez-Cavazos et al. 2014a).

Ramírez-Cavazos et al. (2014a) tested a strain of *Pycnoporus sanguineus*, native from northern Mexico, using a tomato juice as culture medium, resulting in laccase titer of 143,000 U/L. Fenice et al. (2003) obtained a production of 4600 U/L in olive-mill wastewater. Meanwhile, Songulashvili et al. (2007) reported the laccase production using food wastes such as mandarin peelings, wheat bran and soy bran, kiwi fruits, chicken feathers, and ethanol, obtaining a maximum laccase activity of 93,000–97,000 U/L, after the submerged fermentation of wheat bran and soy bran by *Ganoderma* spp. Several agro-wastes such as mandarin and banana peel are substrates rich in lignin carbohydrates and organic acids, which could act as inducers to stimulate laccase production (Osma et al. 2007). In this context, several studies have reported the use of solid supports such as grape seeds (Rodríguez Couto et al. 2006), banana skin (Osma et al. 2007), and groundnut (Couto and Sanromán 2006) as growth substrates for fungi. However, the use of these complex substrates has as drawback the subsequent use/purification of the laccase after fermentation; thus the development of robust protocols that allow the use of crude enzymes (no purification process) in biotechnological applications is required.

3 Laccase-Based Biosensor for Detection of Emerging Pollutants

3.1 Emerging Pollutants in Water Reservoirs

Over the past two decades, the constant presence of emerging pollutants in worldwide water supplies has gained great relevance. This type of pollutants includes a large variety of chemicals used in daily life such as household compounds, personal care products, and drugs for human and animal uses (Daughton and Ternes 1999). Although scientific data have made evident the potential threats of the emerging pollutants to public and ecological health, there is still information that has not been assessed (Horvat et al. 2012) concerning the environmental toxicity, concentration, distribution, and transformation of these compounds in water bodies, which makes their ecological regulation, detection, and treatment very difficult (Deblonde et al. 2011). These pollutants are typically released into the environment via anthropogenic activities such as agriculture practices, industrial, and human discharges (Murray and Ormeci 2012). The emerging pollutants are commonly classified as endocrine disruptors, pharmaceutical compounds, and personal care products (Daughton and Ternes 1999).

The endocrine disruptors are compounds that mimetize the action of hormones in the organisms, causing the alteration of the endocrine system, which has been related to sexual disorders, cancer, and even chronic diseases (Caliman and Gavrilescu 2009; Rezg et al. 2013). Nowadays, the presence of chemicals that exhibit hormone alterations and are involved in the elaboration of plastics and household products has been widely reported, e.g., surfactants, flame retardants, parabens, and plasticizers (Rodriguez-Mozaz et al. 2004). Meanwhile, the chemicals present in personal care products and considered as emerging pollutants encompass disinfectants (triclosan), conservation agents, fragrances (celestolide, tonalide, galaxolide), and UV screens (octyl-dimethyl-PABA, octyl-methoxycinnamte, homosalate) (Caliman and Gavrilescu 2009). On the other hand, the pharmaceutical compounds, as emerging pollutants, encompass human and veterinary drugs that have been widely found in water supplies such as antibiotics, nonsteroidal anti-inflammatory drugs, and beta-blockers (Deblonde et al. 2011). Numerous studies have reported the presence of personal care products, pharmaceutical compounds, and endocrine disruptors in several water supplies at alarming concentrations (Meisenheimer et al. 2002; Mompelat et al. 2009; Einsiedl et al. 2010; Lapworth et al. 2012). Teijon et al. (2010) reported a monitoring survey of pharmaceutical compounds in the water sampled from a WWTP and from the aquifer that is recharged by the discharge of this WWTP, resulting in the detection of these chemicals at concentrations of μg/L.

A large number of analytical protocols have been developed for the detection of these kind of pollutants, mainly by chromatography and spectroscopy techniques, since these methods are extremely accurate and capable to detect very low concentrations; however, they require complicated pretreatment sample, trained personnel, high operating costs, and they lack on-site applicability (Teijon et al. 2010). In this context, the need for portable analytical instruments, capable to field monitoring with high selectivity, sensitivity, and short assay times, has promoted the design of new devices such as biosensors (Marco and Barceló 1996).

A biosensor is an instrument capable of measuring a specific target molecule in a sample, taking advantage of its affinity toward a specific bio-element of recognition (bioreceptors, e.g., immunoreagents, enzymes) (Dzyadevych et al. 2008). There are three essential elements that comprise a biosensor (Fig. 2.2). The bioreceptor interacts specifically with the analyte present in the sample, producing a biochemical

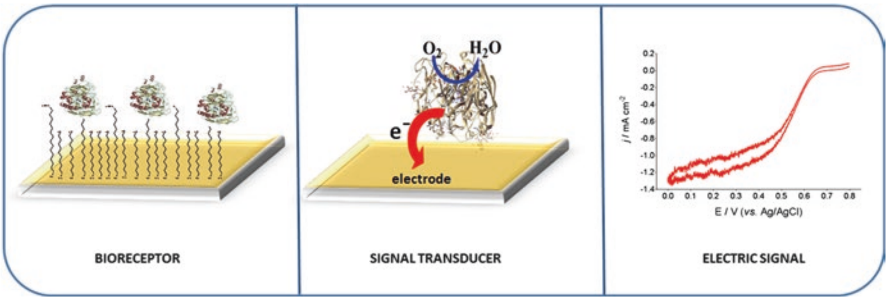

Fig. 2.2 Essential components of a biosensor: bioreceptor, electrochemical transducer, electric pathway in the production of the biosensor response and typical electrical response of the laccase-based electrode biosensors

reaction derived from this interaction, which is amplified and converted into quantifiable electrical signals (Dzyadevych et al. 2008). The transducer is in intimate contact with the bioreceptor and is responsible for the translation of the biochemical event into an electric signal (Dzyadevych et al. 2008).

The use of enzymes as bioreceptors has been widely employed in biosensing due to their stability and easier control of their recognition properties in comparison with other biomolecules such as antibodies or cells (Rogers 2006). In this context, the oxidase enzymes (e.g., tyrosinase, peroxidase, and laccase) appear as good candidates due to their ability to catalyze reactions where electron transference occurs, which can be used as the transduction principle. Laccase enzymes have some benefits over the other oxidases, which make them highly interesting for biosensing applications; their thermostability, versatility to react with a wide range of substrates, no cofactors are needed to perform the catalysis and the formation of water as by-product are some of these advantages (Munteanu et al. 1998).

3.2 Immobilization Methods

In general, an efficient protocol of immobilization is developed to facilitate the biomolecule recovery and reusability. Thus, the immobilization of the bioreceptor would prolong the life of the biosensor and assure its work stability (Liu et al. 2006). The method of immobilization to apply must provide the best conditions to assure the highest stability of the bioreceptor and this will depend on the inherent properties of the recognition element; the operational requirements of the measurement, the target molecule, and the transduction principle are also important factors to take into account in the design of a biosensor (Singh et al. 2008). The immobilization methods most commonly employed for laccase attachment are covalent coupling, adsorption, cross-linking, encapsulation, and entrapment (Fig. 2.3) (Fernández-Fernández et al. 2013).

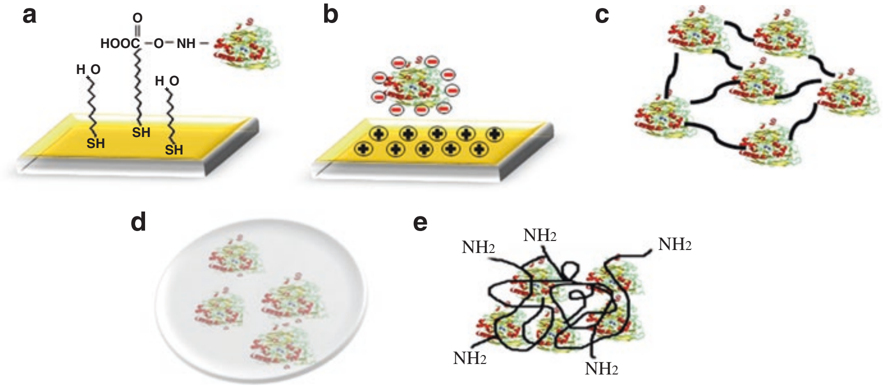

Fig. 2.3 Immobilization methods employed in biosensors. (**a**) Covalent coupling, (**b**) Adsorption, (**c**) Cross-linking, (**d**) Encapsulation, (**e**) Entrapment

The covalent coupling requires the chemical reaction between the functional groups in the carrier with the biomolecule (mainly, through the amino acid residues in their structure) forming covalent bonds; however, it is important to avoid the binding of the enzyme through amino acids within the active site, since that would compromise the biocatalyst activity, resulting in their inhibition (Arroyo 1998). The immobilization via adsorption takes places by weak interactions between the biomolecules and the solid support, e.g., Van der Waals interactions or ionic forces; despite the simplicity and inexpensive requirements of the protocol, this scheme presents the disadvantage of an unsteady fixation of the bioreceptors under pH changes, modification of the polarity or the ionic strength alterations, resulting in the leakage of the molecules (Brady and Jordaan 2009).

The cross-linking involves the generation of intramolecular links within the molecules of the enzyme (Arroyo 1998). Cross-linked enzyme crystals present high catalytic activities and operational stability; however, high quantities of highly purified enzyme are required; under this scheme, it is important to maintain the pH and salt concentration stable (Bryjak et al. 2007). Encapsulation refers to the confinement of the biomolecule within semipermeable spheres made of polymers (Rochefort and Kouisni 2008); meanwhile, entrapment is based on the retention of the enzyme within a polymeric grid; this method assures the integrity of the enzyme structure, however it presents diffusion problems and constant loss of the biomolecules due to differences in the size of the pore in the grid (Ibarra-Escutia et al. 2010).

3.3 Transduction Principles

3.3.1 Electrochemical Transducers in Environmental Applications

Electrochemical transducers refer to the element in a biosensor that translates the transference of electrons occurring during the reaction of an enzyme (immobilized on an electrode) with a substrate (Marco and Barceló 1996; Thévenot et al. 2001).

Laccase enzymes possess mechanical properties and electron transference abilities that make them excellent bioreceptors in electrochemical biosensors (Table 2.1).

The electrochemical transducers in biosensors are able to measure conductometry, potentiometry, and voltammetry principles (Thévenot et al. 2001). Conductometric methods monitor the development of a redox reaction through changes in the conductivity of an electrolytic solution, as a response of the charged products formed by the interaction of the bioreceptor with the analyte (Ronkainen et al. 2010). Potentiometric biosensors measure the changes of potential due to a biochemical reaction occurring between two electrodes (either, reference or indicator electrodes) (Thévenot et al. 2001). Voltammetric measurements are based on the changes of the current during a biochemical reaction on a working electrode; meanwhile, the voltage applied is varied (Chawla et al. 2012). Amperometry is a classification of voltammetry; under this scheme, the potential is maintained constant during the reaction measurement (Thévenot et al. 2001).

Chen et al. (2015) reported the immobilization of laccase in a nanocomposite of gold nanoparticles by a cross-linking method, achieving a direct electron transfer that resulted in a highly sensitive biosensor . Meanwhile, Das et al. (2014) immobilized a *Trametes versicolor* laccase in a nanocomposite matrix comprising of osmium tetroxide on poly 4-vinylpyridine, multiwalled carbon nanotubes; obtaining a current response against pyrocatechol, a genotoxic and mutagenic phenol, in the concentration range of 3.98–16.71 nM and a limit of detection of 2.82 nM with a sensitivity of 3.82 ± 0.31 nA/nM. Vianello et al. (2004) immobilized a *Rigidoporus lignosus* laccase onto a gold carrier obtaining a detection limit of 0.5 mg/L. Tang et al. (2006) reported the immobilization of an hybrid bioreceptor, consisting of horseradish peroxidase and laccase enzymes for the detection of *E. coli* density through the redox reaction of polyphenols generated by the metabolism of the *E. coli*. On the other hand, the development of new nanomaterials has improved the electrochemical analysis performed by biosensors. In this context, Mei et al. (2015) prepared a mixture of palladium and copper to cover a support of graphene oxide in order to build a platform for laccase immobilization; obtaining a detection limit of 2.0 mM for catechol. According to Rodríguez-Delgado et al. (2015), amperometric methods are highly employed in laccase-based biosensors; meanwhile, conductometric and potentiometric methods are not very often employed for this use.

3.3.2 Optical Transducers in Environmental Applications

Optical absorption and fluorescence emission measurements have been extensively used to characterize colored or chromophoric compounds. Based on this concept, the spectroscopic properties of by-products formed by laccase catalysis are employed for the detection of target molecules in optical biosensors (Zoppellaro et al. 2001). Laccase has the ability to produce a reddish dye when catalyzes the coupling oxidation of phenols in presence of 3-methyl-2 benzothiazolinonehydrazone (MBTH) (Setti et al. 1999). Based on this reaction, Abdullah et al. (2007) reported the detection of catechol at concentrations as low as 0.33 mM. The

Table 2.1 Electrochemical biosensors using laccase as bioreceptor for detection of pollutants

Laccase sources	Immobilization method	Working electrode	Target molecule	Sensing parameters	Matrix	References
Coriolus hirsutus	Covalent coupling	Gold	Catechol	Dynamic range 1–400 µM	Synthetic	(Gupta et al. 2003)
				Sensitivity 15 µA/mM		
Cerrena unicolor	Electrodeposition	Platinum	Hydroquinone	Dynamic range 2.0–60 µM	Synthetic	(Jędrychowska et al. 2014)
				Sensitivity 2.34 ± 0.11 µA/mM		
Not reported	Entrapment	Nafion/laccase glassy carbon	Catechol	Dynamic range 0–7 µM	Real samples	(Chen et al. 2015)
Trametes versicolor	Entrapment	Platinum	Phenol	Dynamic range 0.40–6.0 µM	Wastewater	(Timur et al. 2004)
			Catechol	0.20–1.0 µM		
			l-DOPA	2.0–20 µM		
Aspergillus niger			Phenol	Dynamic range 0.40–4.0 µM		
			Catechol	0.4–15 µM		
			l-DOPA	0.4–6.0 µM		
Coriolus versicolor	Covalent coupling	Indium tin oxide	Polyphenolic compounds *of E. coli* metabolism	1.6×10^3 to 1.0×10^7 cells/mL	*E. coli* solutions	(Tang et al. 2006)

(continued)

Table 2.1 (continued)

Laccase sources	Immobilization method	Working electrode	Target molecule	Sensing parameters	Matrix	References
Pleurotus ostreatus	Electrostatic attachment	Indium tin oxide	Phenol	Limit of detection 0.5–4.5 μM	Wastewater	(Kushwah et al. 2011)
			Catechol	0.4–15 μM		
Coriolus hirsutus	Adsorption	Graphite	Catechol hydroquinone	Limit of detection 2 μM	Synthetic	(Yaropolov et al. 1995)
Coriolus versicolor	Entrapment	Glassy carbon/chitosan	Catechol	Dynamic range 1.2–30 μM	Synthetic	(Liu et al. 2006)
				Limit of detection 0.66 μM		
Rigidoporus lignosus	Covalent coupling	Gold	1,4-Hydroquinone	Sensitivity 3 nA/μM	Olive oil wastewater	(Vianello et al. 2004)
Trametes hirsute	Entrapment	Glassy-carbon/ Cetyl ethyl poly(ethyleneimine)/ Nafion	Hydroquinone	Dynamic range 0.1–3.0 μM	Synthetic	(Yaropolov et al. 2005)
Trametes versicolor	Adsorption and covalently coupling	Graphite	Catechol	Dynamic range up to 0.1 mM	Synthetic	(Portaccio et al. 2006)
				Sensitivity 196.0 μA/mM		
Trametes versicolor	Entrapment	Gold	Catechol	Dynamic range 0.67–15.75 μM	Synthetic	(Xu et al. 2010)
Pleurotus ostreatus	Entrapment	Glassy carbon	Phenol	Dynamic range 0.5–4.5 μM	Synthetic	(Kushwah and Bhadauria 2010)

Ganoderma sp. Rckk02	Covalent coupling	Gold with cooper nanoparticles-carboxylated multiwalled carbon nanotubes-polyaniline	Polyphenol/guaiacol	Dynamic range 1–500 µM	Synthetic	(Chawla et al. 2011)
				Sensitivity 0.694 µA μM^{-1} cm^{-2}		
Trametes versicolor	Entrapment	Glassy carbon	Pyrocatechol	Dynamic range 3.98–16.71 nM	Real samples	(Das et al. 2014)
				Sensitivity 3.82 ± 0.31 nA/nM		
Cerrena unicolor	Electrolytic deposition	Platinum	o-Amino phenol catechol phenol	Not reported	Real samples	(Cabaj et al. 2011)
Cerrena unicolor	Entrapment	Platinum	4-tertbutylcatechol	Dynamic range 2–89 µM	Not reported	(Kochana et al. 2008)
			4-methylcatechol	0.21–15 µM		
			3-chlorophenol	0.98–7.9 µM		
			Catechol	0.2–23 µM		

detection was made by spectrophotometric measurements of the reddish compound formed from the coupling of MBTH films (stacked in nafion silicate) to radicals quinone and/or phenoxy produced by laccase enzymatic oxidation (Abdullah et al. 2007). Sanz et al. (2012) studied the reaction of phenol catalyzed by *Trametes versicolor* laccase, using a polyacrylamide film sensor immobilized with the enzyme, obtaining a limit of detection of 0.109 mM.

Surface plasmon resonance (SPR) phenomenon is also an optical principle employed for biosensing, since almost two decades ago. SPR measurements are performed at a fixed angle of incidence and the monitoring is done by detecting changes in the amount of the reflected light, which is correlated to changes in mass on the surface due to binding events (between the bioreceptor and the analyte) (Estevez et al. 2012). In this context, Surwase et al. (2016) reported a first attempt for the detection of phenolic compounds in water by using laccase enzyme as bioreceptor in a SPR device.

4 Laccase as Biocatalyst for Removal of Emerging Pollutants

Several studies have reported the presence of endocrine disruptors, personal care products, and pharmaceutical compounds in diverse water bodies at important concentrations (Snyder et al. 2003; Caliman and Gavrilescu 2009). Thus, the presence of these pollutants in water supplies has become an important issue in terms of treatment technologies for water cleaning, mainly because of the highly resistance of these compounds to common removal techniques. Advanced oxidation techniques based on UV/ozone exposure have obtained efficient yields of removal/inactivation (Esplugas et al. 2007); however, these processes are expensive (Lloret et al. 2012) and in some cases the by-products generated are more toxic than the parent pollutant (Sein et al. 2008). Therefore, laccases appear as strong biocatalysts to be employed in bioremediation treatments, since can react under mild conditions with a broad substrates range, encompassing recalcitrant pollutants, and generate nonharmful by-products. Fukuda et al. (2004) demonstrated the removal of the endocrine activity of the by-products from bisphenol A; catalyzed by *Trametes villosa* laccase (Fukuda et al. 2004). Likewise, Cabana et al. (2007a) reported nonestrogenic activity in the products of reaction by the catalysis of bisphenol A, triclosan, and nonylphenol by employing *Coriolopsis polyzona* laccase.

The removal of emerging pollutants from water supplies using laccase could be achieved under several schemes: (1) free enzyme; (2) immobilized enzyme; and (3) cells from culture broths.

Several studies have reported the use of free laccases for emerging pollutants removal. Yang et al. (2013) reported 27% of diclofenac elimination. The total elimination of estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol (Auriol et al. 2006, 2007, 2008), and oxybenzone (Garcia et al. 2011) was achieved by *Trametes versicolor* laccase. Almost complete removal of 2,4-diclorophenol was observed by free laccase at acidic pH (Jia et al. 2012), temperatures around 30 and 50 °C (Zhang et al. 2008; Gaitan et al. 2011; Qin et al. 2012; Xu et al. 2013), and using elevated concentrations of enzyme (Zhang et al. 2008). Jia et al. (2012) investigated the degradation of 2,4-dichlorophenol by a photocatalytic–enzymatic treatment, achieving 90% within 2 h with the coupled degradation process.

The use of mediators is also common during enzymatic degradations; these compounds are small-size molecules that can extend the ability of an enzyme to react toward noncommon substrates; also are stable and reusable by various cycles (Majeau et al. 2010). ABTS, 1-hydroxy-benzotriazole (HBT), nitroso-2-naphthol-2,6-disulfonic acid (NNDS), Syringaldehyde, 4-Acetylamino-TEMPO 4-hydroxy-TEMPO, Violuric acid (VIO), and p-Coumaric acid are some of the mediators most widely employed for laccase catalysis (Majeau et al. 2010). Ji et al. (2016) reported the elimination of carbamazepine using as mediators p-coumaric acid, syringaldehyde, and acetosyringone, obtaining 60% of degradation after 96 h. Meanwhile, Margot et al. (2015) assessed the potential of laccase to remove sulfamethoxazole and isoproturon with three mediators: ABTS, syringaldehyde, and acetosyringone, showing complete transformation within a few hours. Almost total biotransformation of diclofenac by laccase was also obtained using 1-hydroxybenzotriazole (Nguyen et al. 2013), syringaldehyde, and violuric acid (VA) (Lloret et al. 2010, 2013).

In terms of immobilization, Krastanov (2000) studied the degradation of β-naphtol, observing a complete removal after a hybrid treatment with laccase from *Pyricularia oryzae* and tyrosinase. Lante et al. (2000) immobilized *P. oryzae* laccase on a polyethersulfone membrane, obtaining 18% of β-naphtol removal. Le et al. (2016) provided a novel immobilization technique for laccase on copper alginate for real wastewater treatment, showing 89.6% of triclosan removal after 8 h treatment. Nguyen et al. (2014) reported an enzymatic membrane reactor for the degradation of bisphenol A and diclofenac, obtaining >85% and >60% removal, respectively, by laccase from *Aspergillus oryzae*. Meanwhile, Chen et al. (2016) immobilized laccases on the surface of yeast cells for treatment of bisphenol A (46% removal after 6 h) and sulfamethoxazole (47% removal after 30 h of treatment). Nevertheless, there are just a few works that address the degradation of emerging pollutants in a real matrix and under real reaction conditions (pH, temperature, ionic strength); this is important to consider since some matrix components could decrease the laccase activity and therefore decrease the degradation yield. Rodríguez-Delgado et al. (2016) tested the biotransformation of the micropollutants: diclofenac, 5,7-diiodo-8-hydroxyquinoline, β-naphtol, and 2,4-diclorophenol using laccase from *P. sanguineus* CS43 in groundwater samples, observing a reduced bioconversion for β-naphtol and 2,4-diclorophenol in the real samples in comparison with the synthetic buffer matrix. Biotransformation of bisphenol A, 4-nonylphenol, 17-α-ethynylestradiol, and triclosan were tested in groundwater, as well (Garcia-Morales et al. 2015).

The presence of some ions in a reaction matrix has been reported to induce structural modifications in the active site of the enzyme (Zilly et al. 2011). For instance, halide anions have been related to the interference on the transference of electrons within the active site of the laccase enzyme (Enaud et al. 2011; Margot et al. 2013);

meanwhile, cyanide and calcium provoke the separation of the copper atoms from the enzyme (Cabana et al. 2007b). Kim and Nicell (2006) observed that bisphenol A biodegradation was adversely affected by nitrite, thiosulfate, and cyanide.

5 Future Perspectives and Conclusions

The use of laccases as bioreceptors in biosensors promotes the development of new technologies for environmental monitoring in terms of pollutants screening. However, the immobilization of the bioreceptor is a crucial step in biosensors design, thus more research has to be focused on (1) the creation of new materials for support purposes that diminish the loss of the enzymes once they are immobilized; (2) the improvement of the current immobilization methods to assure an oriented binding of the enzyme in order to safeguard the integrity of the active site, resulting in the recovery of the enzymatic activity once the immobilization was performed; and (3) the use of genetic modifications to extend the laccase stability under difficult catalysis conditions, assuring the recycling of the bioreceptor and thus the lifetime of the biosensor.

The exploitation of laccase enzymes as element of bioremediation opens up enormous possibilities in terms of future treatment technologies for water cleaning. However, unlike biosensor applications, an elevated amount of enzyme is required for bioremediation. Therefore, one of the crucial issues that avoid large-scale use of the laccase is related to the high cost of enzyme production; thus further research should be focused on diminishing the cost that represents the growth mediums for the microorganisms during the laccase production. Furthermore, future research needs to look at (1) the test of the interaction of hybrid treatments in bioremediation, e.g., photocatalytic–enzymatic, enzymatic–enzymatic, whole cell–enzymatic, etc., (2) the study of the structure and toxicity of the by-products formed by laccases catalysis under different reaction conditions, and (3) the implementation a pilot-scale process, where the laccase treatments would be performed under real reaction conditions (pH, temperature, and matrix composition), which consider possible matrix interactions over the catalysis.

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Chapter 3
Biofuels for Sustainable Development: A Global Perspective

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Abstract The quest for sustainable and environment friendly energy sources has become the pressing need of the present. Declining petroleum reserves, worldwide demand for energy, and undesirable effects of greenhouse gas emissions have led to increased worldwide interests in biofuels. This has resulted in the enormous use of biofuels an alternative choice that can link energy security with environment conservation without essentially compromising with the nourishment of the people. Substantial research and development programmes in biofuels for sustainable development have been initiated by many countries. Although sustainable development does not build the world which can be said to be well equipped for the future generations, it establishes a foundation on which the future world can be built upon. Biofuels, the sustainable energy system may be regarded as a cost-effective, trustworthy, and environmental friendly system that efficiently make use of local resources. Biofuels have additionally been lumped into first-, second- and third-generational categories. We use first-generation biofuels in our fuel tanks today. However, the use of biofuels does not imply that its production, conversion and use are sustainable. Biofuels are relatively similar to hydrocarbons and feature some of the similar emission problems like that of standard fossil fuels. If proper care is taken in their production and distribution, they can, nevertheless, be more environmentally friendly. Biofuels are an inexhaustible resource since the stock can be replenished through agriculture. One of the main detractors to the use of biofuels is that setting aside land for biofuel crops means less land for food production. Some foreign countries have said that it is unethical to use crops for biofuel when global hunger is an ever present problem. This chapter emphasizes on the fact that by establishing energy management, the role of renewable energy sources and their modern advances ought to take more importance as a way for contributing energy supply and supporting energy conservation strategy, even though traditional sources, like that of oil, natural gas and coal meet maximum energy demand for the time being. The study concludes with the say that the rise in the use of biofuels at global front is inevitable.

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It thus focuses on the challenge to increase appreciably the production of biofuels by using innovative technologies, which are commercially viable and sustainable.

1 Introduction

Worldwide transportation is responsible for 25% of energy demand and nearly 62% of oil demand; generation of electricity involves single largest consumption of fuel in the world. More than 60% of power generated comes from fossil fuels. Fossil fuels were generated after millions of years after the anaerobic decomposition of prehistoric organic matter buried under the ground. Stocks of fossil fuels are limited, due to the fast consumption of the fuel and due to industrialization, stocks of fossil fuels are depleting very fast. Limited availability has become the reason for its high cost in due course of time. Environmental consequences of fossil fuels and concerns about exhausting petroleum supplies, high oil prices and the need to mitigate the greenhouse effect have spurred the search for renewable transportation biofuels. Biofuels have emerged as an important alternative fuel on world stage. It belongs to renewable category of fuels. The biofuels have environmental benefits, are economically competitive, and can be produced in large quantities.

Biofuels are defined as any hydrocarbon fuel which are obtained from organic matter in a short span of time such as days, weeks or even months from contemporary biological processes like agriculture aerobic digestion, unlike fossil fuels which are obtained by geological process after millions of years. Biofuels may exist in solid, liquid, or gas state. Wood, charcoal, and waste baggasse are solid; ethanol, methanol, propanol, butanol and plants oil are liquid; methane gas and syngas are examples of gaseous biofuels. The production of biofuels requires energy to grow crops and convert them to biofuels. Subsequently, there is increasing interest in the use and production of fuels obtained from plants or organic waste. Biofuels are biomass materials that are directly used as solid fuel or transformed into gaseous or liquid fuels which can be easily stored, so that the harnessed energy can be released through combustion when needed. Biofuels may simply be perceived as liquid or gas transportation fuels derived from biomass. Various biomass raw materials such as energy crops, agricultural residues or forest products can be used to produce biofuels. There is thus a boom in biofuel industries in many developing countries. As the demand for biofuels increases, they have enormous biomass resources which are becoming more valuable. Bioenergy is obtained from these fuels due to the carbon present in them which are generated by carbon fixation process. In this process, atmospheric carbon dioxide is converted into the organic compounds which are found in living organism. Different compounds are produced through this process such as protein, fat and sugar. Any of these may act as source of energy in biofuels. Biofuels are obtained from different sources such as starch feedstock, cellulose feedstock like food crops, wood, sawdust, grasses, waste from domestic and industrial processes, organic part of municipal waste, animal fat and vegetable oil of rapeseed, mustard, soybean, sunflower, palm, jatropha plant, dried manure, waste from farming, animal husbandry, forestry, algae, fungi and different microbes.

Increasing interest has been dedicated to bioconversion of biomass into fuel ethanol, which is considered as an alternative to fossil fuels and the cleanest liquid fuel (Li et al. 2009). Biofuel, on the other hand, is derived from biomass, which can be produced eventually year after year via sustainable farming practices. This indicates biomass and biofuel are renewable. Since last three decades, there has been acceleration in the research on improving production of biofuels for both ecological and economical reasons. The recuperation is primarily for its use as an alternative to petroleum-based fuels. Despite the fact that biofuel can be synthesized by chemical processes such as reacting ethylene with steam (Anuj et al. 2007), biofuel is a renewable energy source produced mostly by the sugar fermentation process (Oyeleke and Jibrin 2009). Biofuels may be considered as hope of energy security that stands as an alternative fuel. They are responsible for providing 2.7% of world fuels which is used for road transport. In 2010, biofuel production was 105 billion litres which was approximately 17% more in comparison to 2009 production level. International energy agency has set the target of 25% of fuels used in transport to be obtained from biofuels by the year 2050 to minimize the dependence on petroleum and coal. In 2011, there was provision of blending of biofuel in 31 countries and 29 states. Bioethanol and biodiesel have maximum consumption in the world. In 2010, the overall world production of bioethanol was 86 billion litres with USA and Brazil as the topmost bioethanol producing countries. Both of these countries generate 90% of bioethanol of the world. Brazil tops the list when we compare the consumption of biofuels in the world; it has a provision of 95% blending of ethanol with gasoline. In other countries, blending with ethanol are found between 10 and 15%, usually blending has to be decided by the performance of engine and exhaust emission with respect to engine load and speed. USA and Australia have provision of 10% ethanol blending in gasoline. India has approved the 5% blending of biofuels in petrol. Ethanol fuel blends are extensively sold in USA. The most common blend comprises of 10% ethanol and 90% petrol (E10). To run on E10 the vehicle engine require no modification and vehicle warranties are also not affected. Only flexible fuel vehicles can run on E85 which has up to 85% ethanol and 15% petrol blends (Tanaka 2006). European Union is the largest producer of biodiesel which accounts for nearly 53% of world production in the year 2010. In France, 8% biodiesel blending can be used in all vehicles using diesel as fuel. Similarly Russia holds 22% of world forest and it is the world leader in the supply of biomass. According to the latest estimate of Grand View Research, it is expected that global market of enzyme may reach $17.5 billion by the year 2024 by the exponential demands of bioethanol (enzymes are used in synthesis) emerging from many developing countries. Extensive research is also being carried out in India on biofuel. Some experts are of the view that vegetable oil blending of linseed, neem and mahua can be tried in diesel engine in different proportion. The rise in the use of biofuels is inevitable. However, biofuel production is questioned from a number of angles. The concern arises on the implications of its emissions, and increased prices of corn that is used for biofuel production, with a particular implication on food security (Wikipedia 2008). Biofuels may in days to come offer a viable substitutes, but “the implications of the use of biofuels for global security as well as for economic, environmental, and public health need to be further evaluated” (EEA 2006).

2 Historical Perspective of Biofuels

Before the use of petroleum products for cooking, heating and transport, biofuels were the first liquid fuels used which included vegetable oils, animal fat, ethanol from crop, etc. (Bailey 1975). However the shift from biofuels to petroleum products (kerosene, gasoline) took place in 1860s. Until the mid-nineteenth century, biofuels were used as primary fuels for cooking. In the twentieth century, biofuels were used for automotive fuels, and in the mid- to late-twentieth century, for internal combustion engines. Various forms of liquid energy derived from renewable plant material have been known and extensively used for many years. In early 1700s, vegetable oils and fats were used to lit the lamps on the main streets in European and American cities (Crosby 2006). The US Congress levied a tax of $2.08 on each gallon of alcohol as a part of the Revenue Act as a payment for the US Civil War in 1862. The tax diminished the competition from biofuels and offered an important boom for the petroleum industry. As a means to promote rural development and national self-sufficiency in the decades before World War I, Germany created the world's first large-scale biofuels industry. The government also encouraged the use of many household appliances like spirit lamps, water heaters, irons and cooking stoves. All these appliances had ethanol as a fuel. By 1902, approximately 95,000 stoves and 37,000 spirit lamps using alcohol as a fuel were manufactured in Germany (Tweedy 1917). At the beginning of the twentieth century, the apparent scarcity of oil resources was the major rationale for British interest in biofuels, and a 1907 British commission noted that "a famine in petrol appears to be inevitable" (Motor Union 1907). The hopefulness about biofuels was so widespread that book entitled "Modern Inventions for students even in 1915" had a chapter entitled "Alcohol Motors and the Fuel of the Future" (Johnson 1915). During the period of 1900–1930s, the Ministry of Agriculture fully supported the French ethanol fuel programme. As a result, biofuel production increased from 2.7 million gallons to 5.7 million gallons in 1900 and 1903 respectively. Eventually, the production grew to 8.3 million in 1905. The main reason was to support French sugar beet markets and to restrict the excessive growth of other crops. The increase in oil imports from the USA and Russia, coupled with the lack of domestic oil deposits, posed another significant challenge. Between the seventeenth and early nineteenth century, number of research scholars made endeavour to construct some type of internal combustion engine (Cummins 1989). In 1826, a U.S. engineer Samuel Morey developed the first internal combustion engine which made use of ethyl alcohol and turpentine (camphene—a volatile fuel), a carburettor and a spark-ignition piston engine. The engine was used to drive a small boat at 8 miles per hour up the Connecticut River. However, only one prototype engine was ever built by Morey as he stayed relatively unknown and was never able to attract financing from any source (Farell 1915; Goodwin and Duryea 1931; Hardenberg 1992). In 1876, the first four-stroke internal combustion automobile engine which used alcohol as the fuel was invented by German inventor

Nicolaus August Otto. Rudolph Diesel displayed his first engine running on peanut oil in 1900. The Ford Model T, which was first made public in 1908, was also designed to run on ethyl alcohol.

Between 1907 and 1909, the U.S. Geological Survey, the U.S. Navy and the U.S. Department of Agriculture performed fuel tests on over 2000 engine and concluded that ethanol has octane rating double that of gasoline's octane rating and thus ethanol could be used in high-compression engine (Lucke and Woodward 1907). In 1920–1930s, most industrial and developing countries produced biofuels, specially by blending ethanol from starch crops or sugar into gasoline. Likewise, ethanol obtained from paper processes, and vegetable oils for diesel engines were also common. During this period, Brazil and Philippines actively participated in the production of biofuels (ethanol from sugarcane). The Brazilian distilleries which produced fuel-grade ethanol increased in number from just 1 in 1933 to 31 by 1939 and to 54 by 1945. The production alcohol used as fuel increased from 100,000 litres in 1933 to 51.5 million litres in 1937. In China, during 1930s a blend of 55% alcohol, 40% benzene and 5% kerosene (Benzolite) was extensively sold (Kovarik 2013). After the war in 1946, in India, nearly eight million litres of alcohol was used, which increased to nine million in 1948. Apart from this, another 20 million litres were used in blends, out of billion litres of gasoline used in 1951. Becoming conscious of possible "food or fuel" conflicts, India prohibited the use of grains and root crops as feedstocks for biofuels. However, in addition it also felt that the power alcohol industry needs to be protected from petroleum interests. Twenty percent blending were feasible as per the 1948 "Indian Alcohol Act" mandated, however, it was not extensively adopted (United Nations 1952).

In the late nineteenth and early twentieth centuries, the low-cost petroleum took the main stage pushing the customary biofuels derived from renewable resources, into niche markets. But in emergency during fuel shortages or surplus agricultural produce, many countries opted to protect biofuel markets through imposing tax policies or by making blending mandatory. In contemporary years, critical question has framed the larger context of energy research, pertaining the impacts of biofuels on food rights, climate change, biodiversity and sustainability.

3 Generations of Biofuels

Biofuels are obtained when conventional gasoline is blended with energy-rich organic compounds such as ethanol, propanol, butanol and ethyl or methyl ester of fatty acids of vegetable oil of rapeseed, mustard, soybean and palm to give bioalcohol and biodiesel. Biofuels are classified into three categories depending on the sources from which these energy-rich organic compounds are obtained.

3.1 First-Generation Biofuels

They are the fuels which are obtained by traditional production techniques. These fuels are obtained from mainly starch/sugar-based feedstocks which are mainly food crops. Generation of fuel is relatively easy and less expensive and commercially viable. Examples of feedstock are sweet sorghum, sugarcane, sugar beet, sweet potato, corn, agriculture waste, by-products of food industry and waste from domestic and municipal sources.

3.2 Second-Generation Biofuels

They are the fuels which are obtained from latest production techniques and they are obtained from cellulose-based feedstocks. Generation of fuels is an expensive and time-consuming process and not commercially viable but are found to be technically viable. Feedstocks are cellulose-based agro, animal husbandry, forestry waste. Cellulose-based biomass is very difficult to convert into biofuels because cellulose is a complex sugar polymer having very high molecular weight; it has crystalline structure due to which it is resistant to hydrolysis. Main components of cellulose feedstock are cellulose, hemicelluloses and lignin. Cellulose accounts for 40–60% and hemicelluloses for 20–40% weight of biomass. Cellulose contains six-membered glucose unit and hemicelluloses contains both five- and six-membered sugar units in their structures. Second-generation fuel technology has made it possible to convert cellulose into biofuel. This process involves two steps. First step is the breakdown of cellulose to smaller sugar units through the hydrolysis step in the presence of acid, and then second step is the fermentation of these small sugar molecules into alcohol by the action of yeast. First step is technically challenging and not commercially viable due to which less ethanol is generated through cellulose-based second-generation biofuels. These cellulose-based biomasses are one of the most abundant materials in nature, and their use also does not threaten the food security. If we can tap this energy potential then huge difference in energy scenario can be expected.

3.3 Third-Generation Biofuels

These fuels are obtained from non-conventional sources such as oil from jatropha plant, algae, fungi and different microbes. Nowadays, research is going on in the direction of using oil from jatropha as biofuel due to the advantages associated with jatropha plant, such as prevention of soil erosion and soil improvement. It is to be noted that the energy-rich organic compounds such as alcohol and oil are obtained by all the three categories mentioned above. For example, ethanol for gasoline blending can be obtained from all the three categories to give bioethanol. Similarly, vegetable oils are obtained from all categories for blending to give biodiesel.

3.4 Advanced Biofuels

The lines between an advanced and a traditional biofuel are blurred in the sense that a fuel that has limited energy density, but can be grown on arid land and have little impact on greenhouse gas emissions is going to be highly valued despite its poor performance.

Lignocellulose: Lignocellulose is a derivative of plant biomass that contains cellulose and lignin. Lignocelluloses can be broken down into ethanol; however, it cannot be effortlessly achieved. Over the years, scientists have developed a number of methods of producing ethanol from lignocelluloses, although the techniques are not particularly cheap. During 2007 the cost of a gallon of ethanol produced from cellulose was roughly U.S. $7/gallon compared to the $1–3/gallon for ethanol produced from corn. The advantage of making use of cellulose for a feedstock is mainly because of the fact that it is generally the leftover, inedible part of crop plants. Paper and other cellulose components make up roughly 70% of all landfill waste, in addition to waste generated from agricultural products. When these wastes decompose, they produce methane gas, which is a warming gas and 21 times more potent than carbon dioxide. Thereby, converting this waste material to ethanol might have a net positive impact on the environment. Finally, lignocelluloses yield about 80% more energy than is consumed in growing the plant and converting it to ethanol. Estimates suggest that there can be 115% reduction in greenhouse gas emission over the long term by converting cellulose into ethanol.

Jatropha-based biofuels: Many research groups in the world are conducting research on *Jatropha curcas*. It is a poisonous tree that produces seeds which are an important source of oil used in biofuel generation. Oil obtained from jatropha tree is used in the blending of biodiesel. Jatropha tree is easily grown in both low and high rainfall areas. It can be very easily cultivated anywhere like canal, roadside, railway tract and found to be suitable for alkaline soil also. Jatropha is found to be helpful in the prevention of soil erosion and improve the quality of soil after cultivation. Indian former president Dr. APJ Abdul Kalam was a strong supporter of Jatropa based biodiesel. Much of the research based on jatropha is based on how to increase the yield of jatropha cultivation through genetic manipulation, soil science and improved horticulture practices.

Algae-based biofuels: Algae are also found to contain the natural oil content. These oil-rich algae after extraction can be processed into fuel and the dried remainder can be further processed into ethanol. Advantage with algae-based fuel is that algae culture does not threaten the food crop and food productions since cultivation of algae neither requires land nor fresh water because algae are grown on waste water pond. Algae are cultivated for 5–8 days and then harvested. Then algae are scraped, dried and then carbohydrates are extracted. The carbohydrate is converted into natural sugars and via fermentation process after the action of enzyme, sugar is converted into butyric, lactic acid and acetic acid. Finally, butyric acid is converted into butanol through fermentation process. This conversion process is less expensive and found to be more efficient. Butanol which is obtained is a better fuel than ethanol in terms of efficiency. This process not only provides better fuel but also helps in the purification of polluted water bodies. Algae takes up the excess of nitrogen and phosphorous (from fertilizers) from the polluted water bodies and makes it suitable for the growth of marine flaura and fauna. Worldwide water bodies are threatened by huge water pollution levels, this algae cultivation technique can make the huge difference.

Fungi-based biofuels: Fungi can play a significant role in the production of biofuels. Recently, group of Russian scientists have claimed to isolate some lipid from single cell fungi, which they claimed that isolated lipid can be converted into biofuel in an economic and very efficient manner. More fruitful research is likely to appear in near future based on this fungi 'Cunninghamella *japonica*'. Several other fungi believed to convert cellulose into biofuels have been isolated. This suggests that in near future fungi-based biofuel generation cannot be ruled out.

4 Types of Biofuels

Biofuels can be classified according to source and type. They may be derived from municipal solid wastes (MSW), municipal sewage, industrial wastes, animals' manures, agricultural crop and forestry residues. In general, they may be classified as solid, liquid and gaseous biofuels. Biofuels can be obtained in different forms and they have quite a number of utilities.

4.1 Solid Biofuels

These are the most common types of fuels. Some of the commonly used solid biofuels are as follows:

Refuse derived fuel: This is the fraction produced after mechanical and thermal treatment from MSW. It mainly contains paper and plastic residues and is chiefly used as a fuel in industrial applications (cement works, etc.).

Briquettes: These are produced from virgin biomass during a thermo-mechanical process. They are used in industrial combustion applications (e.g. drying, steam or hot water production, etc.) and in central heating systems as a fuel.

Pellet: The production of Pellets is chiefly based on thermo-mechanical or physico-chemical method of compaction of finely chopped lingo-cellulosic biomass obtained from forestry or agricultural residues. Pellets have the same applications as that of briquettes.

Wood: This category includes the wood produced from forests as well as the forestry and agricultural residues. The wood and its residues are chiefly used in generation and co-generation of power, industrial heating applications (cement works, etc.), and central heating systems (Petrou and Mihiotis 2007).

Sewage: It is produced from the municipal or industrial sewage cleaning process and used in power generation applications.

Industrial waste: They are by-products of different industrial processes. Classic examples are residues of wood industry, residues of cotton industry (gins), etc. They are used in industrial heating systems and generation or co-generation of power applications.

4.2 Liquid Fuels

4.2.1 Bioethanol

Probably the most common type of biofuels found and used around the world are obtained by the fermentation reaction of microorganisms over sugar. The sugar substrate can be obtained from molasses corn, sugar beets, wheat and various starchy foods. Apart from fermentation, enzyme digestions, distillation and drying are some additional methods used for production of ethanol. It is used as fuel for automobiles and also for heating purposes at homes. Physical properties (in particular density) of bioethanol are similar to gasoline which makes it suitable to blend with gasoline. Bioethanol is obtained by the blending of gasoline with ethanol. Generally ethanol is blended in different proportions; some countries like Brazil allow mixing up to 95% and some have low blending provision of 10–15%. E10 (10% bioethanol and 90% gasoline) and E85 (85% bioethanol and 15% gasoline) are common blends of bioethanol. Brazil is one of the major consumers of bioethanol. Ethanol for bioethanol fuel is made by fermentation of carbohydrates which are found in the sugar or starch-rich crops such as wheat, corn, sugar beet, and sugarcane. Bioethanol obtained by this method is called the first-generation bioethanol. If ethanol is obtained from cellulose-based non-food materials such as agriculture waste, grasses then it will be called the second-generation bioethanol. Ethanol from starch feedstock are obtained in three steps which are fermentation of sugar, distillation of ethanol followed by dehydration process to get 99.5% dry ethanol. But in cellulose feedstock one additional step is required which is the hydrolysis of cellulose into smaller sugar units. Hydrolysis is done in presence of acid or enzyme. Dehydration step is done to get the ethanol which is 99.5% dry and is very suitable for gasoline blending. Fermentation process involves chemical reaction where sugars are converted into ethanol and carbon dioxide in presence of yeast. Ethanol concentration which is attained by fermentation depends upon the type of yeast we are using and operating condition. Bioethanol functions mainly as an additive to improve the emission quality of vehicles. Advantage with ethanol is that it can be blended with gasoline in any proportion. Energy content of ethanol is roughly 65% of that of gasoline. It means large volume of bioethanol will be required to generate the same amount of energy which gasoline generates. But this can be balanced by the enhanced efficiency provided by bioethanol. Bioethanol burns with clean energy because it is oxygenated fuel and show complete combustion, but it is responsible for corrosion in engine and emits significant amount of ozone and thus causes smog. Ethanol is mainly used but propanol and butanol are also used for blending with gasoline to give biopropanol and biobutanol.

4.2.2 Biodiesel

Biodiesel has been popular mainly because of its clean nature of burning which emits very low amount of polluting gases. Biodiesel can be used as a neat fuel or in blends with diesel in such a machine with or without modifications. Biodiesel is not only used in automobiles but it is also used in commercial equipments. Biodiesel can be used as the source of fuel in all the diesel engines that were designed after 1994. Europe is the largest consumer of biodiesel. Biodiesel is the substitute of diesel. Its composition is similar as that of diesel. Chemically biodiesels are ethyl and methyl ester of different fatty acids, these fatty acids are produced by the heating of oil and fat (from oil feedstock) with alcohol in the presence of sodium hydroxide where trans-esterification occurs and biodiesel are formed along with glycerine. Then this biodiesel is blended with diesel, pure oil and fat are not blended with diesel due to fact that they are highly viscous and due to different chemical characteristic and combustion pattern of oil/fat from diesel. Trans-esterification is just the replacement of glycerol which is a trihydric alcohol with another alcohol. Biodiesel feedstock includes oil and fat from animal and vegetable sources such as rapeseed, soyabean, mustard, sunflower, palm, coconut, jatropha, etc., and alcohols. Oil from feedstock is obtained by mechanical press extraction and solvent extraction methods. In mechanical press methods, oil plants are heated at a temperature of 110 °F and then it is pressed and crushed for oil to ooze out of feed stock, but in solvent extraction method oil plant is treated with solvent and oil comes in solvent from which oil is separated by distillation method. Solvent extraction method is costly but oil which is obtained is of very high purity. Pure biodiesel if used alone has an ability to reduce the level of emission by 60% in comparison of what pure diesel emits. It is an oxygenated fuel that means ratio of oxygen is more in comparison of other hydrocarbon fuels so its burning will be much cleaner and combustion of fuel will be complete which will reduce the emission of particulate matters, carbon monoxide, hydrocarbon and sulphur compounds. According to the estimate of World Energy Organization (WEO), ozone forming potential of biodiesel is about 50% less in comparison to diesel. Cancer-causing potential of biodiesel particulate matter is also very less which is about 90% less than that of diesel in accordance with the experiment done by University of California, USA. Biodiesel is biodegradable in comparison to fossil diesel, using biodiesel is less hazardous as it is having a flash point of 148 °C, and on the other hand fossil diesel is having a flash point of 52 °C. Energy content of biodiesel is slightly less than fossil diesel and it causes corrosion. Biodiesel can be used alone or in blend with diesel. Blending of diesel with biodiesel (which are obtained from oil and fat) improves the lubricancy, just 1% blending by biodiesel can improve lubricancy by 65% and this raise the cetane value. But biodiesel is associated with high production cost, more likely to attract moisture and poor low temperature property. The most common blend of biodiesel in Europe is called B20, B2, B1 which are 20%, 2% and 1% blending of diesel with biodiesel. For use of these blending no modification in engine is required.

4.2.3 Bioethers

These types of biofuel can improve the performance of engines when considering fuel/oxygen factor. They can enhance the levels of fuel/oxygen. This factor ensures spontaneous and high temperature burning. Overall, the engine performance can be enhanced as a result of this.

4.3 Gaseous Fuels

Gaseous biofuels are produced during the biomass gasification process that is either a thermal or a microbial degradation of biomass substances.

4.3.1 Biogas

It is the methane gas that is generated when organic matters are digested in presence of microbes called anaerobe in anaerobic condition (in absence of oxygen). Farmers collect the animal dungs and expose it to anaerobic digestion to get biogas. It is a very rich source of methane gas. Main uses include generation of electricity, cooking purpose, water heating, etc. Entire organic biodegradable substances like wood and paper that are collected from the waste bin can be used as the substrate for anaerobic digestion. All such apparent refuse materials can actually produce useful biogas.

4.3.2 Syngas

Syngas can also be used as a source of fuel in a number of equipments such as turbines, diesel engines, and combustible engines. It is the outcome of partial combustion of biomass and it includes gases like hydrogen and carbon monoxide. It is a mixture of carbon monoxide gas, carbon dioxide gas, hydrogen gas and other hydrocarbons which are produced by the incomplete combustion of biomass (in the presence of insufficient oxygen). Syngas can be converted into diesel substitute through the Fischer Tropsch process. It is also used in generation of electricity and in the synthesis of hydrogen gas and methanol.

5 Advantages and Disadvantages of Biofuels

5.1 Advantages

The advantages of biofuels are that they aid in reduction of the emissions of gases responsible for producing the greenhouse effect, chiefly CO_2 emissions (Finco 2012). The organisms from which biomass comes, during their lives absorb CO_2 equivalent to the amount emitted after biomass is burned. This shows that there is 100% renewal of biomass used and it absorb equal amounts of CO_2. In some of the cases, biofuels through biogas combustion can certainly contribute towards regional development and sustainability, like that in the case of cogeneration power systems. Two of the best indicative cases are that of the power generation systems in India (Mukhopadhyay 2004) and Cuba (Isla de Juventud). These units are operated with local biomass and thus contribute to development of the local communities and their power supply systems. Used biomasses are reliable alternatives to fossil fuels, such as kerosene and diesel. The research studies in India show that 80% of biofuel users noted an economic benefit from the system of greater than \$1/month, when 40% of the population of this area had a monthly income of \$20. In any case, biofuels' contribution to local development and sustainability depends upon the type of biomass used.

5.2 Disadvantages

The land use changes and intensification of cultivation following the increased mass production of biofuels such as biodiesel and bioethanol may cause new green house gas emissions and affect the biodiversity, the soil quality, and the natural resources (Finco 2012; Perimenis et al. 2011). The emission of CO_2 captured in biomass and the soil into the atmosphere takes place as a result of the deforestation or clearing of grasslands for biomass cultivation. The most severe problem regarding use of biofuels' is the increase of food market prices. Because of the increased use of cultivation land for biomass' production, there is a decrease in food production. In addition, food prices rise because farmers prefer to produce products with certain prices. A typical example of the problem regarding food prices was the corn price in the USA which was used extensively for production of ethanol. Likewise, Europe is another example of the continuous rise of prices of agricultural produce (Greece). In 2006–2007, soy was sold for 180 euros/ton and corn for 170 euros/ton to chicken egg producers in Greece. In 2007–2008, the prices increased by approximately 60% to 300 and 270 euros/ton, respectively. This however, led to increase in price of eggs. As a consequence of such market's behaviour, demand for biomass, which is conventionally used as a raw material in various industrial sectors (such as paper and wood industry), increases and causes a consequent rise in its price. Biofuels also cause environmental impact, such as acidification and ozone layer depletion. Some another disadvantages pertaining to the supply chain of solid biofuels production are dioxin emissions and heavy metals (Pb, Hg, etc.). This problem is principally associated with Refuse Derived Fuel and constitutes a conflict of interest among the various stakeholders. Energy plants in which many pesticides and fertilizers are used are cultured in an intensive way. This cause eutrophication and eco-toxicity due to the contamination of surface waters. More likely biofuel attracts moisture and are less suitable for use in low temperatures.

6 Compatibility of Biofuels with Existing Infrastructure

Many developing nations lack the transportation, institutional regulatory and service infrastructure to support biofuel industry. Unlike traditional fuels, better-quality advanced biofuels must be compatible with infrastructure of existing fuel, distribution systems and engines. High cost must not be incurred for alterations of tanks, pipelines, pumps or ships. As such, compared to other solutions, advanced biofuels should be a cost-effective alternative in electric or gas-fuelled vehicles. The biofuels available in the market depend on the speed of advancement in technology and nature and will require growth and potential modifications in the modes of transportation and distribution of liquid fuels. Nevertheless, it is unlikely that the investments will precede investments in biofuels production for the reason that development banks will not tender financing unless demand for the product (course of technological innovation to commercialization) is clearly identifiable.

Present technologies make it viable to the transport sector to provide first-generation biodiesel, ethanol or biogas to be blended with fossil fuels. By 2020, second-generation biofuels derived from lignocelluloses which are under development should be available in the market. Research is going on for the improvement of their conversion efficiency. A tenfold increase compared with current cost-effective capacities might cause them to be highly competitive. Within bioenergy policies, transportation field is fast-developing and represents a main source of anthropogenic greenhouse gas emissions; importance has been placed on biofuels for transportation. Biofuel combustion emits less greenhouse gases throughout their life cycle as compared to fossil fuels, considering that part of the emitted CO_2 returns to the atmosphere where it is fixed from by photosynthesis in the first place. However, to assess the compatibility of biofuels with the existing infrastructure it is important to explore the following:

Scalability: To meet the increasing worldwide demand for energy there is a surplus need of millions of tons of biomass annually for manufacturers of biofuels. Eventually to produce enough fuel there will be a need for evaluation of technologies to produce advanced biofuels that could use the existing manufacturing infrastructure. However, discovering the best option may take much time as many a times the technology that is viable in the laboratory often cannot be successfully implemented to economic commercial production.

Sustainability: The advanced techniques for manufacturing advanced biofuels must be quite sustainable. For this there is a need to understand the impact of production and use of biofuels on environment and socio-economic aspects related to land, water and biomass use.

Cost: One of the pressing needs is to have an economical large-scale production of biofuels. The manufacturers producing biofuels from biomass need to cut down the costs of cultivating, harvesting and transporting biomass. To enable rapid acceptance in market, the foremost requirement for the advanced biofuels must be its compatibility with existing facilities without demanding modification in the vehicles and secondly it must meet consumer expectations for both price and performance as well. For example if we consider the utilization of ethanol as motor fuel, a frequent argument that arises is that it reduces dependence on oil imports. There are concerns on additional issues involving ethanol distribution and infrastructure. Ethanol-blended gasoline has a tendency to split in pipelines. Additionally, ethanol is corrosive and has the tendency to damage existing pipelines. Therefore, there is a limitation that, pure ethanol and ethanol blended gasoline cannot be transported by pipeline. Thereby, the existing distribution system for ethanol is dependent on rail cars, tanker trucks, and barges. However, these transport modes lead to higher prices than pipeline transport. Thus there is a potential need to replace all susceptible pipeline components with, hard components, which are likely to be expensive, and could further increase ethanol transportation costs.

7 Performance and Emission Characterization of Biofuels

Many researchers have evaluated various performance parameters like thermal efficiency, specific fuel consumption, power output of compression ignition engine using biodiesel and blends of biodiesel and petro-diesel as fuels. The studies have found 20–60% emission reductions of CO_{2eq} on the utilization of first-generation biofuels as compared to fossil fuels. Whereas, the expected reductions for potential future commercialized second-generation biofuels are in the range of 70–90% of CO_{2eq} compared to fossil fuels (FAO 2008). Due to the variety of feedstock and conversion processes used, and the diverse sites of production and consumption, the first-generation biofuels show large range of emission reductions. Many research studies have reported on biofuel/biomass burning contributions to ambient air in China (Zhang et al. 2008, 2010; Sang et al. 2011). Emissions due to biofuel are very significant and have substantial impact on regional air quality in Asia. The study conducted by Streets et al. (2003) found that the high biofuel emission regions were mainly located in central and East China, and Southern and Southeast Asia. It also estimated that in Asia, the major biofuel emissions occurred from the combustion of agricultural waste, woods, and animal waste (dung). The average annual biofuel consumption from both anthropogenic and natural sources in Asia was estimated to be 730 Tg, with forest burning, crop residue open burning and grassland/savanna burning accounting for 45, 34 and 20%, respectively. However, regionally, forest fires in Southeast Asia dominated. Of the total biofuel consumption in Asia, the contribution to emissions in different countries were found to be China 25%, India 18%, Indonesia 13%, and Myanmar 8%.

Rachel Evangelene Tulip and Radha (2013) investigated the performance and emission characterization of internal combustion engine. The results of the study showed increase in CO emission of B20 than diesel, while B40 and B60 were found to reduce significantly. The smoke and CO_2 emissions were estimated to be to some extent lesser than diesel fuel. In all the blends of mustard biodiesel, an increase in NOx emissions was found as compared to diesel. The emission increases with the increase in blend ratio. Due to the higher cetane number of the biodiesel hydrocarbon emissions reduced significantly. Due to the enough oxygen content the brake thermal efficiency of B20 to some extent was higher than diesel. B40 and B60 were found to be inferior to B20.

The performance and emission characteristics of Neem oil biodiesel blends (NOME-diesel blend) in a DI engine were investigated by Nabi et al. They reported that in comparison to conventional diesel fuel, the NOME-diesel blend showed reduction in emissions as well as smoke and CO, while NOx emission was found to increase with diesel-NOME blends. With EGR 15% NOME-diesel blend showed lower NOx emissions in comparison to mineral diesel (Nabi et al. 2006). Avinash and Atul studied the effect of NOME-diesel blend on performance and emissions of the four-stroke, constant-speed, single cylinder, water-cooled, direct injection diesel engine (http://www.iitk.ac.in/erl/Index_files/Manuscript_IITK.pdf). The study found that with increasing load carbon monoxide emissions also increased. More carbon monoxide is produced due to lack of oxygen at higher load wherein richer fuel–air mixture is burned. At lower loads, carbon monoxide emissions for biodiesel blends were somewhat same to that of mineral diesel. The biodiesel blends showed considerable reduction in carbon monoxide emission at higher load. Increase in the NO emission was found for the biodiesel fuelled engines in comparison with mineral diesel. At all loads, the smoke opacity for biodiesel blend fuelled engines was found to be lower than mineral diesel.

Subbaiah et al. (2010) investigated the overall proficiency along with exhaust emission characteristics of a direct injection diesel engine which used traditional diesel fuel, rice bran oil biodiesel, a mix of rice bran oil biodiesel and diesel and three blends of diesel-biodiesel-ethanol as fuel over the whole range of load on the engine. The investigational outcome showed the maximum brake thermal efficiency when 15% ethanol in diesel-biodiesel-ethanol blends was used. With the increase in ethanol percentage of diesel-biodiesel-ethanol blends, the temperature of exhaust gas and the intensity of sound from the engine reduced. With more percentage of ethanol in blends of diesel-biodiesel-ethanol, the smoke and carbon monoxide emissions reduced significantly. The unused oxygen with 5% ethanol in blend of diesel-biodiesel-ethanol was found to be lower as compared to that of conventional diesel fuel. With the increase in ethanol percentage of diesel-biodiesel-ethanol blends, the NOx, HCs, and CO_2 emissions were found to increase but the hydrocarbon emissions were lesser than in the case of diesel fuel. The study was conducted to explore the impact of mixture of rice bran oil biodiesel and diesel and three blends of diesel-biodiesel-ethanol fuel. The brake thermal efficiency was found to amplify whereas, the emissions of CO, hydrocarbons decreased. Likewise, the sound and smoke emitted by it also decreased. Thus to improve the performance and reduce the emissions of a diesel engine, the rice bran oil biodiesel can be used as an additive to mix higher percentages of ethanol in diesel-ethanol blends.

Ozer Can et al. (2004) examined the effects of ethanol addition on the performance and emissions of a four-stroke cycle, turbocharged indirect injection diesel engine at varying fuel injection pressures. The study showed reduction in CO, soot and SO_2 emissions, with increased NOx emissions after the ethanol addition. Another study reported slight increase in the emissions of carbonyls and NOx with considerable reduction in the emissions of particulate matter and tetrahydrocannabinol with the use of biodiesel ethanol-diesel blends (Xiaobing Pang et al. 2006). Srikanth et al. (2013) investigated experimentally the performance and emission characteristics of a single cylinder diesel engine fuelled with diesel fuel, fish oil biodiesel and diesel-biodiesel-bioethanol blends. The results showed maximum thermal efficiency of 13.22% greater than conventional diesel fuel and 10.11% greater than fish oil biodiesel. With the blend of 75% diesel, 10% biodiesel and 15% bioethanol (DE15B10), at maximum load, the CO emissions were found to reduce by 50% than the conventional diesel fuel. In diesel-biodiesel-ethanol blends, the hydrocarbon emissions and smoke opacity were found to increase with increasing ethanol percentage, whereas the emissions of oxides of nitrogen decreased to a considerable extent.

8 Impact of Biofuels: Environmental Benefits

For more than a century, ethanol has been used as biofuels, however, its environmental benefits have attracted worldwide interest since last 2–3 decades. Many nations have produced biofuel with limited destruction to the environment. The old methods of traditionally burning the fields before harvest, so as to fertilize the fields with ash and remove the leaves, have been eliminated as a result of advancement in fertilizers and natural pesticide. Although environment pollution due to smoke is avoided, this may however, lead to environment pollution because of runoff from fields. Some researchers have pointed out that nitrogen flow in lakes, streams and other water bodies may increase as a result of increase in biofuels production. Biofuel is an eco-friendly fuel (El Diwani et al. 2009). Although biomass biofuel may not be a complete solution to waste management and prevention of climate change, it certainly contributes towards it. In addition, it also contributes towards fuel security.

Studies have suggested that significant reductions in CO_2 emissions may occur due to increased use of biodiesel and ethanol-blend fuel compared to conventional gasoline. According to Forge (2007), although Green House Gas (GHG) emissions over the full ethanol life-cycle may vary dramatically depending upon the energy sources used to produce the ethanol, analyses of GHG emissions showed that biofuels produce fewer emissions than fossil fuels. In Canada, manufacturing plants where corn and wheat are used to produce ethanol are fuelled by natural gas and thus are responsible for fewer GHG emissions as compared to American plants which are fuelled by coal or other fossil fuels in manufacturing ethanol. Thus environmental benefits of using ethanol compared with gasoline show significant variations. At present times in Canada, if 5% of conventional fuels are replaced with biofuels, it may relatively have negligible impact on reducing GHG emissions across the country. However, if 10% of the fuel would utilize corn-based Ethanol (E-10 blend) in all vehicles, GHG emissions would lower by approximately 1%. Forge (2007) thus pointed out that in order to have a real impact on a Canada's total GHG emissions, there is a need to target certain types of biofuels, such as biodiesel and cellulose-based ethanol.

9 Global Perspective

Biofuels are most commonly used globally, for transportation and cooking stoves. However, research is going on for developing environmentally sound techniques that would minimize the waste disposal problem. With the aid of imaging technology, researchers from Washington University in April 2008 have developed out a way to convert manure into biomass. They found that continuous and vigorous mixing aids microorganisms convert agricultural waste into alternative form of energy, thereby giving farmers a simplified way to manage their waste (Business Line 2005). Agriculture is reported to be the new energy sector due to the increasing use of bio-refineries mainly in the USA, the European Union (EU) and in the BICS (Brazil; India, China and South Africa) group (Le Monde 2007).

Under the new EU level proposals (European Commission 2012) two restrictions were introduced which included i) limiting food/agricultural based biofuels of first generation to 5%, and ii) support conditions. After 2020 only those biofuels would receive financial help which contribute to considerable GHG savings and would not produce from crops generally used as food/feed. However, by focusing on the development of the second- and third-generation of biofuels (like lignocellulosic ethanol, Bio-SNG, synthetic biofuels) this problem may be solved. These biofuels will not compete with food production and will use feedstock including lignocellulosic material, waste and residues (Perimenis et al. 2011) or stimulate production of algae origin biodiesel (Harvey and Pilgrim 2011). However, Harvey and Piligrim (Harvey and Pilgrim 2011) emphasized that unlike Brazil and the USA, for the development of the second-generation biodiesel, such as biomass to liquid (necessary to meet the more stringent GHG reduction requirements from 2017) Europe has not seen larger state-of-art innovation programme. The second-generation biofuels, produced from wastes and agricultural residues, offer a cost-effective advantage apart from various advantages (Lee and Lavoie 2013). Besides, first-generation biofuels produced from organic waste are generally considered as sustainable, since they do not have significantly effects on the land-use, food prices, etc. (European Biofuels Technological Platform 2009).

The third-generation biofuels which are produced from cyanobacteria and microalgae have potential advantages of high biomass productivity and the ability to grow in cultivation ponds over biofuels which are produced from plants (National Academies Press 2013). Latvian researchers also carried out studies in this field (Sondors 2013). However, some of them argued that along with potential environmental and social advantages, algal biofuels could result in negative environmental and detrimental effects in addition to significant resource inputs, which usually is true for all types of energy production (Georgianna and Mayfield 2012). In spite of this, algae are almost recognized to be ideal organisms for developing the very productive and robust crop strains that are essential for cost-effective and feasible production of biofuel (National Academies Press 2013). As a whole, biofuels derived from biomass are not the best way for storing solar energy, since the stored solar energy for biodiesel produced from rapeseed is less than 0.1%, for bioethanol it is less than 0.2%, and for biogas it is around 0.3% (Georgianna and Mayfield 2012).

U.S.A.: In US basically the ethanol fuel is produced from corn. Corn is a very energy demanding crop, which creates only 0.9–1.3 energy units of ethanol on utilization of one unit of fossil-fuel energy. As per the 2007-12-19 U.S. Energy Independence and Security Act, 2007, American fuel producers have to use minimum of 36 billion gallons of biofuel by 2022. This is almost five times increase greater than existing levels. There has been decrease in the export of food which has caused increase in the grain prices all over the globe. In 2012 half of the production cars of General motors were capable of running efficiently using E85 fuel. Two new plants for the ethanol fuel were built at Coskata Inc. Theoretically, the process using E85 fuel has been claimed to be five times more efficient in energy as compared to corn-based ethanol, but it is in development stage and has not been proven to be cost-effective in a free market. The greenhouse gas emissions were reduced by 86% on using cellulose-based ethanol as compared to 29% reduction using corns (Wikipedia 2008).

Brazil: Ethanol fuel is available throughout Brazil. It is the second largest producer and exporter of ethanol in the world. The world's first sustainable biofuels economy and the biofuel industry leader is considered to be in Brazil (Reuters 2008). Brazil along with the US account for 70% total ethanol production of the world (Sanchez 2007) and nearly 90% of ethanol is used for fuel (World Bank 2008) which lead the industrialized world in global production of ethanol. In 2006 Brazil produced 16.3 billion litres of ethanol which represents 33.3% of the world's total production and 42% of the world's ethanol was used as fuel (World Bank 2008). In 2003, the country's ethanol programme offered employment to nearly 700,000 persons. Brazil cut down import of oil by a cumulative undiscounted total (US$50 billion) in 1975–2002 (Lovins 2005). The production of ethanol is largely intensified in the Central and Southeast regions of the country, including the main producer, São Paulo State. These two regions were solely responsible for nearly 90% of Brazil's ethanol production in 2004 (Macedo et al. 2004). The government statistics

Brazil produced around 17.471 billion litres of ethanol in 2006, 23 billion litres in 2007 and in 2008, the Companhia Nacional de Abastecimento (Conab), expected a production growth of 14.97% and 19.46%, bringing the total ethanol production ranging from 26.45 to 27.9 billion litres (Wikipedia 2008).

China: The government has made E10 blends mandatory in five provinces of China that have 16% of the nation's passenger cars. In Southeast Asia in 2007, Thailand also made usage of 10% ethanol mix in gasoline mandatory. In Malaysia and Indonesia likewise, the palm oil industry planned to supply an increasing segment of national diesel fuel requirements. In Canada, the government aimed for 45% of the country's gasoline consumption to contain 10% ethanol by 2010 (Wikipedia 2008).

India: In line with the Bioethanol programme, presently E5 blends are efficiently used in most parts of the country, eventually, targeting to extend the requirement to E10 and then E20. Jatropha oil is produced from the seeds of the *Jatropha curcas* plant that grow in wastelands throughout the country. Its oil is an excellent source of biodiesel. India is very much interested in reducing its reliability on coal and petroleum. However, to meet the ever increasing energy demand and encouraging Jatropha cultivation was a crucial part of India's energy policy. Large plots of waste land were selected for Jatropha cultivation and were supposed to provide much needed employment to the poor (CJP 2006).

Colombia: Colombia made it mandatory to use 10% ethanol in all sold conventional fuels in cities with populations exceeding 500,000. In Venezuela, the state oil company supported the construction of 15 sugar cane distilleries, as the government made a E10 (10% ethanol) blending mandatory (Wikipedia 2008).

Europe: The European Union in its biofuels directive, 2006 had set the target that by 2010 each member state should try to achieve a minimum of 5.75% biofuel utilization of used traffic fuel. By 2020 the figure is expected to be 10%.

France: Among the EU States, France is the second largest consumer of biofuel. According to the Ministry of Industry, France's consumption has increased by 62.7% of which the biodiesel represents the biggest share (78%), far ahead of bioethanol with 22%.

Germany: Germany is the largest European biofuel consumer, with a consumption estimate of 2.8 million tons of biodiesel, 0.71 million ton of vegetable oil and 0.48 million ton of bioethanol (Wikipedia 2008). The biggest biodiesel German company is ADM Ölmühle Hamburg AG, which is a subsidiary of the American group Archer Daniels Midland Company.MUW (Mitteldeutsche Umesterungswerke GmbH & Co KG) and EOP Biodiesel AG are the major producers of biofuel. The German sugar corporation, Südzucker is a biggest challenger in terms of bioethanol production.

Spain: Abengoa, the Spanish group, via its American subsidiary Abengoa Bioenergy, is the European leader in the production of bioethanol (Wikipedia 2008).

Sweden: In 2005, oil phase-out in Sweden by 2020 was announced by the government. Also Stockholm introduced a fleet of hybrid ethanol-electric Swedish buses (Wikipedia 2008).The Sweden government had initiated the work to end up oil dependency. One-fifth of cars in Stockholm specifically run on ethanol fuels.

United Kingdom: In 2005, the Renewable Transport Fuel Obligation made an announcement regarding renewal of 5% road vehicle fuel. In 2008, the Royal Society report stated that biofuels were failing to reduce GHG emissions significantly. This would lead to environmental damage. The only solution to it was to put proper Government policies in proper place (Wikipedia 2008).

10 Biofuels Towards Sustainable Development

Biofuels development on large-scale regarding food versus fuel has raised many environmental concerns in respect to deforestation, water scarcity, water pollution. It also has added impact on socio-economic considerations. Considering these impacts, there is a need for developing sustainable criteria and certification of standards for biofuels production and trade. A detailed assessment of the costs and advantages of different biofuels used in transport has been given by Zah et al. (2007). However, more such studies on these aspects are essential for assessing different biofuels. Because of the growing competition for land and use of biomass, the potentials for energy crops are very limited. As some of the biofuels are very costly compared to other alternatives, some have negative impact on the environment, all biofuels used for transportation cannot be considered as being sustainable. Nonetheless, there is a large prospect for biofuels for transportation which must be developed in accordance with other objectives for achieving sustainability in a way by alternative use of biomass for heat and electricity generation.

With the use of integrating technologies, the energy and environmental issues can be overcome with the help of the second-generation biofuels (e.g. sustainable and profitable biodiesel produced from microalgae). In combination with photonics and biotechnologies, large quantities of algal biomass required for biodiesel production could be grown in photo-bioreactors. Some research studies have pointed out that the direct hydrothermal liquefaction technique is quite energy proficient in production of biodiesel from algae without much requirement to lower the water content of the algal biomass. However, more research in this area and accurate economic assessments of biofuel production is essential. The integrated biomass-production conversion system includes microalgal production at thermal power plants (thermal power plants used for treatment of wastewater and emission control, CO_2-sequesteration, integration of an internal heat exchanger network and utilization of high pressure and high temperatures from the conversion reactor for power generation). The residual biomass obtained from oil extraction could be used partially as source of small amounts of other high-value microalgal products, and as high protein animal feed. Microalgal oil has the prospective of chemically converting it into kerosene, which is the vital component of jet fuel. Jet fuel in present times accounts for nearly 8% of the total petroleum use with not many renewable alternatives. Microalgal biofuels are more advantageous over ethanol biofuel and biodiesel. Ethanol is not dense enough with only one half the energy per volume of the jet fuel. Biodiesel although has about 80% the energy density of kerosene, but solidifies at the low temperatures at high altitude flights. Thus, microalgal biofuels are a sustainable energy resource, however, the challenge will be the economics of production. Some companies have already started with the commercial-scale production of microalgal biofuels.

11 Conclusion

The main advantages of using biodiesel are its renewability, better quality exhaust gas emission, its biodegradability and the organic carbon present in it which is photosynthetic in origin. It does not contribute to a rise in the level of carbon dioxide in the atmosphere and consequently to the green house effect. Thus, if biofuels production is to create economic growth without jeopardizing our natural capital and social equity, it must be developed in the framework of well thought-out national policy. Accordingly, it is incumbent on each country to define its own agenda, taking advantage of the demand from the developed countries as a means of solving their own problems and creating new opportunities for sustainable rural development. There is likely room in the marketplace for all biofuel generations, with each broadening the feedstock and technology options and improving fuel performance. The challenge is to increase substantially the production of biofuels by using innovative processes and technologies, which are commercially viable. The key challenge for developing the next generations of biofuels is acquiring economical feedstock which contributes 80–90% of the final fuel price for most processes and is critical to the economic viability of future generations of biofuels. Regulatory support is also required to enable the commercialization of innovative bioenergy technologies. Given that biofuel production is inevitably increasing, a comprehensive assessment of the environmental impacts of biofuel production, and the identification of measures to reduce these impacts, is required at local to regional scales.

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Chapter 4
Greening the Indian Transport Sector: Role of Biodiesel

Dipesh Kumar, Bhaskar Singh, and Kuldeep Bauddh

Abstract India being the world's fastest growing major economy requires to substantially boost up the availability of energy for sustaining high rate of economic growth. Critical dependence on imported forms of fossil energy can be detrimental in the long run. With around 51% of the total demand for petroleum products, the transportation sector is likely to be among the most vulnerable sectors to petroleum supply shocks. In order to improve energy security and also to reduce the emission intensity of GDP, indigenously developed renewables are urgently required. Considering the quantum of diesel use in India and associated environmental degradation, biodiesel appears to be a very promising alternative which can be used and distributed by utilizing existing infrastructure. The Government has taken serious note of the situation and has introduced policies for developement and use of biodiesel. However, the biodiesel industry in India is still in its infancy requiring careful examination and addressing of policy lacunae.

1 Introduction

The world is confronted with twin challenges of growing energy demand for sustained and accelerated economic growth and climate change along with environmental degradation that arise out of traditional practices of energy production and use, i.e. combustion of fossil fuels (Edenhofer et al. 2011, McMichael et al. 2007). The problems associated with use of fossil fuels have been discussed at length (Lewis and Nocera 2006). Some of the major problems include: limited availability, spatially variable distribution, emission of GHGs, and degrading ambient air quality (Dresselhaus and Thomas 2001).

India has attracted significant market attention as a country, having very high prospects of rampant economic growth and having maintained the rate of growth at around 7% per annum since 2000 despite global economic slowdown

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(Binswanger-Mkhize 2013). The projected rate of growth is even higher but the availability of energy for driving the wheels of economy can potentially become a bottleneck in the unimpeded growth of the country (Gunatilake et al. 2014). India is currently the largest consumer of commercial energy (Chontanawat et al. 2008) and projections estimate an increase in India's primary energy demand to around 1469 MTOE by 2030 (De Fraiture et al. 2008). Oil is the second largest source of energy in India as it accounts for roughly 30.5% of the total primary production and there has been a continuous surge in demand for oil at a CAGR of around 6.12%, while at the same time demand for coal (accounts for roughly 51.8% of the total production) is witnessing a decreasing trend (MoPNG 2015).

Absence of required transportation-related infrastructure could possibly become one of the major nemeses for the growth of the country (EBTC 2013). Transportation system is one of the vital organs of an economy as it facilitates trade of goods between regions and motorized mobility of people across regions. Well-developed transportation system is often used as an indicator of economic well being of the society. In addition to personal level communications, transportation system provides access to a wide diversity of social and economic services (Prideaux 2000). Being one of the significant contributors to the GDP, the automotive sector is also one of the largest consumers of energy. The share of automotive industry in GDP is likely to grow up to 12% from the current contribution of 6.7% by 2026 (EBTC 2013). More than 25 million people were employed by the automotive industry in the past decade and it is further likely to create ten million additional jobs by 2022.

India is having one of the world's largest transportation infrastructures and it is heavily dominated by road transport. The vastness and dominance of the road transport infrastructure can be gauged by the presence of an extensive road network of over 47 lakh kilometres responsible for over 80% of passenger and over 65% of freight movement (EBTC 2013). In the past few decades, there has been a tremendous surge in vehicle ownership (Fig. 4.1) (Planning Commission of India 2003a).

Indian transport sector, driven primarily by road transport and powered almost exclusively by internal combustion engine technology, is heavily dependent on availability and affordability of oil which overwhelmingly powers around 95% of vehicles. Interestingly, out of the total demand for crude oil in India (227 MMT) during 2013–2014 around 83% (189.24 MMT) of the demand was met by imported crude oil (Ministry of Statistics and Program Implementation 2015). Ninety-five per cent of the vehicles plying on roads are powered by internal combustion engine employing either diesel (high-speed diesel) or petrol as fuel. The extent of dependency of Indian transport sector on diesel and petrol can be fathomed from the fact that 99.6% of petrol and over 70% of the total diesel demand during 2013–2014 stemmed from transport sector (MoPNG 2015). The demand for diesel is substantially (c.a. four times) higher than that for petrol as diesel has several other sectoral demands (e.g. railways, agriculture, industries, electricity, etc.). The extensive use of compression ignition internal combustion engines can be attributed to the following: high durability, reliability, high efficiency, low operation cost (Zelenka et al. 1996), and relatively low cost of diesel in comparison to petrol. Diesel drives the commer-

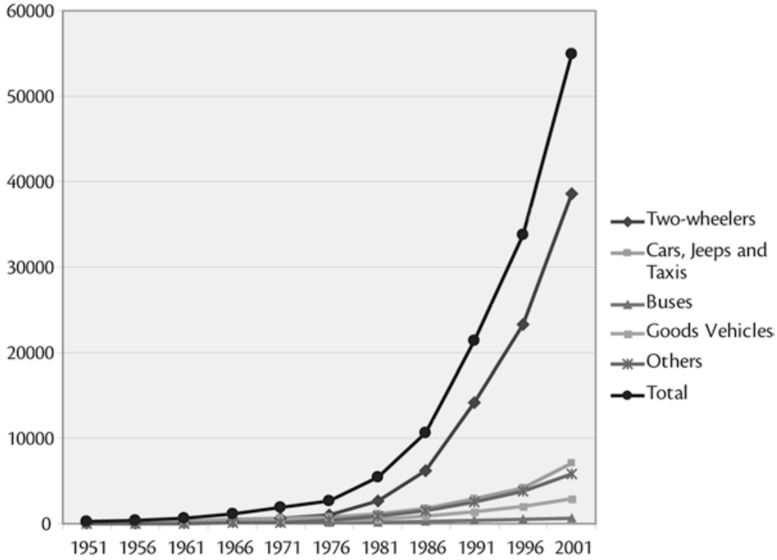

Fig. 4.1 Trend of growth for different vehicle types in India (Planning Commission of India 2003a)

cial transportation system in India: power, trucks, railways, buses, ships, electricity generators, and several off-road vehicles and equipments of agricultural and industrial utility (MoPNG 2015).

Critical dependence on imported forms of energy makes us highly vulnerable to supply and price shocks. The dependence on imported crude currently stands at nearly 83% of the total demand (Ministry of Statistics and Program Implementation 2015) and the figure is soon likely to cross 90% unless some aggressive policy interventions are introduced. Expenditure incurred on importing crude oil during 2013–2014 stood at a staggering 864,875 crores (MoPNG 2014). The expenditure came down to 687,416 crores during the next financial year (MoPNG 2016), but it was largely due to the falling demand for crude oil as China is going through a period of economic recession while the US has started using locally available shale oil (Bowler 2015). Fluctuating prices of crude oil in international market affects our day-to-day life in several ways (Parikh et al. 2009). Low prices increases the affordability of oil but it also undermines efforts aimed at enhancing energy security, energy efficiency and energy conservation and discourages investments in fossil alternatives (Baumeister and Peersman 2013). The Government of India has proposed to cut down the quantum of imported petroleum by 10% by 2020 in order to enhance energy security by utilizing locally available renewables (MoEF 2015). Transport sector accounted for roughly 23% of the total (worldwide) CO_2 emissions in 2007. The rate of increase in CO_2 emission by the transport sector is growing at a faster rate than the total CO_2 emissions. Transportation-related energy consumption is responsible for around 13% of the country's total GHG emissions. As per IEA report 2009, the total emission of CO_2 during 1990–2007 grew by 38% (20.9 Gt in 1990 to 28.8 Gt in 2007), while during the same period emissions from the transport sector witnessed an increase of about 45% (5.58 Gt in 1990 to 6.63 Gt in 2007) (IEA 2009). CO_2 emissions from the transport (road) sector between 1985 and 2005 grew at an alarming CAGR of 5.7% and emission of oxide of nitrogen from road transport grew at CAGR of 6.1% (Garg et al. 2006). The world's demand for energy in the transport sector is projected to grow at 2% per annum and majority of this demand is expected to arise from developing countries. The Indian automobile industry is expected to grow at around 10% per annum in line with economic growth and population increase. The demand for energy and CO_2 emissions are likely to shoot up by as much as 80% than existing levels by 2030 (Kahn Ribeiro et al. 2007). And this increase in demand for energy and externalities associated with combustion of fossil fuels are to be attributed mostly to the road transport.

Vehicular exhaust is one of the significant contributors to the degrading ambient air quality in cities throughout the globe. Combustion of fossil fuels in internal combustion engines produces a hot gaseous mixture. The composition of the gaseous mixture depends on the constituents of the fuel, air to fuel ratio, design of the engine, load, age and maintenance of the vehicles and several other factors. Complete combustion of carbonaceous fuels leads to the emission of CO_2 and H_2O while its incomplete combustion leads to the formation CO, soot and unburnt hydrocarbons. Depending on the fuel composition and temperature of the combustion chamber, emission of sulphur-di-oxide and oxides of nitrogen can also take place (Marland and Rotty 1984).

2 Drivers of Green Transport System

Indian transport sector is one of the largest in the world. Increase in population and income are two major drivers responsible for increase in demand of transportation needs. Limited availability of fossil fuels and shortcomings associated with their use has led to the exploration and development of alternative fuels and technology that are sustainable in the long run. Some of the major drivers for adoption of green transport system are: (1) limited availability of fossil fuels, (2) spatial variability in fossil reserves, (3) degrading ambient air quality, and (4) climate change. Transport system in most of the developing countries is crippled by several problems and the situation varies from one country to another and also varies greatly within a country (Iles 2005). Poor conditions of road, traffic congestion, use of outdated emission control systems, poor fuel quality, and poorly maintained old vehicles have led to the emissions of noxious pollutants. Air pollution is a major killer and is responsible for several million premature deaths every year (Cohen et al. 2005) and an overwhelming majority of these are being reported in India and China (Lelieveld et al. 2015). Critical dependence on imported petroleum for meeting our energy needs

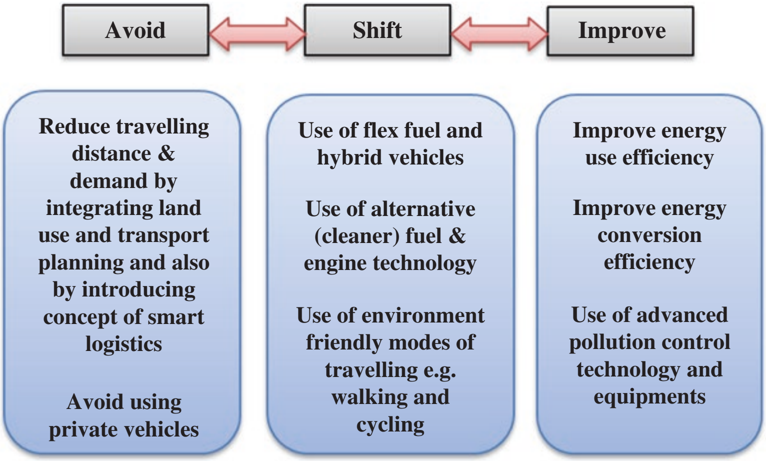

Fig. 4.2 Approaches for green transport system

has negative impacts on the economy and environment (Asif and Muneer 2007). Economic growth and rising income has led to substantial increase in number of privately owned vehicles which has proportionally translated to an increase in fuel demand and emission of CO_2 and air pollutants.

Congestion due to vehicles, climate change and air pollution affects the quality of life and the overall functioning and productivity of the society. Article 21 of the Indian constitution mandates a healthy and pollution-free environment to its citizens and hence it is a constitutional obligation for the Government to ensure pollution-free environment (Lee 2000). Exposure to air pollutants may lead to several ailments and Government expenditure on public health amounts to 4.05% of its GDP (Patil et al. 2002). This expenditure can somewhat be offsetted by introducing pollution control measures, and transport sector being one of the major culprits immediately needs introduction of pollution control measures (Cropper et al. 1997). Figure 4.2 depicts some of the possible ways to achieve a green transport system.

3 Role of Biodiesel

Biodiesel consists of a mixture of esters (Fatty Acid Alkyl Ester) derived via transesterification of vegetable oil or animal by facilitating their reaction with an alcohol (usually methanol) in presence of a catalyst (Ma and Hanna 1999). The idea of using vegetable oil as a fuel for diesel engine is as old as the diesel engine itself. Dr. Rudolph Diesel was the inventor of diesel engine and he designed it to run on a variety of fuels including vegetable oil. He had even showcased his engine running on peanut oil in an exhibition at Paris (Bhattacharyya and Reddy 1994). After the death of Dr. Diesel, the petroleum industries were able to make inroads and subsequently the design of the diesel engine was modified to run on a by-product of gasoline refining which was later named as diesel (Klein 1990). Once again there is a global shift toward the use of biomass for production of biofuels. This shift is driven by dwindling availability of fossil fuels and the shortcoming associated with their use for energy production (Alonso et al. 2010). Since the design of the diesel engine was modified to run on diesel, the existing compression ignition internal combustion engines will experience problems like poor atomization, incomplete combustion and choking when using straight vegetable oil (SVO) as fuel (Sidibé et al. 2010). These problems are to be attributed to the relatively high viscosity of straight vegetable oil. Four major approaches for reducing the viscosity of vegetable oil have been identified: blending, pyrolysis, microemulsion, and transesterification. Among these transesterification is the most common method for reducing the viscosity of vegetable oil. Transesterification involves conversion of each triacylglycerol molecule of the vegetable oil/animal into three molecules of fatty acid alkyl esters (Biodiesel) with the release of one molecule of glycerol (Ma and Hanna 1999).

Perhaps the most significant advantage offered by biodiesel is its miscibility in diesel, and various blends of biodiesel and mineral diesel can be prepared for use in diesel engine (Tat and Van Gerpen 1999). For blends up to 20% biodiesel no engine modification is required and thus it can directly be used in diesel engine. Considering the quantum of diesel used in India (four times petrol), there is an urgent need to look for alternatives (MoPNG 2014). Taking this into account the Government of India through its national policy on biofuels (2009) has proposed to blend 20% biodiesel in high-speed diesel by 2017 (National Policy on Biofuel 2009). The Government has also decided to cut down the import of crude oil by 10% by 2022. Using blends of biodiesel and high-speed diesel can help meet this ambitious target of reducing import dependence. Further, the Government through INDC (Intended Nationally Determined Contribution) has decided to cut down the emission intensity of GDP by 33–35% relative to 2005 levels (MoEF 2015). This ambitious target can be achieved by using a mix of measures such as increasing the share of renewable in its energy mix, improving energy efficiency and conservation measures, and by creation of carbon sinks. Increasing the share of renewable to 40% of the total energy mix and creation of carbon sinks to the tune of 2.5–3 billion tonnes (CO_2 equivalent) by 2030 are among the major policy interventions proposed for meeting this target.

Planting biodiesel feedstocks on marginal land/wasteland can help meet these targets. The plants while growing will take up CO_2 from the atmosphere and plant biomass can be used for the production of biofuels (including biodiesel from oil) and other bioproducts. As these measures on their own will not be sufficient for meeting the targets of INDC, hence these measures need to be supplemented with energy conservation and energy efficiency improvement measures.

Biodiesel has cleaner emission profile than diesel (Fig. 4.3) and can help improve the degrading ambient air quality (Lapuerta et al. 2008). With stringent emission norms being imposed on vehicles and measures to reduce the content of sulphur

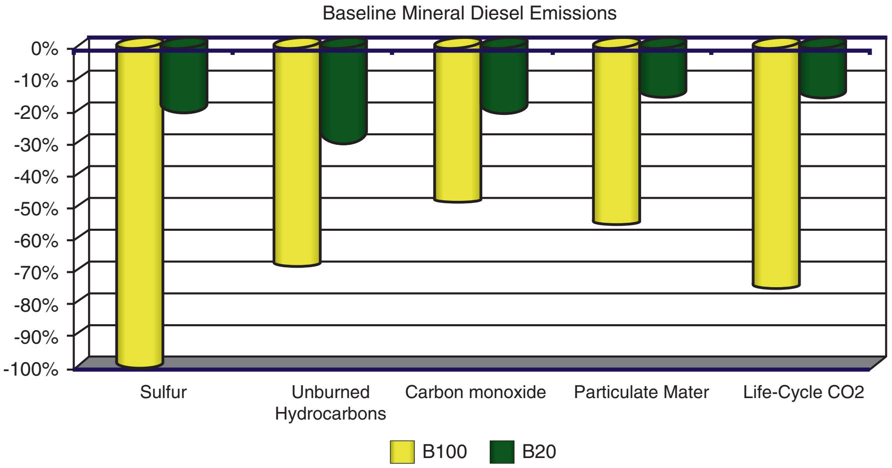

Fig. 4.3 Per cent change in emissions when using biodiesel in diesel engine (Lapuerta et al. 2008)

Table 4.1 Maximum permissible levels of sulphur in diesel (Mishra and Goyal 2014)

Bharat stages	BS II	BS III	BS IV	BS V	BS VI
Total sulphur (max. in mg/kg)	500	350	50	10	10

and aromatics in diesel (Table 4.1), the cost of diesel is highly likely to increase in future (Johnson 2011). Reducing the concentration of sulphur in diesel negatively affects the lubricity of the fuel and thus diesel use in compression ignition engines may need to be supplemented with lubricating agents (Herbstman et al. 1996). Biodiesel offers high lubricity and it is virtually devoid of any sulphur and thus using blends of biodiesel and diesel can offer economic and environmental advantages. The proportion of oxygen in biodiesel is relatively high (≈11%) which facilities its complete combustion and hence emission of CO and unburnt hydrocarbons is low (Barnwal and Sharma 2005).

Currently, BS IV is in effect (only in few selected cities) and the Government has decided to bypass BS V and will directly enforce BS VI throughout the country by April 2020. Sixteen per cent (90 mha) of the geographical area of the country has been categorized as wasteland having minimal fertility. Some of the second-generation biodiesel feedstocks such as *Jatropha curcas* and *Millettia pinnata* have minimal moisture and nutrient requirements such that they can be grown even on wastelands (Azam et al. 2005). These plants improve the fertility of the soil by means of improved soil organic matter content and biological nitrogen fixation (*Millettia pinnata* is a legume) and can potentially effect wasteland reclamation. These reclaimed parcels of land can again be put to productive use. Azam et al. (2005) explored the potential of 75 oil seed bearing plants for cultivation on wasteland for the production biodiesel in India. According to Azam et al., Jatropha and Karanja (*Millettia pinnata*) are among the most suitable oil seed plants for production of biodiesel.

Production of biodiesel from first-generation feedstock (edible grade oil) is impractical as India is among the top importers of edible grade oil and it may give rise to food vs fuel controversy, while biodiesel production from third-generation feedstock (single cell oil) is currently way more expensive and its sustainability is repeatedly being questioned. Thus second-generation feedstocks appear to be the most promising feedstock for India (Naik et al. 2010). Retrofitting existing vehicle fleet to CNG will incur substantial cost and the coverage of CNG refuelling stations is low which will again result in long queues at the refuelling stations. Increasing the coverage of CNG distribution lines and refuelling stations will incur additional infrastructure-related expenditure (Kathuria 2004). Low biodiesel blends (≤B20) on the other hand can be used in existing diesel engine without requiring any engine modification (Ma and Hanna 1999) and can be dispensed in blended forms by the existing diesel refuelling stations. Several researchers have reported a positive effect on rural economy as biodiesel plantations are likely to provide employment opportunity to the local community besides increasing their income and can also help reduce migration of people for livelihood needs (Bender 1999).

In addition to its use as a fuel for diesel vehicles, biodiesel can also be used to generate electricity using modified generators. Close to 300 million households in India do not have access to electricity due to their remote locality rendering their connectivity to grid difficult while there are even larger numbers of household which face frequent load shedding (Kamalapur and Udaykumar 2011). Decentralized electricity can be produced locally by using biodiesel as fuel for modified diesel generators. In 2007, Winrock international India set up a decentralized power plant for electrification of Ranidhera, a small hamlet of around 105 households in Kabirdham district of Chhattisgarh. The electrification of households was achieved using power generated from straight Jatropha oil as fuel for modified generators (Gmünder et al. 2010).

Combustion of fossil fuels is one of the leading factors responsible for introduction of pollutants in the air and subsequent degradation of its quality and is also one of the major contributors to the emission of GHGs (Woodwell et al. 1978). As per WHO report released in July 2016, 10 out of 20 most polluted cities in the world are in India including Gwalior (2nd), Allahabad (3rd), Patna (6th), Raipur (7th), Delhi (11th), Ludhiana (12th), Kanpur (15th), Khanna (16th), Firozabad (17th), and Lucknow (18th) (WHO 2016). The study analysed the average annual concentration of PM 2.5 in 3000 cities of 103 counties. The report mostly blamed the industrial and automobile sector for the hazardous levels of PM 2.5 in the air. The previous WHO report (2014) had ranked Delhi as the world's most polluted city. Taking serious note of the situation, the Government of Delhi introduced several measures to control air pollution including restricting the entry of heavy duty diesel vehicles in the city, imposition of pollution tax on polluting vehicles, banning the registration of diesel vehicles of over 2000 cm^3 capacity and introduction of odd–even rule. Emission profile of biodiesel is cleaner than diesel (Fig. 4.3) and its use in blended form translates proportionally in terms of emission reductions.

4 Policy Framework

Biofuel industry in India is still in its infancy and for sustenance and growth of the industry fiscal measures, fuel mandates and other forms of political interventions are critical (Ravindranath et al. 2011). The developmental objective of India focuses on economic growth and development along with equity and overall well being of its population. Until alternatives of fossil forms of energy based on indigenously available renewables are developed and used, India will remain vulnerable to international geopolitics affecting the supply and demand of petroleum (National Policy on Biofuels 2009). Utilization of biofuels is also compelling in view of the stringent emission standards being imposed on automobiles to curb pollution of air and for reducing the emission of GHGs.

In July 2002, the Planning Commission of India (now known as Niti Ayog) set up a committee on development of biofuels. The committee in its report released in July 2003 recommended progressive movement towards the use of biofuels. Five per cent blending target of biodiesel in high-speed diesel was proposed by the report beginning 2006–2007 which was to be increased gradually up to 20% in 2012–2013 (Planning Commission of India 2003b). For achieving these targets, national mission on biodiesel was proposed. The national mission was recommended in two phases in which the first phase was to be a demonstration phase in which large-scale plantations of non-edible oil seed plants were proposed and it was to be implemented by 2006–2007. The second phase was largely a self-sustaining follow-up project in which the acreage under non-edible oil seed plantations were to increase substantially so as to meet 20% blending target by 2011–2012. For the first phase of national mission on biodiesel, jatropha plantations on around 400,000 ha were suggested along with development of jatropha nursery, seed procurement and extraction centres, installation of biodiesel production (transesterification) plant and blending and marketing of biodiesel. State and non-state actors involved in the program achieved varying degrees of success. The second self-sustaining phase was about increasing the acreage of land under jatropha plantation to the tune of 11.2 mha in order to meet 20% blending target by 2011–2012 (Planning Commission of India 2003b). Unfortunately, the area under jatropha plantation could merely reach about half a million ha (Biswas et al. 2010). Jatropha plantations were also brought under the ambit of flagship programme NREGA. In between (October 2005) the Ministry of Petroleum and Natural Gas (MoPNG) came out with purchase policy for biodiesel according to which the OMCs were to purchase biodiesel from 20 procurement centres located across the country. The minimum purchasing price (MPP) of biodiesel was set at 26.50 per litre which turned out to be around 20–40% lower than the actual production cost. It hampered the production and sale of biodiesel. Several public sector OMCs and private firms have signed memoranda of understanding (MOUs) with state governments for establishment of plantations on wastelands and some of the companies have contracted with farmers but there are very few success stories despite the incentives offered by the Government. Factors such as land ownership issues, marginal land holdings, use of low yield cultivars, infrastructure inadequacy for collection of seeds and oil extraction, low yield under low input/suboptimal conditions and failure in capacity and confidence building were also responsible for impeding the growth of biodiesel industry (Rajagopal 2007).

The Union cabinet approved the national policy on biofuels in September 2008 which sets an indicative target of 20% blending of biodiesel and ethanol in high-speed diesel and petrol respectively by 2017. An element of flexibility has been introduced by including provisions for periodic review of blending targets depending on the availability of bioethanol and biodiesel (National Policy on Biofuels 2009).

The policy focuses on plantations of non-edible oil seed plants (Jatropha and Karanja) on degraded, fallow and wasteland in forest and non-forest area. Contract farming for privately owned wasteland by introducing MPP was also allowed. Provision for periodic revision of MPP for providing a fair price to the farmers was proposed as a major policy instrument. Depending on the techno-economic viability, the potential of over 400 non-edible oil seed bearing plants were proposed to be exploited.

The blending of bioethanol in petrol was mandated, while that of biodiesel in high-speed diesel has not been mandated by law and were merely of recommendatory (an indicator of Government's preferences) nature (Rajagopal 2007) and depending on the availability of biodiesel in due course of time its blending was proposed to be mandated. Central taxes and duties were not proposed to be levied on biodiesel. Excise and custom duty exemptions were to be offered for biodiesel production equipments and engines running on biodiesel. Further, in order to keep the cost of indigenously produced biodiesel, lower duties and taxes were proposed on imports.

There are around 20 large biodiesel production plants in India capable of transesterifying edible as well as non-edible grade vegetable oil. Currently, the total commercial biodiesel production is only around 140–300 million litres due to the lack of feedstock availability, and production plants do not operate for most of the year. Unorganized sectors such as irrigation pumps, diesel gensets, mobile towers, etc. remain the major purchasers of biodiesel. Low MPP has impeded the commercial sale of biodiesel. Low availability of feedstock, inflation, and absence of efficient marketing channels has led to higher production cost of biodiesel (TERI 2015). Taking note of the condition, the Government is reportedly mulling for enhancing the MPP of biodiesel to 34 per litre. The Government has recently taken several important steps for boosting the biodiesel industry in India. Biodiesel manufacturers are now allowed to directly sell biodiesel to bulk consumers such as defence establishments and Indian railways (TERI 2015). Interdepartmental consultations are being held for allowing 5% blending of biodiesel for bulk consumers. The Government has further notified biodiesel standards (IS 15607-05) (Table 4.2) in line with American (ASTM-D6571-08) and European (EN 14214-08) standards considering the ambitious blending targets (Raheman and Pradhan 2012). According to few reports, the Government is also mulling to introduce standards for B100 fuel (neat biodiesel) and vehicles. The Government's decision to cut down import of petroleum by 10% (by 2022) can also prove to be a boost for biofuel industry.

Table 4.2 Fuel standards for diesel and biodiesel

Fuel property	Diesel[a]	Biodiesel[a]	Biodiesel[b]
Fuel standard	ASTM D975	ASTM D6751–08	IS 15607-05
Higher heating value, Btu/gal	~137,640	~127,042	–
Lower heating value, Btu/gal	~129,050	~118,170	–
Viscosity, cSt (40 °C)	1.3–4.1	4.0–6.0	2.5–6.0
Ester content, %	–	Not mentioned	96.5
Acid number, mg KOH per gm	–	0.50	0.50
Oxidative stability @110 °C	–	Not mentioned	6 h (min.)
Specific gravity, kg/l (15.5 °C)	0.85	0.88	–
Density, lb/gal (15.5 °C)	7.1	7.3	–
Carbon, %	87	77	–
Hydrogen, %	13	12	–
Oxygen content, %	0	11	–
Sulphur content, %	0.0015 max	0.0–0.0024	50 mg/kg (max)
Flash point, °C	60–80	93	120
Cloud point, °C	−35 to 5	−3 to 15	–
Pour point, °C	−35 to (−15)	−5 to 10	–
Cetane number	40–55	48–65	51

[a] National Renewable Energy Laboratory 2009, [b] Raheman and Pradhan 2012

In spite of several positive policy measures, the biodiesel industry in India is still in its infancy. Lack of sufficient infrastructure and poor institutional setup has failed the previous policy measures, and achievement of the proposed blending targets of national biofuel policy also appears to be a remote possibility. Absence of uniform policy throughout the country, different tax regimes, administrative control on movement of biodiesel across state borders are equally responsible for the debacle. Almost 2,500 crores of investment by the industry is lying idle as several factors have led to restricted growth of the industry and there is no commercial market for biodiesel (TERI 2015). Government regulations only allow selling of biodiesel to OMCs at a pre-decided price (lower than the production cost) and there are transport restrictions across state borders. Considering the growing demand for diesel, biodiesel industry has immense potential to grow but the pricing policy is restricting investments in the sector. The petroleum ministry decides the price of biodiesel depending on the prevailing retail price of diesel. In order to bring price parity the Government needs to allow selling of biodiesel directly to consumers and the pricing of biodiesel should be freed from diesel. Policy-based measures should also consider the externalities of using fossil fuels while considering its alternatives.

Clearly, there is immense scope for biodiesel industry in India as we will remain highly vulnerable to petroleum supply shocks and energy insecurity until indigenously produced renewables are promoted and used. Poor policy framework and improper implementation of the existing policies with little foresight have led to restricted growth of the industry. Policy support framework required along the supply chain for promoting biodiesel industry is shown in Fig. 4.4. The automobile

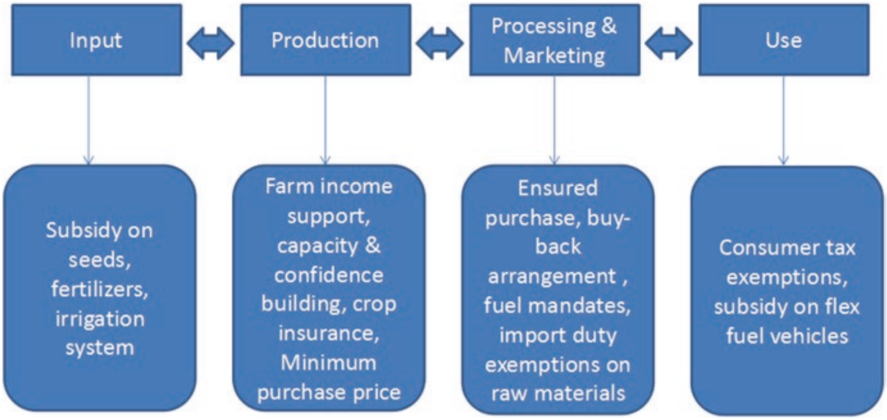

Fig. 4.4 Policy support for biodiesel along the supply chain

industry too has equally important role to play in developing a green transport system as fuel-based measure alone may not be able to entirely address the concerns related to our existing transportation system.

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Chapter 5
Microalgae Biofuels: A Green Renewable Resource to Fuel the Future

Mylsamy Prabhakaran, Venkataraman Sivasankar, Kiyoshi Omine, and M. Vasanthy

Abstract Intensive investigation and spectacular attention have been currently on microalgae fuels because they are the only sustainable alternate, inherently renewable, economical, and eco-friendly fuels unlike fossil fuels which are fast depleting. Microalgae are rapidly growing organisms and contain high amount of oil content when compared to season-depending terrestrial and food crops containing low percent dry weight of oil. The oil content of microalgae, a remarkable feature could be tuned higher especially under stress conditions as nutrient deficiency, in particular nitrogen. It is quite explicit and plausible that the conversion of microalgae into liquid fuels facilitates a sustainable, long-term fuel production circumscribed in an environmentally attuned manner. As the world population reaches ten billion mark and the sequels of negative environmental, economic and social impacts, the compromise based on first-generation biofuels seems quite irremediable. To surpass the existing problems, microalgae biofuels are envisaged as rather promising and potential due to high photosynthetic efficiency and growth rate in the development of about 70% of lipid content within the cells depending on species. Photo-bioreactors substitute the open raceway ponds for curtailing the problem of contamination and evaporation in open ponds. The production of value-added byproducts after biofuel extraction could also be the best outcome of microalgae residues.

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1 Introduction

The term “Biodiesel” was very first coined in the Magazine of Power Farming, which states, “…the two fuels, Biodiesel and distillate are virtually identical…” (Power Farming Magazine 1984). Later, Knothe (2001) reported that the Chinese paper published in 1988 (Wang 1988) used the word Biodiesel. In the present trend, researchers are searching for a substitution of an alternate, inherently renewable, economical, and environmental friendly fuels against the finite fate of fossil fuels. Due to very high demand and overexploitation of unsustainable resources like fossil fuels, the fossil fuel reservoirs are estimated to deplete in another 60 years (Stephenson et al. 2010). Although the production of biodiesel from food crop plants (corn and soybeans) is appreciable, the resulting consequences such as shortage of food and expensive extraction seem tricky to achieve sustainable growth. Microalgae serve for the third-generation feedstock and are able to provide huge amount of biofuel than the second-generation crops without troubling the environment. Based on the report of Chisti (2007), the production of about 1200–10,000 gallons of oil per acre from algae could be possible against the 48 gallons per acre and 18 gallons per acre of soy and corn respectively. Microalgae, the photosynthetic microorganism, involves in the conservation of solar energy into chemical forms by photosynthesis and have greater growth rate than plants (Ndimba et al. 2013). These require simple nutrients and are able to live under diversified conditions by facilitating the growth of algae in areas which are not supported under mainstream agriculture (Slade and Bauen 2013). It is estimated that from the production of 20 g m^{-2} day^{-1} of microalgae, about 30% conversion of liquid fuel is feasible with a required land area of 4.47×10^5 km^2 for the fuel replacement through total transportation. The merits of biofuels (like ethanol) based on the beneficial aspects to climate and CO_2 reduction against petroleum fuels, concluded by the International Institute for Sustainable Development (ISDD 2015), are predominantly zero. There have been unique ways which emerge continuously to design eco-friendly technologies for the production of renewable energy from algal biomass. In addition, their favorable carbon life cycle and a renewed focus on rural economic development are highly conspicuous (Sivakumar et al. 2011). Some species of microalgae produce more than 50% of their dry mass as long chain hydrocarbons that can be converted into biodiesel (Sivakumar et al. 2010; Niehaus et al. 2011). Microalgae can be grown in open raceway ponds or in plastic tanks called bioreactors with little more sunlight, heat, and water (Carvalho et al. 2006). Photobioreactors (Costa and de Morais 2011; Tang et al. 2011; Chen et al. 2013) allow essentially pure culture of microalgae for extended durations and enable a large quantity of microalgae biomass (Menetrez 2012) which are commonly not seen in the case of open raceway ponds. Various researchers have documented the successful production of microalgae biomass using photo-bioreactors (Lee et al. 2010; Molina Grima et al. 1999; Pulz 2001).

2 Biofuel

Biofuels can be derived directly from plants and indirectly from organic wastes (Aksoy et al. 2011). The precursors of renewable biofuels, most often referring to plants, microalgae or plant-derived materials have inherent carbon through the process of photosynthesis. Biomass to biofuel conversion is made possible by thermal, chemical, and biochemical conversions in solid, liquid, and gaseous forms.

The two liquid bio-renewable transportation fuels are bioethanol and biodiesel. Bio-ethanol is an alcohol, resulting through the fermentation process by utilizing carbohydrates and cellulosic biomass. It is usually chosen as a gasoline additive to increase the octane number and to improve vehicular emissions in Brazil and used for high compression ignition (CI) engine for heavy vehicle transport in India and United States. On the other hand, biodiesel, a product of trans-esterification process (oil and fat) is preferred as a diesel additive to reduce particulate and hydrocarbon emissions from diesel engines and extant in Europe, India. United States and Brazil are the global leaders in biofuel production, which accounts for about 90%. The goal of International Energy Agency is to meet more than 25% of the global demand with biofuels by 2050 to reduce the dependence on non-renewable fuels, such as petroleum and coal. It is conspicuous that the automotive industries get flourished due to the production of biofuels across the world. As a token of complementing this fact in 2010, Brazil redesigned its automobile strategy in 79% of car production with hybrid fuel system of bio-ethanol and gasoline.

Based on production technologies, biofuels are classified as first-generation biofuels (FGBs), second-generation biofuels (SGBs), and third-generation biofuels (TGBs).

2.1 First-Generation Biofuels (FGBs)

First-generation biofuels are extracted from crop plants such as food and oil crops which include sugarcane, sugar beet, vegetable oils, and animal fats (Himmel et al. 2007). However, the execution of first FGBs seems to be more complex and limited to commercial production and transportation due to its competition with food crops and agricultural land (Adenle et al. 2013). Major problem of FGBs is the availability of farming lands to produce oil crops. But the amount of biomass cannot replace a major quantity of the fossil fuel used in the transportation sector due to the less yield which makes it impractical for production on a large scale (Lang et al. 2001). The second important issue with the first-generation biofuels is the competition between fuel and food. Most of the biofuels are produced directly from food crops hence the demand for biofuel crops increased and it affects the food production (Karp and Richter 2011; Abdelaziz et al. 2013a, 2013b; Leite and Hallenbeck 2012; Leite et al. 2013; Popp et al. 2014). The shortage of food has been blamed for the increase in food cost across the globe over the last couple of years (UN Report 2007).

2.2 Second-Generation Biofuels (SGBs)

Second-generation biofuels are derived from biomass sources which include agricultural, forest harvesting and wood processing residues, non-edible components from food crops along with non-food crops such as miscanthus, tobacco seed, mahua and jatropha (Alam et al. 2012), wheat straw, corn, wood and energy crop using advanced technology (lignocellulosic fuels). Lignocellulosic biomass varies among species but generally consists of about 25% of lignin and 75% of carbohydrate polymers, cellulose and hemicelluloses (Aristidou and Penttila 2000). The second-generation biofuels are extracted from lignocellulosic biomass after a series of physical and chemical treatments, unlike conventional technology adopted for the production of first-generation biofuels. The conversion of cellulose into ethanol is feasible when the cellulose is separated from lignocelluloses catalyzed by enzymes. However, the production costs of SGBs are expensive. Based on the composition of organic matters, the fuel extraction technology differs from the first-generation biofuels to second-generation biofuels.

2.3 Third-Generation Biofuels (TGBs)

The third-generation biofuels are based on algae, whose oil is able to produce diversified fuels, such as diesel and certain components of gasoline due to the characteristics of microorganisms. The key to algae's potential as a renewable fuel source lies in the high productivities of algal biomass and an acre of produced algae can produce 9000 gallons of biofuel. Achieving the potential for these high productivities in real-world systems is a key challenge to realize the promise of sustainable and affordable algal biofuels. Algae are low-input, high-yield feed-stock to generate biofuel and could be the best replaced for fossil fuels (Mihaela et al. 2013; Karaj and Muller 2014; Khayoon et al. 2012; Hossain and Davies 2012; Li et al. 2013; Ziolkowska and Simon 2014).

Microalgae have been noted as one of the best non-edible feedstock for producing biodiesel compared to oil crops such as soybean, rapeseed, and even oil palm (Table 5.1). This is due to the number of advantages like higher oil productivity (at least 15–20 times higher oil yield per hectare than conventional crops), rapid growth rate (biomass doubling within 3.5 h during exponential growth), and no requirement of arable land as it can be grown in marginal areas. Algae can effectively utilize less water than the oilseed crops and do not need fresh water. It is very advantageous that algae can tolerate saline or brackish water and minimum land area is enough to produce large cultivation of biomass. Biomass from marine, cellulosic, and other non-food sources are prominent to substantiate higher productivity, lower greenhouse gas emission, and enhanced sustainability as compared to the first-generation biofuels (FGBs).

Table 5.1 Comparison of oil yield between biofuel sources

Crop	Oil yield (L/ha)
Corn	172
Soybean	446
Canola	1190
Jatropha	1892
Coconut	2689
Oil Palm	5950
Microalgae[a]	136,900
Microalgae[b]	58,700

[a]70% oil (by wt) in biomass
[b]30% oil (by wt) in biomass

3 Microalgae Biofuel

Microalgae, a technical synonym for seaweed, are known to contain large amounts of lipids within their cell structure, and so they are increasingly becoming an interest as a biofuel feedstock. There are approximately 30,000 known species of microalgae. Microalgae is a very heterogeneous group containing prokaryotic (similarity with cyanobacteria called blue-green algae) and eukaryotic (diatoms) photosynthetic microorganisms with a simple structure that allows them to grow rapidly and exist in fresh and marine water (Li et al. 2008). There needs further exploration regarding the blue-green species and their application aspects as well. Those algae species which can produce potent lipid contents could be screened and applied for energy production on commercial basis. The development of high lipid contents at favorable compositions facilitates the ease of cultivation and processing. The microalgae system of cultivation is carried out using different aqueous ponds/reactors and temperatures. Nevertheless, the optimal cultivation temperature to grow algae is recorded around 20–30 °C. The constituents of microalgae biomass are lipids, protein, and carbohydrates (Kobayashi et al. 2013; Milano et al. 2016). Lipids, accumulated within the cells of microalgae (~20–70%) highly depend on the microalgae species and cultivation conditions, and are extracted for the purpose of deriving biodiesel. Although some microalgae strains are able to generate 70% of lipids, it has not been achieved at industrial scale as it could be achievable up to 30%. An economically viable microalgae strain needs to be very essential for the production of algae fuel applied in power generation.

Some microalgae species are known to produce and accumulate hydrocarbons up to 30–70% of their dry weight, and their oil production rate is 1000 times higher as soybeans grown on the same amount of land (Kong et al. 2007). The oil content in microalgae can exceed up to 80% of the weight of the dry biomass, but generally achieved oil levels are from 20 to 50% (Metting 1996). Microalgae cells can double every few hours during their exponential growth period (Metting 1996). The largest seaweed, giant kelp is known to grow as fast as 50 cm/day by reaching a length up to 80 m (Thomas 2002) and leaves the signature as a promising crop for human use.

Marine microalgae seem to be rather idealistic for biodiesel production in India because of the geographical coastline of 7517 km (or 4700 miles) which could be best suitable for marine microalgal mass cultivation and commercialization. Currently, the nation is struggling with fresh water resource depletion, scarcity and instability of petro-diesel price hike. Hence the choice of biodiesel production from marine microalgae could be a more reliable fuel resource and better alternate to fossil fuels. Marine microalgae have greater lipid productivity compared to freshwater and easy to mass production coupled with realization that a high salinity prevents extensive contaminants lead to produce lipids with high purity. The marine microalgae populations are dominated by phytoplankton as suspension in seawater. This is termed as bio-fouling when boats or piers are used for colonizing the surface. Some important biodiesel producing microalgae reported in literature (Chisti 2007; Rodolfi et al. 2009) are shown in Table 5.2. The outline of biodiesel production is represented in Fig. 5.1.

Table 5.2 Habitats and oil content of some microalgae

Microalgae	Oil content (% dry weight)	Habitat
Botryococcus braunii	25–75	Fresh water/estuary
Chlorella vulgaris CCAP 211/11b	19.2	Freshwater
Chlorella sp.	28–32	Freshwater
Scenedesmus sp. F&M-M19	19.6	Freshwater
Scenedesmus sp. DM	21.1	Freshwater
Chlorococcum sp. UMACC112	19.3	Freshwater
Monodus subterraneus UTEX 151	16.1	Freshwater
Neochloris oleoabundans	35–54	Fresh water
Isochrysis sp.	25–33	Marine
Nannochloropsis sp.	31–68	Marine
Monallanthus salina	>20	Marine
Crypthecodinium cohnii	20	Marine
Tetraselmis sueica	15–23	Marine
Dunaliella primolecta	23	Marine
Phaeodactylum tricornutum	20–30	Marine
Nannochloris sp.	20–35	Marine
Cylindrotheca sp.	16–37	Marine
Pavlova salina CS49	30.9	Marine
Skeletonema sp. CS252	31.8	Marine
Chaetoceros muelleri F&M-M43	33.6	Marine
Pavlova lutheri CS182	35.5	Marine
Chaetoceros calcitrans CS178	39.8	Marine
Nitzschia sp.	45–47	Marine
Schizochytrium sp.	50–77	Marine

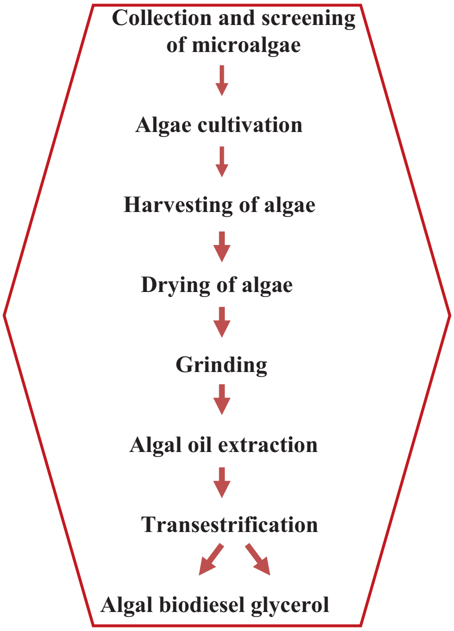

Fig. 5.1 Outline of microalgae-based biodiesel production

4 International Biofuel Production: Current Scenario

Renewable resources are more evenly distributed than fossil and nuclear resources, and energy flows from renewable resources are more than three orders of magnitude higher than current global energy use. Today's energy system is unsustainable because of equity issues as well as environmental, economic, and geopolitical concerns that have implications in the future (UNDP Report 2000). Large scale of biofuel production required continuous feedstocks availability. A number of feedstocks exist in the world for biofuel production including energy crops (Miscanthus, Jatropha), waste oil (Food processing waste oil), agricultural residues (straw, corn stover), forestry residues, and novel feedstocks such as microalgae (FABRI 2008). Based on International Energy Agency (IEA) reports, USA and Europe decided to displace petroleum fuels upto 6% with biofuel. But the production of conventional biofuel requires enormous land area. For the substitution of gasoline with biodiesel, the crop land requirement for USA and Europe are about 13% and 15% respectively. The crop land requirement for the substitution using ethanol is reported to be 5% and 8% for Europe and USA respectively. It has been committed by the Government of United States to increase the bioenergy by threefolds in the time span of next 10 years using biofuels (FABRI 2008). An optimistic way of approach is reported by Sustainable Energy, Ireland (SEI) that an estimated 100 ha of microalgae production would be achieved by 2020 (Bruton et al. 2009).

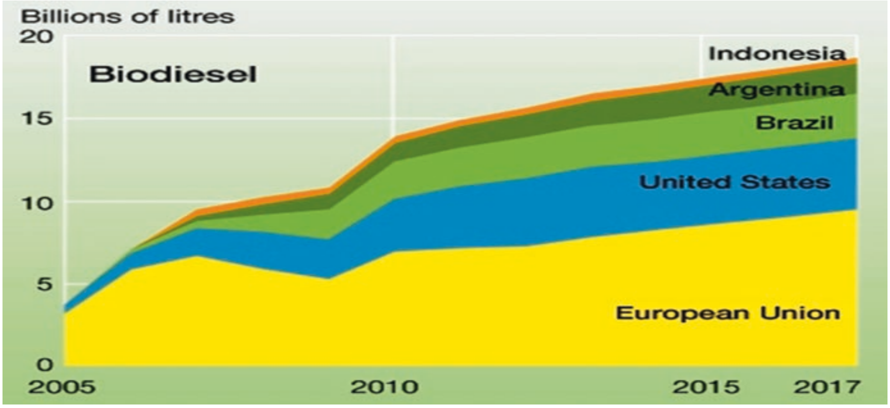

Fig. 5.2 World biofuel production

According to EurObserv'ER Biofuels Barometer 2012, the biofuel consumption rate of 3% increase between 2010 and 2011 was registered with 13.6 million tons of oil equivalent used in 2011 as compared to 13.2 million tons in 2010. A graphical view of global biodiesel production from 2010 to 2017 (Fig. 5.2) depicts that Indonesia and Argentina are the leading biodiesel producers (~15–20 billion liters) followed by United States, Brazil and Europe (FABRI 2008). In Japan, a recent magazine reported that Isuzu Motors Co. Ltd. and Euglena collaborated in a DeuSEL project which aims at the practicability of microalgae-based biofuel using euglena microalgae strain in a bus called DeuSEL by 2018 (http://www.algaeindustrymagazine.com).

5 Screening of Potent Microalgae Strains

Screening of algae is to identify and maintain promising algal specimens for cultivation and strain development. During the screening process for potent strains, microalgae were subjected to purification by serial dilution followed by plating technique. The individual colonies were isolated and inoculated into bold basal media (BBM) for freshwater microalgae, F2 liquid media (Guillard and Ryther 1992) and CHU 10 for marine microalgae. These media were enriched with nitrate and phosphate salts by the addition of $NaNO_3$ (1.5 g L^{-1}), $NaHPO_4$. H_2O (0.04 g L^{-1}), and GeO_2 (10 mg L^{-1}) for preventing the interference of diatoms (Sialve et al. 2009). Then the plates were left to incubate under light intensity at 120 μmol photons m^{-2} s^{-1} for 12:12 h Light/Dark at 25 °C and were regularly checked for the presence of microalgae, if any (Guillard and Ryther 1992). The isolated microalgae cultures are shown in Fig. 5.3.

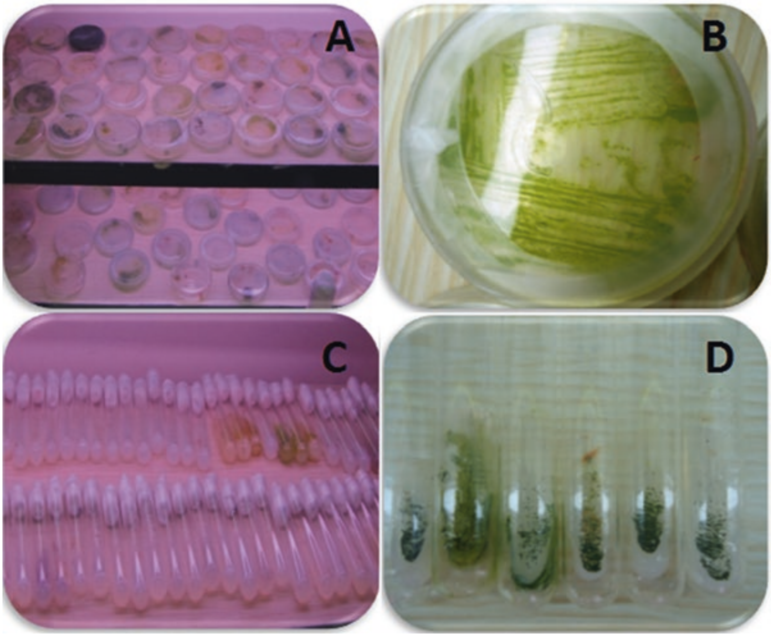

Fig. 5.3 Isolation of microalgae from collected water samples: mother inoculum plates (**a**), Pure culture plates of microalgae (**b**), Mother inoculum slant (**c**), Pure culture slant of microalgae (**d**)

6 Indoor Maintenance of Microalgae Culture

The microalgal stock cultures were maintained in conical flasks (borosil, 500 mL) containing the medium of 200 mL with cotton plug (Fig. 5.4) and then sub-cultured in fresh medium every 3–4 weeks. To loosen the cell mass and to prevent them from sticking to the bottom of the flask, 0.5 cm of glass beads were added to each culture flask followed by manual shaking on a regular basis to keep the algae dislodged in suspension. The cultures were maintained in a room at constant temperature (25 ± 0.2 °C) equipped with banks of cool-white fluorescent lights providing an irradiation of approximately 70 μmol photon m^{-2} s^{-1} on a 12:12 h L:D cycles (Lorenz et al. 2005).

7 Lipid Accumulation and Induction in Microalgae Under Different Stress Conditions

Microalgae yielding lipids are classified as neutral lipids, polar lipids, wax esters, sterols and hydrocarbons as well as phenyl derivatives such as tocopherols, carotenoids, terpenes, quinines and pyrrole derivatives such as chlorophyll. Microalgae

Fig. 5.4 Indoor microalgae culture maintenance at laboratory

lipids can be categorized as storage lipids (non-polar lipids and triacylglycerol) and structural lipids (polar lipids, phospholipids, and sterols). Research investigations on lipid induction techniques in microalgae including nutrients stress (nitrogen and/or phosphorus starvation), light irradiation, pH, temperature, heavy metals, and other chemicals are well established. In response to certain environmental conditions, some microalgae could efficiently modify the lipid metabolism (Thompson 1996). The production of lipid content in microalgae usually varies in the range of 1–70% of dry mass depending on species which could be up to 90% in certain environments (Metting 1996).

Microalgae can trigger itself for lipid production, when put into stressful condition as nutrient deprivation, the microalgae change the use of carbon uptake from reproduction to energy storage in the form of oils (Piorreck et al. 1984). Under nitrogen deficient environment, the strain of algae is stressed from 22 to 58% in its oil content per dry mass (Sheehan et al. 1998). *Dunaliella salina* has been extensively studied for its low temperature modification of lipid composition (Thompson 1996). Thompson (1996) studied the significance of about 20% increase in unsaturated lipids from 30 °C to 12 °C. On subjecting the cells of the diatom, *Nitzschia laevis* to high salt concentration (10–20 g L^{-1}), high amount of ω-3-fatty acid called eicosapentaenoic acid (EPA, $C_{20}H_{30}O_2$) is produced (Chen et al. 2008). In addition, the degree of unsaturation among fatty acids of neutral and polar lipid fractions is found to exhibit a sharp escalation due to the increase of salt concentration. The production of fatty acids by various algae is pertinent to the induced stress which either fulfills the requirement of different fatty acids or fatty acids with altered compositions. Another diatom called *Stephanodiscus minutulus* could accumulate TAGs with a decrease in polar lipids due to the nutrient starvation under limited silicon, nitrogen or phosphorous concentrations and noticed for the entire nutrient-hunger cultures (Lynn et al. 2000). The green alga called *Chlamydomonas moewusii* reacts to the nutrient limited environment by way of decreased PUFA C16:3, C16:4 and PUFA C18:3. On the other hand, the overall concentrations of C16:1 and C18:1 FA were observed with an increasing trend (Arisz et al. 2000). In a study on the lipid induction of 25 algae species reviewed by Sharma and Schuhmann (2012), the nutrient stress was based on nitrogen (limitation and medium), phosphorous and silicon starvations and, the dominating outcomes were realized from the studies with an increase in total lipids, TAGs, and unsaturated FAs. However, nitrogen starvation-based induction is the most successfully applied platform for the escalation of lipids in a time span of 2–5 days where slow growth rate and cell count was registered (Widjaja et al. 2009).

8 Microalgal Cell Disruption Methods

Lipids extraction from the intracellular site of the microalgae cells is primary and an important step in biofuel production. Economical way of approach is well appreciated by avoiding the large use of organic solvents. The overall yield of biodiesel largely depends on both cell disruptions and the method employed. Established kinds of methods for cell disruption such as autoclaving, bead-beating, microwave application, sonication, osmotic shock, and 10% addition of sodium chloride are usually employed to identify the most effective method. Microwaves can shatter cells via shock induced by high-frequency waves (Virot et al. 2008; Engler 1985). Sonication breaks cell wall and membranes through cavitation effect, and has been the widely used one to disrupt microbial cells (Lee et al. 1998; Geciova et al. 2002). Bead-beating causes direct mechanical damage to the cells, based on high-speed spinning with fine beads (Lee et al. 2010). Rakesh et al. (2015) investigated and reported that the microwave oven method was a simple, effective, and promising alternative among cell disruption methods especially for *Botryococcus* sp. MCC31.

9 Extraction and Trans-esterification of Lipids from Microalgae Dry Biomass

During the lipid extraction process, the decomposition of organic molecules is avoided by carefully choosing the methodology which suffices completion. Several organic solvents in the extracting process of lipids are used viz. chloroform/methanol (Bligh and Dyer 1959; Folch et al. 1957), hexane/isopropanol, dichloromethane/methanol (Graeve et al. 2002), acetone/dichloromethane, dichloromethane/ethanol, and diethyl ether (El-Shoubaky et al. 2008). Although a commendable lipid extraction around 99% can be achievable, the expenditure of the process seems unconvincing (Metzger and Largeau 2005).

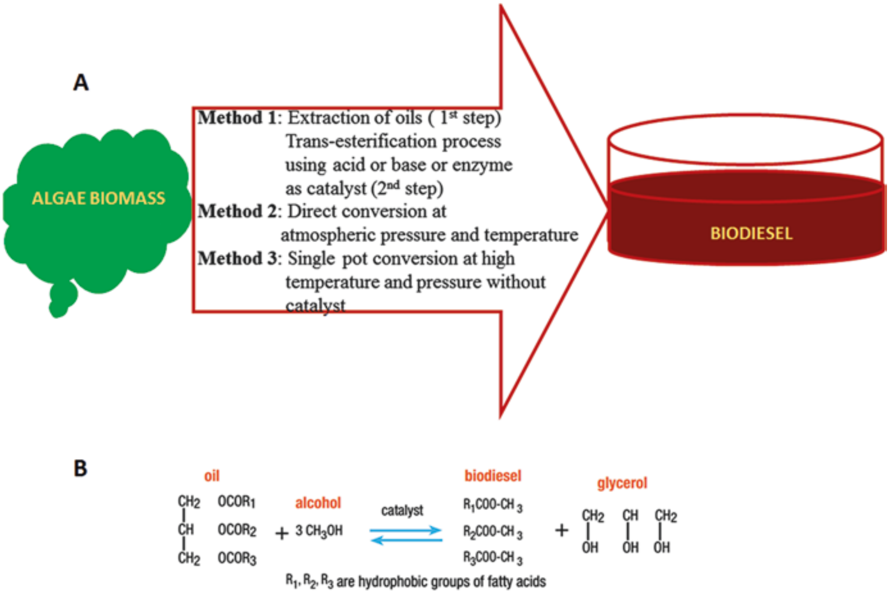

Fig. 5.5 General methods for the conversion of algae biomass to biodiesel (**a**). The trans-esterification process (**b**)

Microalgae lipids are mainly composed of 90–98% triglycerides, smaller amounts of mono- and diglycerides, free fatty acids and residual lipids like phospholipids, phosphatides, carotenes, and sulfur compounds. The conversion of fatty acids to fatty acid methyl esters (FAMEs) is made possible by trans-esterification process using lipids obtained from solvent extraction. The trans-esterification reagent is prepared by dissolving 0.3 g sodium hydroxide in 2 mL of ethanol and added to the oil and left for 16 h. The trans-esterified lipid extracts from microalgae species are shown in Fig. 5.6. The production of fatty acid ethyl esters (FAEEs) is advocated to have slightly higher cetane numbers, improved low temperature operability and greater oxidative stability than FAMEs.

There are three generally adopted methods to perform the production of biodiesel from algae mass as shown in Fig. 5.5a. The process of trans-esterification (Fig. 5.5b) enables the conversion of microalgae oil into fatty acid methyl esters (FAMEs) using alcohol as significant substrates in the presence of acid or alkali or enzyme as catalyst (Canakci and Vangerpen 1999; Du et al. 2008; Mutanda et al. 2011). The commonly used alcohols are methanol, ethanol, propanol, butanol, and amyl alcohol. The most widely used substrate at affordable cost is methanol. However, the toxicity of methanol raises additional consequences like transportation and storage (Zanin et al. 2013). The combined process of oil extraction and trans-esterification is reported to be economical for the overall biodiesel production and referred as the in situ trans-esterification process (Haas and Wagner 2011). Researchers attempted for the production of biodiesel from various algae such as

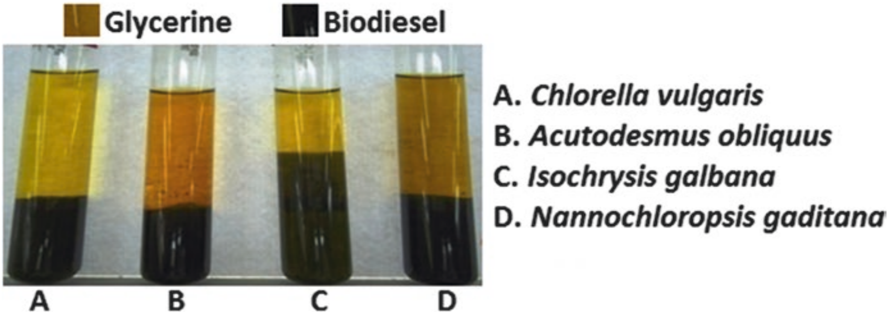

Fig. 5.6 Trans-esterified lipid extracts from selected microalgae

Schizochyrtium limacinum (Johnson and Wen 2009), *Chlorella* sp. (Ehimen et al. 2010), *Spirulina* sp., and *Chlorella vulgaris* (Velasquez-orta et al. 2012) by in situ trans-esterification process at different conditions and organic solvents. Ethanol is considered attractive than methanol due to its long-term sustainability, less toxicity and safety (Demirbas 2009). The acid-catalyzed conversion of free fatty acid (in feed stocks) to esters is plausible but the rate of conversion of triglycerides to methyl esters is too slow (Gerpen 2005) than catalyzed under alkali conditions. The alkali-based catalyzed trans-esterification process is about 4000 times faster than acid-based process (Fukuda et al. 2001) and has gained prominence across the world.

10 Large-Scale Cultivation of Microalgae

10.1 Open Raceway Pond Cultivation Method

Open raceway pond (Fig. 5.7a–c) and photo-bioreactors (Figs. 5.9 and 5.10) are the best methods used for culturing large volumes of microalgae (Grima et al. 2003). These ponds appear circular and not deeper than 30 cm because of light penetration which is approximately possible up to 300 mm of water. For constant mixing of algae biomass and to avoid sedimentation, a paddle functioning is enabled. Due to circulatory motion of the biomass, the continuous mixing reduces the shading effect. Raceway ponds are prone to contamination by other microalgae as well as microorganisms such as rotifers that consume microalgae. Even though open systems successfully carry out the production of microalgae on a large scale, the known species of appreciable growth are small in number (Borowitzka 1999). The main advantage associated with the raceway ponds is cost, whereas the photo-bioreactors offer better productivity with high cost (Chisti 2007).

Chlorella vulgaris and *Acutodesmus obliquus* were grown in improvised CFTRI medium and *Isochrysis galbana* and *Nannochloropsis gaditana* were grown in F2 medium with the incubation temperature ranging from 20 °C to 30 °C with natural

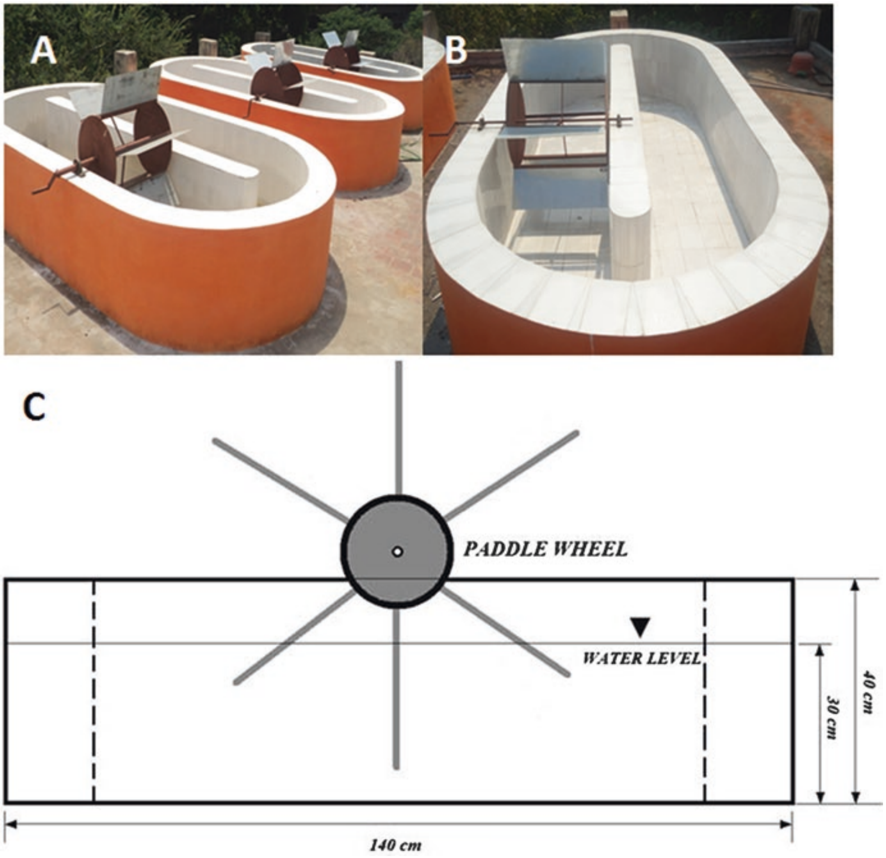

Fig. 5.7 Race way pond-overview in series (**a**), inside view (**b**), cross-sectional view (**c**)

day/night cycle (20,000–75,000 lux). Prior to the culture inoculation, these ponds were cleaned and the medium was prepared using filtered water and also to be ensured that the depth of the medium does not exceed 15 cm. Microalgae inoculums were added into the medium and grown daily with thrice manual stirring and sampling (Fig. 5.8a–d) for the algae species such as *Chlorella vulgaris* (A), *Acutodesmus obliquus* (B), *Isochrysis galbana* (C), and *Nannochloropsis gaditana* (D). Aliquots were drawn for recording various parameters such as cell growth, water level, pH, salinity, and temperature. Any loss of water through evaporation could be compensated with 7 L of media per day till the end of experimentation. At the end of 30 days, the biomass was harvested as shown in Fig. 5.9a–d. These microalgae are cosmopolitan genus with small globular cells. It includes strain with a high temperature tolerance as some strains can grow between 15 °C and 40 °C (Chisti 2007). The algal strains grow autotrophically in an inorganic medium and in mixotrophic and heterotrophic conditions as well (e.g., with addition of acetic acid and glucose). At present, autotrophic production of microalgae and their high growth rate prevents the other microalgae contamination in open pond system.

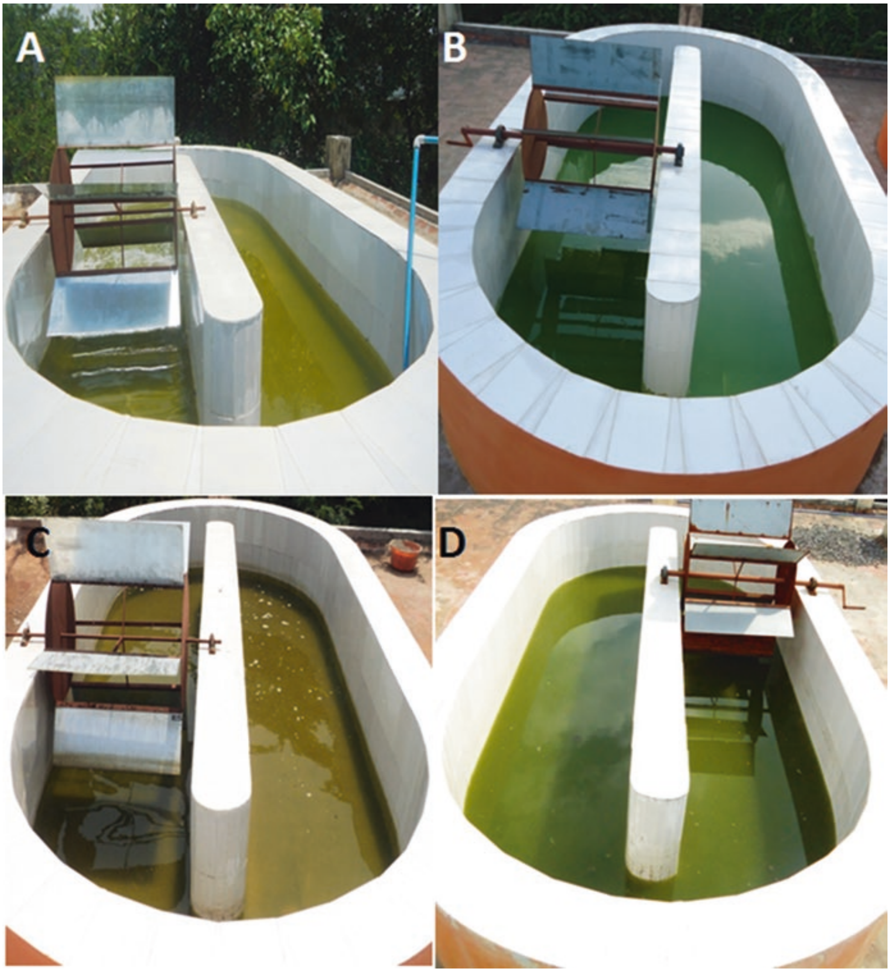

Fig. 5.8 Microalgae cultivations in raceway pond—*Chlorella vulgaris* (**a**), *Acutodesmus obliquus* (**b**), *Isochrysis galbana* (**c**), and *Nannochloropsis gaditana* (**d**)

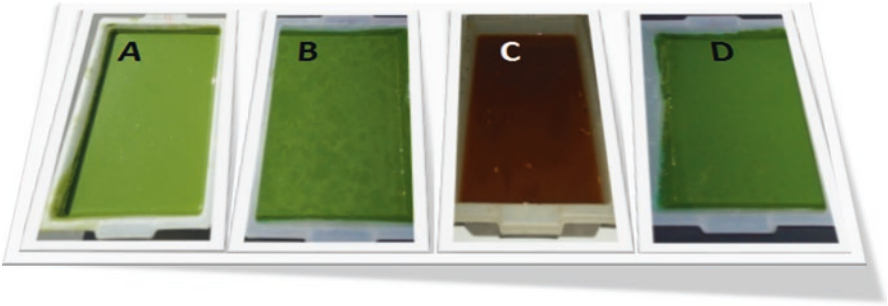

Fig. 5.9 Slurry of selected microalgal strains—*Chlorella vulgaris* (**a**), *Acutodesmus obliquus* (**b**), *Isochrysis galbana* (**c**), and *Nannochloropsis gaditana* (**d**)

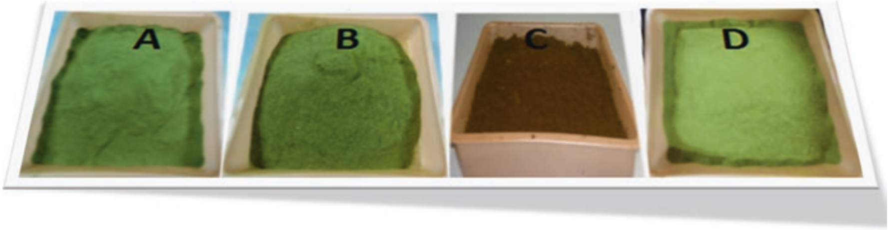

Fig. 5.10 Algae biomass of selected strains—*Chlorella vulgaris* (**a**), *Acutodesmus obliquus* (**b**), *Isochrysis galbana* (**c**), and *Nannochloropsis gaditana* (**d**)

Algal cultures in the open raceway ponds were allowed to settle for 24 h and the supernatant medium was siphoned off. Then the resulting biomass was solar-dried and harvested (Fig. 5.10a–d).

10.2 Photo-Bioreactor and Cultivation Method

The other commonly encountered system to grow microalgae is a closed photobioreactor (PBR). In a closed reactor system, the circulation of water through pumps is enabled such that alga is cultivated in suspension under artificial light or sometimes waste heat. The continuous monitoring of nutrient and gas levels is pertinently achieved. High productivity, low contamination, efficient CO_2 capture, continual runs, and controlled algae growth merit the PBR process. Nevertheless, the drawbacks often suggested are high capital and operating costs. In view of cost, many design and operational challenges are ahead for revisions.

The design challenges under consideration worldwide are:

- Fouling and cleaning of any system consisting of external and internal walls
- Prevention of light penetration through PBR due to accumulating dirt (external) or algae (internal)
- Ensuring the optimum photosynthetic efficiency through mixing
- Maintaining turbulent flow by pumping or sparging with gases through energy supply
- Maintaining the positive energy balance for the entire process by minimizing the parasitic energy load

The structural design, in particular the depth of PBR needs to be in such a way of allowing sufficient light to penetrate the reactor system. The reactor (PBR) system with poor design restricts light accessibility and does not facilitate algae productivity. Equally algae suffer photo-inhibition due to overexposure to sunlight. In addition, the reactor design must support effective mixing of CO_2 and other nutrients where CO_2 bubbling is another way of maximizing its capture and reducing the mixing costs. Carbon is the key element and its composition is about 45% in microalgae.

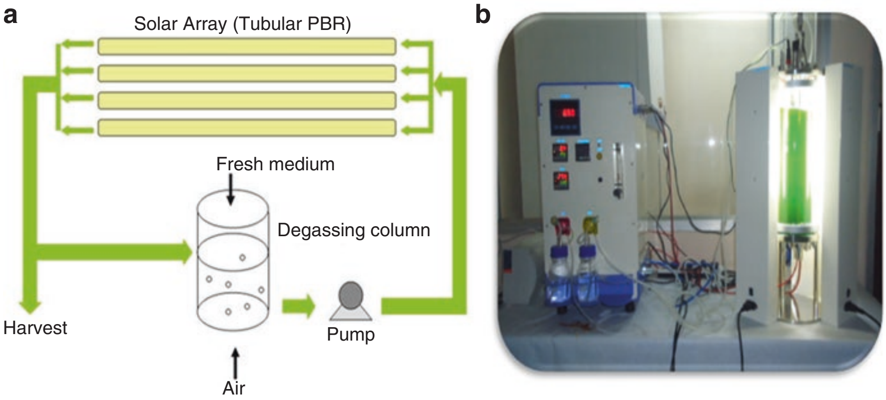

Fig. 5.11 Tubular photo-bioreactors (**a**). Airlift photo-bioreactor for microalgae cultivation (**b**)

It is an estimable 1.65 kg of CO_2 requirement essential for the production of 1 kg of microalgae based on mass-balance approach (Berg-Nilsen 2006). As a matter of fact, PBR design may not be an agreed "one-size-fits-all" approach because the thriving climatic conditions will be different for microalgae in different systems. Photo-bioreactors at reduced cost are designed with simple materials such as transparent pipes by utilizing solar lighting and gravity feeding of the growth medium.

The vital components viz. light especially from the solar radiation and temperature influence the growth of microalgae. Locations such as tropical and equatorial regions which vary roughly between 35°N and 35°S are corroborated to be inclusive to grow high biomass. The third component is nutrient supply. The availability of phosphorous (as phosphate) less than 0.2 μmol L^{-1} and nitrogen (as nitrate) less than 2 μmol L^{-1} facilitates the growth of algal medium (Rasmussen 2007). Diatoms require silicon (as silicate less than 2 μmol L^{-1}) content, which is the growth limiting factor in addition to N and P and seems to be feasible as a result of freshwater run-off from land to sea in the form of silicate leaching. However, a plethora of opportunities for all the nutrients is rather possible by way of run-off fertilizers from cropland which ultimately end up in rivers and estuarine systems.

The mostly tubular design photo-bioreactor used (Fig. 5.11a) for microalgae cultivation has a number of clear transparent tubes, usually aligned with the sun's rays. These tubes are generally less than 10 cm in diameter to maximize sunlight penetration. The medium broth is circulated through a pump to the tubes, where it is exposed to light for photosynthesis. A portion of the microalgae is usually harvested after it passes through the solar collection tubes, making continuous algal culture possible. In some photo-bioreactors, the tubes are coiled spirals to form what is known as a helical-tubular photo-bioreactor. These systems sometimes require artificial illumination, which adds to production costs, so this technology is only used for high-value products. Either a mechanical pump or an airlift pump maintains a highly turbulent flow within the reactor, which prevents the algal biomass from settling (Chisti 2007).

Photo-bioreactors (Fig. 5.11b) allow the control of various abiotic factors. Light intensity and photoperiod may be controlled depending on the light source (Borowitzka 1999; Chisti 2007). Photo-bioreactors allow the ability to grow monocultures (Pure culture). The factor that may make the greatest contribution to the choice of a photo-bioreactor over a raceway pond is the productivity in which configuration is more viable (Chisti 2007). Microalgae life cycle analysis (LCA) is an important factor in the determination of the system of choice. The net energy return from photo-bioreactors is less than that of raceway ponds more economical (Cambell et al. 2011; Collet et al. 2011). Selecting a suitable microalgae biomass production method for making biodiesel requires a comparison of capabilities between raceways and photo-bioreactors (Pulz 2001). Comparison of raceways and tubular is shown in Table 5.3. In these production methods, the comparison is made for optimal combinations of microalgae biomass productivity and concentration that have been actually achieved in large-scale photo-bioreactors and open raceway ponds. Table 5.4 represents the comparison between the photo-bioreactor and open raceway pond methods of producing biomass of microalgae. This comparison is for an annual production level of 100 tons of biomass in both cases. Both production methods consume an identical amount of carbon dioxide, if losses to atmosphere are disregarded. Photo-bioreactors provide much greater oil yield per hectare as compared to open raceway ponds (Molina Grima et al. 1999; Tredici 1999; Pulz 2001).

A low-cost airlift PBR for marine microalgae culture designed by Xu et al. (2002) registered the volumetric output rate of 0.21 g L^{-1} day^{-1} at the superficial gas velocity of 4 mm per second in the draft tube for *chlorella sp.* in the batch culture. The cultivation of *Skeletonema costatum* using airlift PBR was explored to be superior to the bubble column in terms of specific growth rate of 0.046 h^{-1} with the maximum cell concentration of 4.6×10^6 cells per mL and the productivity of 6.5×10^4 cells per second, influenced by the design which alleviates more light intensity and incidence for the effective utilization of algae (Monkonsit and Powtongsook 2011). The promising mass cultivation of a cost-effective rectangular airlift loop PBR for the production of microalgae was reported by Guo et al. (2015). Jeffryes et al. (2016) attempted an interesting PBR geometry supported by a new mathematical expression that could achieve a creditable annular-plate airlift PBR of 55 L capacity with stupendous energy-efficient production of algae biomass. A coupled model integrating airlift and flat plate reactors was designed for the enhancement of algae production with better light–dark cycling and higher surface to volume ratio of about 7% as compared to the conventional airlift PBRs (Soman and Shastri 2015). Bagnoud-Velasquez et al. (2015) established the possibility of driving the nutrient neutral production of biomass with zero release of waste by efficient water management together with nutrient recovery with the designed FPA–PBR model. Similarly, with the large-sized, flat panel airlift photo-bioreactor (FPA PBR), a sufficient rigidity to continuously culture the microalgae was reported by Lee and Ahn (2015). The culture characterization of *Dunaliella salina DCCBC2* using this model lessened the culture time and continuous culture of about 29% and 19% respectively as compared to the conventional FPA-PBR models.

Table 5.3 Generalized comparisons of two different cultivation methods of algae production

Factors	Open ponds	Photo-bioreactors
Cultivation	Multi-strain cultivation	Well suitable for single-strain cultivation
Contamination	High	Less to none
Cleaning	None	Required due to wall growth and dirt
Controlling of growth conditions	Very difficult	Easy
Temperature	Highly variable	Required cooling
Automatic cooling system	None	Built in
Automatic heating system	None	Built in
Microbiology safety	None	UV
Biomass quality	Variable	Reproducible
Biomass productivity	Low	High
Lipid productivity	Low	High
Light utilization efficiency	Low	High
Air pump	Built in	Built in
Shear	Low	High
CO_2 transfer rate	Poor	Excellent
Mixing efficiency	Poor	Excellent
Water loss	Very high	Low
Evaporation	High	No evaporation
O_2 concentration	Low due to continuous spontaneous out gassing	Build-up occurred requires gas exchange device
CO_2 loss	High, depending on pond depth	Low
Space required	High	Low
Periodical maintenance	Less	More
Capital investment	Low	High
Operating cost	Lower	Higher
Harvesting cost	High	Lower
Scale up technology for commercial level	Easy to scale up	Most of photo-bioreactor models are difficult to scale up due to limitations

11 Advantages of Microalgae-Based Biofuel Production

11.1 *Reduce Carbon Emissions Rates*

Biofuels are considered as the best solution to the cropping-up environmental problems especially the climate change. Carbon emissions from combustion of biofuels are significantly lower than fossil fuels. Usually biodiesel is blended with petro diesel in the ratio 20:80 and named as B20; whereas, 100% pure biodiesel is called B100. Table 5.5 represents the comparative features for B100 and B20 in terms of lessened pollutant emissions (Annual Report 2003–04, Planning Commission, Government of India).

Table 5.4 Comparisons of photo-bioreactor and raceway production methods

Variable	Photo-bioreactor	Raceway ponds
Annual biomass production (kg)	100,000	100,000
Volumetric productivity (kg m−3 day^{-1})	1.535	0.117
Areal productivity (kg m−2 day^{-1})	0.048[a]	0.035[b]
	0.072[c]	
Biomass concentration in broth (kg m−3)	4.00	0.14
Dilution rate (day^{-1})	0.384	0.250
Area needed (m2)	5681	7828
Oil yield (m3 ha−1)	136.9[d]	99.4[d]
	58.7[e]	42.6[e]
Annual CO_2 consumption (kg)	183,333	183,333
System geometry	132 parallel tubes/unit; 80 m long tubes; 0.06 m tube diameter	978 m^2/pond; 12 m wide, 82 m long, 0.30 m deep
Number of units	6	8

[a] Based on facility area
[b] Based on actual pond area
[c] Based on projected area of photo-bioreactor tubes
[d] Based on 70% by wt oil in biomass
[e] Based on 30% by wt oil in biomass

Table 5.5 Comparison of emission rate between biodiesel and diesel

Emissions	B100 pure biodiesel	B20 (20% biodiesel and 80% petroleum diesel)
Regulated emissions (%)		
Total unburned hydrocarbons	–93	–30
Carbon monoxide	–50	–20
Particulate matter	–30	–22
NO_x	13	2
Non-regulated emissions (%)		
Sulfates	–100	–20
Polycyclic aromatic hydrocarbons (PAHs)	–80	–13
Nitrated PAHs (NPAHs)	–90	–50
Ozone potential of HC	–50	–10
Life cycle emissions (%)		
Carbon dioxide	–80	Nil
Sulfur dioxide	–100	Nil

(−) less percentage of emission from biodiesel as compared to 100% petroleum diesel, (+) More percentage of emission from biodiesel as compared to 100% petroleum diesel

11.2 Biofuels: Resemblance and Energy Conservation

Biofuels are simple and familiar to consumers, producers, and policy makers. In the past two decades, ethanol has been used as an additive or as a blend with gasoline. In fact, Henry Ford and Rudolph Diesel respectively the grandfathers for the automobile assembly line and the diesel engine forecasted the dependence of future transportation fuels from plant/algal-based sources. Hazell and Pachauri (2006) acknowledged that every country is able to produce its own fuel such that the dependence of foreign resources for energy can be reduced. Physical and chemical characteristics of biofuels including their liquid state, specific energy density, viscosity, and combustion characteristics are more similar to gasoline or diesel than for alternatives. Biofuel is combustible in existing internal combustion engines with minor changes which further facilitates the blending process at low levels of about 10–20% (De la Torre Ugarte 2006).

12 Conclusion

Microalgae play a quintessential role to the burgeoning biofuels and is a more abundant, affordable, and sustainable feed-stock. It is quite advantageous to establish a parallel foundation for cultivating algae in areas which do not merit for direct agriculture but could promote the access of unique resource utilization of mixed feedstocks. Research and developmental activities venture into strategic investments to support algal biofuels and focus on to minimize the level of risks and uncertainty in pertinent to the commercialization of the algae to biofuel process. It is also rather plausible by achieving the energy contribution from marine algae and is likely to be anticipated in 2020. High input of dedicated research capacity and competence could empower the exploration potential and appropriate technologies in marine algae production and hence derived biofuels.

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Chapter 6
Harvesting of Microalgae for Biofuels: Comprehensive Performance Evaluation of Natural, Inorganic, and Synthetic Flocculants

Sanjay Kumar Gupta, F.A. Ansari, Kuldeep Bauddh, Bhaskar Singh, A.K. Nema, and K.K. Pant

Abstract Microalgal biomass is considered as one of the most suitable alternative feedstocks for the renewable biofuels. Microalgae have several advantages such as ability to grow in harsh environment, comparatively very high productivity, and high lipid contents. Due to such potentials, microalgal biomass is preferred over the convention biofuel feedstocks. The concentration of microalgal biomass typically ranged between 0.5 and 1 kg/m^3 in the raceways or open pond type cultivation systems and around 5–10 kg/m^3 in the closed photobioreactor-type cultivation systems. The bottleneck of the algal biofuels is the harvesting of microalgae biomass from diluted culture media. Irrespective of the density of the algal biomass, the water content in microalgal culture exceeds 99% that makes the separation process lengthy and energy intensive. This largely determines the economic viability of microalgae-based biofuels and by-products. Among various techniques used for the harvesting of microalgal biomass, coagulation and flocculation have been found very effective and inexpensive; however, the choice of the coagulant depends on the use of harvested biomass for desired end products. The success of microalgae har-

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vesting by flocculation requires thorough understanding about the nature of the flocculants, its molecular weight, mode of interaction, etc., along with the understanding about the algae species to be harvested. Harvesting of microalgae by coagulation and flocculation has its own advantages and disadvantages; however, being simple and cost-effective, it is one of most preferred techniques especially if the biomass is used for biofuels.

1 Introduction

1.1 Microalgae: A General Overview

The size of microalgae generally range between 2 and 50 μm in diameter and the optimum dry biomass concentration range from 0 to 0.5 mg/L. in poorly managed algal cultures in open ponds and raceways, whereas the concentration of microalgae may range between 1 and 2.5 mg/L. in laboratory-controlled conditions. The typical cell loading ranges between 0.1 and 4 wt%. Moreover, the microalgae cells possess negative charges; therefore, such cells remain in the suspended forms due to electrostatic repulsion. That is why the harvesting of microalgae faces several challenges.

From past few decades microalgal biomass have gained much consideration as a promising feedstock of various types of biofuels due to its inherent potential of faster growth rate and accumulation of lipids and other important metabolites (Morweiser et al. 2010; Tsukahara and Sawayama 2005; Olguín 2012; Ansari et al. 2015; Balasubramani et al. 2016; Rawat et al. 2016). In microalgal biomass, the protein contents range from 20 to 50 wt%, carbohydrates 10–40 wt%, and lipids 10–70 wt%. However, the concentration of all of these three metabolites can be manipulated depending upon the requirements. The microalgae can grow in very harsh environment and the area-specific biomass yield is very high. Additionally, microalgae are good sequesters of atmospheric CO_2. Many studies have shown its phycoremediation potential, i.e., removal of various types of the nutrients such as nitrogen and phosphorus from water (Di Termini et al. 2011; Sawayama et al. 1998, Wu et al. 2012a; Sriwastav et al. 2014; Gupta et al. 2016).

The wastewater treatment by the use of various types of flocculants is in practice since long (Tenney et al. 1969; McGarry 1970; Sukenik et al. 1988; Buelna et al. 1990; Gupta et al. 2016); however, harvesting of microalgal biomass through flocculation is in practice from past few years (Knuckey et al. 2006; Henderson et al. 2008; Vandamme and Foubert 2010). Due to ease of the process and comparatively cost-effectiveness, the harvesting of microalgae through either natural or chemically induced flocculation got recognition around 10–15 years back. However, the efficiency of the flocculants and flocculation yields depends on several environmental variables, chemical properties of the flocculants, type of water media (i.e., fresh or marine), and more importantly, the species of the microalgae.

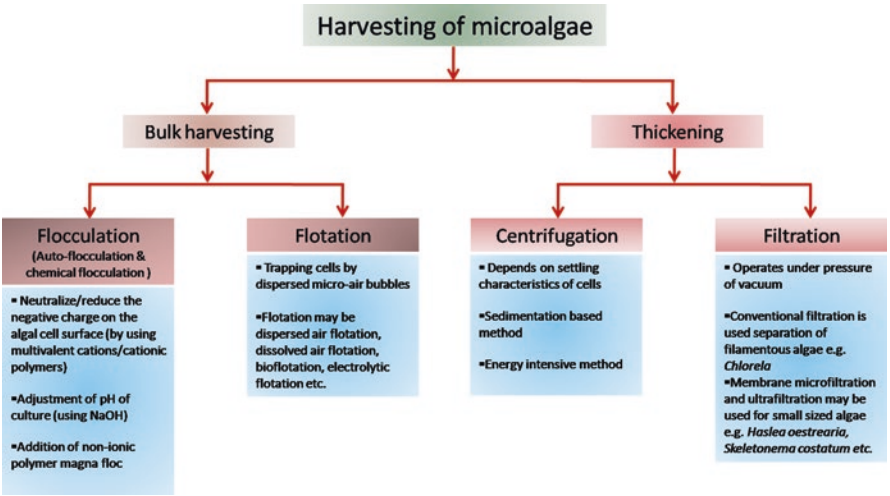

Fig. 6.1 Common methods of harvesting of microalgae

1.2 Harvesting of Microalgae

The harvesting of microalgae from its source is one of the key steps for the production of biofuel at a large scale. The techniques which are efficient and economically viable to harvest make the end product (biofuel) cost-effective (Shelef et al. 1984; Park et al. 2011). Due to the small size, low density and negative charge on the surface, harvesting of microalgae is a challenging task (Molina et al. 2003; Wang et al. 2008; Greenwell et al. 2010). A cost-effective process of production of biofuel from algae is needed to match with the cost of contemporary crude oils. The microalgae can be harvested by a number of methods. Among the existing methods, the two basic and contemporary harvesting techniques are "bulk harvesting" and "thickening". Bulk harvesting is done at large scale which may be done by two processes, i.e., flocculation and flotation, whereas in the thickening, dewatering takes place by centrifugation and filtration of microalgal cells (Fig. 6.1). Bulk harvesting is a large-scale separation of microalgae from the growing medium. The concentration factor ranged from 100 to 800 times and which is dependent on the culture types, species of microalgae and method of harvesting. Bulk harvesting may be achieved by two basic techniques, i.e., flocculation and flotation.

1.2.1 Flocculation

Flocculation is based on the neutralization/reduction of the negative charge present on the surface of algal cell by using flocculants (either natural or synthetic) that agglomerate the algal cells in the form of flocks which allow the cells to settle down in the culture medium. Electrostatic patch mechanism, bridging, sweeping flocculation, etc., are other approaches of flocculation method for the harvesting microalgae (Vandamme et al. 2013). Generally, the multivalent cations and cationic polymers are used to harvest the algae through this process (Molina et al. 2003; Wang et al. 2008). Flocculation is of two types: (i) auto-flocculation and (ii) chemical flocculation.

Auto-Flocculation

In the auto-flocculation, microalgae flocculate naturally without adding any flocculants, which is due to the presence of salts in the culture medium (Ayoub et al. 1986; Knuckey et al. 2006; González-Fernández and Ballesteros 2013; Brady et al. 2014). It has been reported that with the increase in pH of seawater (generally from ~ 8 to 9.5–11), algae starts to autoflocculates due to interactions (electrostatic) between cations such as Ca^{2+}, Mg^{2+}, etc. (Ayoub et al. 1986; Sheehan et al. 1998; Knuckey et al. 2006). In the phosphate enriched wastewater too, microalgae auto-flocculate as calcium phosphate precipitation takes place (Sukenik and Shelef 1984).

Chemical Flocculation

Chemical flocculation is done by adding chemicals like metallic salts (e.g., iron chloride, iron sulfate, aluminum sulfate, etc.), alkaline compound, polyelectrolytes, etc. (Ben-Amotz and Avron 1990; Brady et al. 2014). Polyacrylamide polymers are also common chemical flocculants; however, it provides a chance to contaminate the algal biomass with toxicant acrylamide (Bratby 2006). Biopolymer chitosan derived as a waste material (chitin) from shellfish is an effective flocculent that works efficiently at relatively low pH (Chang and Lee 2012).

1.2.2 Flotation

Flotation is a common method for harvesting of microalgae (Ma et al. 2007; Edzwald 2010; Cheng et al. 2010; Xu et al. 2010). It is a separation technique based on the gravity of solid particles in which air/gas bubbles are attached to the algal cells and are carried to the surface of medium in the form of float from where it may be skimmed out (Edzwald 2010). The efficiency of this process may be increased by using air bubbles (40–80 μm) produced artificially by inserting pressurized air (Edzwald 2010). This approach is known as dissolved air flotation (DAF). The particles float at the top of the culture medium by attaching them to air bubbles. The particles float at the top of the culture medium by attaching them to air bubbles. Further, chemical coagulants like ferric salts, aluminum, etc., may be used to smooth the process flotation. In addition, dispersed air flotation, bioflotation, and electrolytic flotation are few other form of flotation that may also be used to achieve substantial efficacy.

1.2.3 Thickening

Thickening is aimed to concentrate harvested microalgae cells in the form of tick slurry or cake of the cells. The major objective of thickening is to the removal of water to maximum extent from the extracted/collected microalgae cells. The major tools using for thickening are centrifugation filtration and electrical. Centrifugation is an efficient method of thickening but may be applied only at small scale. Filtration is also a potential method of thickening of microalgae cells. It comprises vacuum filtration, deep-bed filtration, cross-flow ultrafiltration, magnetic filtration, etc.

1.2.4 Centrifugation

Centrifugation method is not economically feasible to harvest the algae at large scale due to its energy intensive nature; however, its application is very common in laboratory-based extraction. It depends on the application of centrifugal force; therefore, it takes less time for separation of cells from its growing medium. Pahl et al. (2013) has described that there are five types of centrifuge used to harvest microalgae cells viz. (i) perforated basket centrifuges, (ii) disc stack centrifuges, (iii) imperforated basket centrifuges, (iv) decanters or scroll centrifuges, and (v) hydro cyclones.

1.2.5 Filtration

Filtration process is used to harvest the microalgae having low density. One limitation of this process is blockage of filters; therefore, it requires timely maintenance. Several methods have also been investigated like reverse flow vacuum to tackle the problem of clogging of the filters. The efficiency of conventional filtration methods is quite low but suitable for the large (filamentous) algae like *Spirulina platensis*. For the filtration of smaller algae like *Chlorella*, *Dunaliella*, etc. as well as recovery of the residual cells; membrane microfiltration and ultrafiltration may be used where the cells passed through micropores which make these tools more effective (Uduman et al. 2010; Zhang et al. 2010). Rossignol et al. (1999) have suggested a polyacrylonitrile ultrafiltration membrane suitable for the recovery of algae *Hasleaostrearia* and *Skeletonemacostatum* from marine water. Filter presses, tangential (cross) flow filtration, gravity belt filters, vacuum filters (rotary drum), etc., are some common filtration methods used for the thickening of the microalgae (Pahl et al. 2013).

1.2.6 Electrical Thickening

Electrical thickening is generally based on electrical approaches like electrophoresis, electro-flotation, electro-flocculation, etc. (Pearsall et al. 2011; Show et al. 2013). An electric field is applied to the algae reactor by using metallic electrodes and the thickening of microalgae cells occurs at both electrodes. The growth medium has been reported to affect the electrical thickening (Pearsall et al. 2011). Some of the studies strongly recommend the use of electrochemical harvesting techniques which are characterized by the least energy consumption and least effects over the biochemical constitutes, i.e., fatty acid compositions, carbohydrate, and proteins concentrations in the harvested biomass (Guldhe et al. 2016).

2 Constrains of Microalgae Harvesting

The major constrains of microalgae harvesting are the high energy requirements and the quality of harvested biomass. The sustainability of the algal harvesting depends on its cost-effectiveness (Coons et al. 2014). The traditional harvesting by techniques such as centrifugation and filtration are highly energy intensive, therefore economically challenging for the pilot scale production of low value products from harvested microalgal biomass (Pienkos and Darzins 2009). Various studies have demonstrated that the harvesting yields vary with the type of harvesting (Guldhe et al. 2016, Gupta et al. 2014). Similarly, other techniques such as flotation, electrochemical harvesting need higher capital costs as well as required skilled manpower for achieving optimum outputs. Other than economics, the quality and quantity of the biomass yield entirely depends on the harvesting techniques. It has been observed that quality of the harvested biomass typically depends on the technique used. For example, the microalgal cells get damaged if the biomass is harvested by centrifugation which shortened the self-life of the harvested biomass. While, in the case of harvesting done by flocculation, the contamination of biomass is the major problem. Other than biomass contamination, the macro- and micro-metabolites of the algal cells also get affected during harvesting. Therefore, the harvesting is an important aspect and special attentions are needed for the selection of the harvesting techniques. Majorly based on specific end uses of the biomass and economics, such techniques are being selected.

3 Advantages and Disadvantages of Various Harvesting Techniques

Other than chemical coagulation/flocculation, numerous other techniques such as filtration, centrifugation, auto-flocculation, bio-flocculation, gravity sedimentation, flotation, electrochemical processes, etc., are used for the concentrating microalgal biomass. All of the above said methods have its own advantages and disadvantages. For example, sedimentation is the simplest and highly cost-effective technique of harvesting; however, it is considered less effective for wider varieties of the microalgae as it consumes too much time. Even some of the studies have reported deterioration of the microalgal biomass due to the requirement of longer time in the settling process. Centrifugation is one of the most efficient methods for separating both marine and freshwater microalgae from their culture; however, being energy intensive, it is not economical for low valued products. Moreover, due to high shear forces, the shear-sensitive microalgal species get damaged during centrifugation. For such shear-sensitive microalgal species, filtration is one of the easiest techniques and the recoveries are also high; however, cleaning of membranes is one of the major hurdles. The operational cost of cleaning of membranes, replacement of the membranes, or safeguarding the membrane by fouling/clogging is too high to be economical. Flotation is also a comparatively simpler technology that requires small space and is feasible for pilot scale harvesting. This method is comparatively short time taking and low cost as well. However, the method is unsuitable for harvesting of marine microalgae species. In comparison, electrochemical harvesting can be used for diverse group of macroalgae species. Nevertheless, such techniques are less used due to high capital costs and energy requirements.

In flocculation, generally three types of harvesting are practiced. First is the auto-flocculation and second is the bio-flocculation, both of the techniques have been found effective and inexpensive as well. The major advantages of both of the methods are that the flocculated wastewater can be reused. However, microbial contamination and changes in the biochemical composition of the harvested biomass have been reported in some of the cases. The third type of harvesting is aided through use of chemical flocculants. However, neutralization of the wastewater is required before disposal while using chemical aided flocculation which adds additional costs. Chemical bases coagulation and flocculation has its own advantages and disadvantages. Being simple and cost-effective due to least energy requirements, harvesting by coagulation and flocculation is a most preferred technique (Grima et al. 2003, Knuckey et al. 2006). Additionally being comparatively very simpler among other techniques, there is no requirement of sophisticated instruments or trained manpower. Nevertheless, there are possibilities of chemical contamination of the harvested biomass; therefore, the selection of the flocculants is of prime concern.

4 Harvesting of Microalgae by Flocculation

The flocculation of microalgae is accomplished by the addition of various types of flocculants. Similar to the wastewater flocculation, the optimized doses of the flocculants are added to the selected portion of microalgae culture and rapidly mixed for 5–10 min. The mixture is then allowed for slow mixing for flock developments. The slow mixing is done for around 20–30 min. After that, sedimentation of large algal floc is accomplished by gravitation. The settled slurry is taken out and dewatered. The thickened microalgal biomass is further dried either by sun drying or using hot air oven. The biomass is further used for various purposes, i.e., extraction of valued products, food supplements, fodder, and biofuels. However, the harvested biomass contains traces of flocculant used; therefore, the choice of the flocculants, i.e., natural (cationic starches, chitosan, guar gum, etc.), inorganic (multivalent metal salts), or synthetic organic flocculants (polyelectrolytes, polyelacralycs, etc.) entirely depend on the use of harvested biomass for desired end products. The success of microalgae harvesting by flocculation requires thorough understanding about the chemical nature of the flocculants, its molecular weight, mode of interaction of flocculant with microalgae, pH of the algae culture, etc. Similarly, the information about the algae species to be harvested, its cell size, etc., eases the harvesting and optimum recovery of the biomass. Fig. 6.2 shows an overview of harvesting of microalgae by flocculation and evaluation of biomass for biofuels.

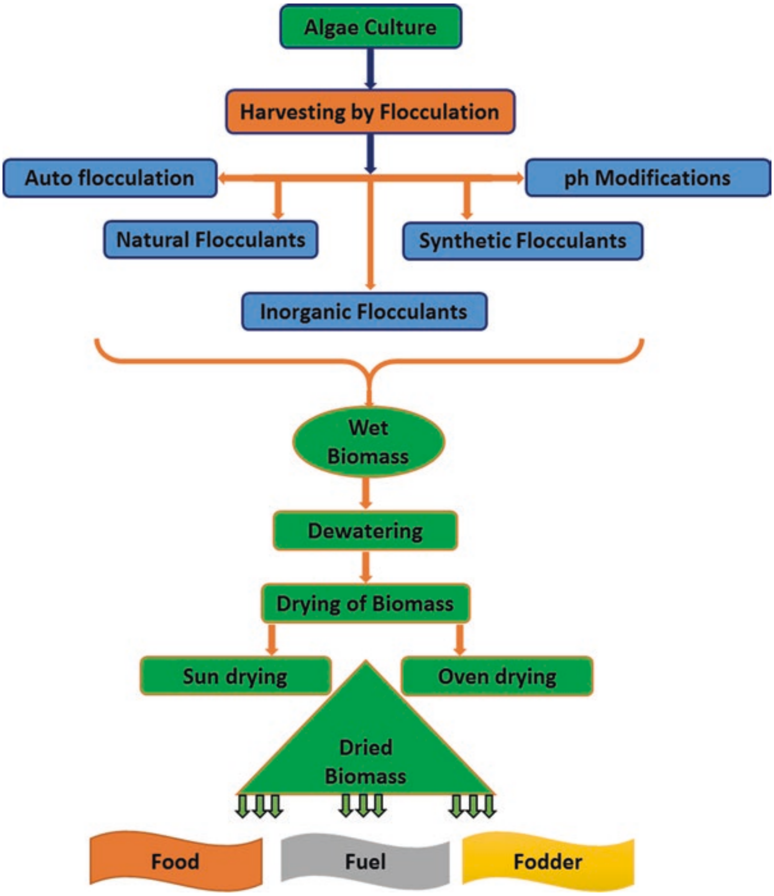

Fig. 6.2 An overview of harvesting of microalgae by flocculation and evaluation of biomass for biofuels

4.1 Mechanism of Algae Coagulation and Flocculation

The flocculation of microalgae is accomplished by addition of various types of flocculants. Similar to the wastewater flocculation, the optimized doses of the flocculants are added to the selected portion of the microalgae culture and rapidly mixed for 5–10 min. The mixture is then subjected to slow mixing for flock developments. The slow mixing is done for around 20–30 min. After that, sedimentation of large algal floc is accomplished by gravitation. The settled slurry is taken out and dewatered. The thickened microalgal biomass is further dried either by sun drying or using hot air ovens. The biomass is further used for various purposes, i.e., extraction of valued products, food supplements, fodder, and biofuels. However, the harvested biomass contains traces of flocculant used; therefore, the choice of the flocculants, i.e., natural (cationic starches, chitosan, guar gum, etc.), inorganic (multivalent metal salts), or synthetic organic flocculants (polyelectrolytes, polyelacralycs, etc.) entirely depend on the use of harvested biomass for desired end products (Borges et al. 2011).

The success of microalgae harvesting by flocculation, i.e., optimum recovery of biomass requires thorough understanding about the chemical nature of the flocculants, its molecular weight, mode of interaction of flocculant with microalgae, pH of the algae culture, etc. Similarly, the information about the algae species to be harvested, its cell size, etc., eases the harvesting and optimum recovery of the biomass.

The basic flocculation mechanism of microalgae is similar to the flocculation of water. Mostly, algal flocs develop by three forces, i.e., charge neutralization, adsorption, and adsorption bridging. As the microalgal cells possess negative surface charges due to presence of amine ground (NH) and carboxylic groups, therefore, in the water medium such cells remain electrostatically stabilized. The electrostatically stabilized microalgal cells remain in the suspension and such stabilization does not get affected by pH which is one of the most unique properties of microalgae (Ives 1959). However, the increasing pH unstabilizes the surface charge that is why some of the microalgae get flocculated and settled down by gravity, which is termed as auto-flocculation. Most of the microalgal species release some organic chemicals which are known as exudates and remain in the culture. Such extracellular organic chemicals reduce the flocculation efficiency of the flocculants (Henderson et al. 2006; Shin et al. 2008). Therefore, overcoming to the effects of algal exudates on flocculation is another hurdle as the higher doses of flocculants are required which poses additional economic burden.

The first and most important phenomenon of microalgae flocculation is the charge neutralization. When cationic flocculants (+ve charges) are added to the algae culture, these flocculants first destabilize and subsequently neutralize the –ve charges of the microalgal algal cell walls. Thus, the flocculation accelerates with charge neutralization. When all the negatively charged algal cells get neutralized with the positively charged flocculants, the flocculation is optimum. At this stage, algal cells aggregate together and form larger algae-flocculant flocs which get settled in due course of time. However, further increases of flocculants doses again destabilize the electrostatic charges, thus, the algal floc and the algal cells again get dispersed in the medium. Various factors such as pH and salinity of algal culture, chemical nature of the flocculant directly affect the flocculation rate. At higher pH, the algal cells automatically get precipitated in high alkaline medium. Sea/brackish waters contain relatively high concentration of divalent metal ions such as $Ca2^+$ and $Mg2^+$ which get precipitated as metal hydroxide. Sukenik and Shelef (1984) observed that at pH >8, the *Scenedesmus dimorphus* auto-flocculated and the flocculation efficiency was recorded over 98%, whereas at lower pH (5–7), any short of flocculation was not observed in sea water culture. However, the shift to high pH does not necessarily results in flocculation for all the algal species (Knuckey et al. 2006; Morales et al. 1985). The difference between various freshwater and marine water algal species may be attributed to the chemical composition of cell walls of various algal species; however, this has not yet been identified. As the flocculation efficiency of a given flocculant varies between species to species of algae, therefore, it is very important to optimize the flocculation doses in each case.

During the growth, microalgae also secrete some of the organic chemicals known as algal exudates. Such chemicals interfere with the charge distribution of the flocculants used for the harvesting; therefore, higher doses of the flocculants are required for optimum harvesting (Vandamme et al. 2012b). As it is now well understood that which type (i.e., marine or freshwater) of algal species secretes more organic compounds, therefore, these aspects need more investigations.

4.2 Factors Affecting Flocculation of Microalgae

Flocculation of microalgae is a typical process which is sensitive to the species harvested. There are several factors which determine the harvesting yields. Such prevailing factors could be flocculant-related, i.e., pH of the water, chemical nature of the flocculant, molecular weight, dose, ionic potential, etc. (Fig. 6.3). Similarly, various factors are related to microalgal culture such as microalgae species, size, concentration, cellular composition, and negative potential of the cell wall. All these factors play a major role in the flocculation processes. Therefore, the dose of a flocculant may vary substantially for two different microalgal species, thus influencing the harvesting efficiency. For example, the flocculation efficiency of Chitosan and Alum is pH-dependent and increases with decreasing pH for *Scenedesmus* sp. whereas, for the same species the flocculation of a polyamine-based synthetic flocculant was pH-independent (Gupta et al. 2014). Similarly, the flocculation efficiency increases with increase of the dose; however, after optimum dose, the flocculation efficiency gradually decreases with increase of flocculant doses. The higher ionic potential and molecular weight are also important factors that influence efficiency of harvesting. High molecular weight flocculants with higher cationic potential attain comparatively higher flocculation of microalgae at lower doses; whereas, higher doses are required for the low molecular weight

Fig. 6.3 Effects of various factors on flocculation of microalgae

flocculants having low cationic potential. The size of the microalgae is also a crucial factor for harvesting of high-density culture of smaller size microalgae where a higher dose of the flocculants may be needed.

4.3 Flocculants Type, Harvesting Efficiencies, and Effects on Biomass

The recovery of biomass from diluted algal cultures in which the dry biomass concentration typically ranges between 0.5 and 5 mg/L. costs more than two-third of the overall biomass production cost (Wu et al. 2012b). In this regard, pre-concentration of microalgal biomass with the help of aid of an appropriate chemical coagulants substantially decreases the cost of the harvesting (Pienkos and Darzins 2009; Brentner et al. 2011). Various types of flocculants are used for the harvesting of microalgae. Majorly, these flocculants can be classified into two groups, i.e., natural and chemical. The natural products are mainly the plant-based materials. The second group is the chemical-based flocculants. Based on their chemical characteristics, the chemical flocculants are also broadly classified further into two groups, i.e., inorganic flocculants and organic flocculants. The organic flocculants are mainly synthetic polymeric compounds having high molecular weight and charges. Based on the ionic properties, the synthetic flocculants may further be classified as anionic flocculants used for the flocculation of cationic molecules and the cationic flocculants which are used mainly for flocculation of the molecules possessing anionic potential. Most of the cationic flocculants are mainly used for the removal of suspended particles, thus have major utilization in wastewater treatments. Such flocculants are also used for flocculation of microalgae as well.

4.3.1 Natural Flocculants

Commonly three types of the natural flocculants are in use. These may be starch derivatives, polysaccharides, or alginates. The natural flocculants may be non-ionic, cationic, or anionic. All of these flocculants are water soluble and once added to the suspension, absorb the suspended particles and forms flock which get settled due to gravity. Depending upon the uses, the natural starches are chemically treated to improve its flocculation properties. For example, natural starch can be converted into anionic form by oxidation or cationic form by amine treatment. Guar gums are the most common examples of the polysaccharide-type flocculant. Most of these natural flocculants are highly effective over lower pH. Other common natural flocculants are namely chitosan, cationic starches, *Moringaoleifera* seeds, gelatin, and strychnospotatorum. Chitosan is prepared by deacetylation of chitin. Chitin is generally obtained from the exoskeleton of crustaceans and/or fungi. Maringa seeds powder is also used as a natural flocculant as it is comprised of dimeric cationic proteins. These cationic proteins act as an excellent flocculant and can absorb and neutralize electrostatic colloidal charges of algal cells.

Effect of Natural Flocculants on Biomass Recovery

Numerous studies have shown effective harvesting of various species of algae (marine and freshwater) with natural flocculants. Chitosan is one of the most studied natural flocculants and numerous studies have reported the flocculation efficiency in the range of 80–95% (Divakaran and Pillai 2002; Riaño et al. 2012; Sirin et al. 2012). Riaño et al. (2012) reported around 92% biomass recovery of *Microalgal-bacterial consortia* with chitosan. A comprehensive evaluation of flocculation efficiency of natural flocculant and biomass recovery is presented in Table 6.1. Similarly, Divakaran and Pillai (2002) also found the flocculation efficiency of around 90%. Other than chitosan, *Cobetia marina* L03, poly γ-glutamic acid, *Moringa oleifera,* etc., have also been found effective in harvesting of *Chlorella* sp. and *Desmodesmus* sp. (Ndikubwimana et al. 2014; Lei et al. 2015). Moringa efficacy has been found in the range of 80–90% for *Chlorella* sp. (Hamid et al. 2014; Teixeira et al. 2012). In a study, Choi (2015) reported 98% biomass recovery of *Chlorella* sp. with eggshell. In another study, 55% flocculation efficiencies were achieved for *A. falcatus* harvested by chitosan (Guldhe et al. 2016). The flocculation efficiency of chitosan is also pH-dependent and therefore for efficient harvesting of microalgae, an optimization of the pH is must (Rashid et al. 2013).

4.3.2 Inorganic Flocculants

Among all types of coagulants and flocculants, the inorganic flocculants are comparatively cost-effective and can be applied for various types of water matrix. Such coagulants are widely used for water and wastewater treatments; however, many studies have shown effectiveness of wide variety of inorganic coagulants for harvesting of various microalgal species. Similar to the water, the algal coagulation and flocculation with such coagulants is based on the sweep-floc type of mechanism. In this type of coagulation and flocculation, the inorganic coagulants such as aluminum sulfate or ferric chloride, etc., forms aluminum or ferric (i.e., metal) hydroxide

Table 6.1 Comprehensive evaluation of flocculation efficiency of natural flocculant and biomass recovery

Microalgae	Natural flocculants	Biomass recovery (%)	Reference
Scenedesmus sp.	Chitosan	90–95	Gupta et al. (2014)
Chlorella vulgaris	*Cobetia marina* L03	72.7	Lei et al. (2015)
Desmodesmus sp.	poly γ-glutamic acid	43–98	Ndikubwimana et al. (2014)
Chlorella sp.	*Moringaoleifera*	90	Hamid et al. (2014)
Mix culture	Ecotan and Tanfloc	90	Gutiérrez et al. (2015)
Spirulina sp.	Chitosan	>90	Divakaran and Pillai (2002)
Oscillatoria sp.	Chitosan	>90	Divakaran and Pillai (2002)
Chlorella sp.	Chitosan	>90	Divakaran and Pillai (2002)
Microalgal-bacterial consortia	Chitosan	92	Riaño et al. (2012)
Chlorella sorokiniana	Chitosan	90	Xu et al. (2013)
Phaeodactylum tricornutum	Chitosan	80–90	Sirin et al. (2012)
Chlorella vulgaris	Eggshell	98	Choi (2015)
Chlorella vulgaris	Modified spent yeast	91	Prochazkova et al. (2015)
Chlorella vulgaris	Moringa oleifera	88	Teixeira et al. (2012)
Chlorella vulgaris	Chitosan	85	Vandamme et al. (2012b)
N. salina	Chitosan	98	Garzon-Sanabria et al. (2013)
M. aeruginosa	Tanin	97	Wang et al. (2013)
N. oculata	Tanfloc SL	97	Roselet et al. (2015)
C. vulgaris	Tanfloc SL	100	Roselet et al. (2015)

precipitate with the calcium and magnesium salts and hydrate, which settle down with the colloidal particles. Though most of the above listed inorganic coagulates are effective in harvesting of the microalgae, the contamination of harvested biomass is of concern. Therefore, the use of such flocculants depends on the end use of the algal biomass.

Effect of Inorganic Flocculants on Biomass Recovery

The inorganic flocculants are mainly metallic hydroxides having polymeric structure. The activated silica and some of the clay materials such as bentonite soils possess the colloidal properties. The coagulation and flocculation with most of these flocculants are mainly based on adsorption and charge neutralization. Being cost-effective, the inorganic coagulants are widely used for wastewater treatments; however, the hazardous waste sludge production is of major concern. In case of the harvesting of microalgae, most of the studies have reported >90% flocculation efficiency of the common inorganic coagulants such as aluminum sulfate, ferric chloride, ferric sulfate, etc. (Sirin et al. 2012; Teixeira et al. 2012; Kim et al. 2015). Similarly, many previous studies have also reported higher flocculation efficiencies of sodium, potassium, and calcium hydroxide for various microalgae species (Table 6.2). Schlesinger et al. (2012) reported 90–95% biomass recovery of *Phaeodactylum* sp., *Nannochloropsis* sp., *Pavlova* sp., and *Isochrysis* sp. with calcium hydroxide. Whereas, the biomass recovery of *Nannochloris* was approximately 66% with sodium and potassium hydroxide. In the case of *Isochrysis* sp., the higher recovery (95%) was recorded with ammonium and potassium hydroxide (Schlesinger et al.

Table 6.2 Comprehensive evaluation of flocculation efficiency of inorganic flocculant and biomass recovery

Microalgae	Natural flocculant	Biomass recovery (%)	Reference
Scenedesmus sp.	Alum	90–95	Gupta et al. (2014)
A. falcatus	Alum	86	Guldhe et al. (2016)
Chlorella sp. KR-1	Ferric chloride & sulfate	99	Kim et al. (2015)
Chlorella vulgaris	Methyl-esterified clay	99	Choi (2016)
Phaeodactylum tricornutum	Aluminum sulfate	83	Sirin et al. (2012)
Chlorella vulgaris	Aluminum sulfate	94	Teixeira et al. (2012)
Chlorella vulgaris	Cationic starch	85	Vandamme et al. (2012b)
Phaeodactylum	Calcium hydroxide	97	Schlesinger et al. (2012)
Nannochloropsis	Calcium hydroxide	95	Schlesinger et al. (2012)
Pavlova	Calcium hydroxide	90	Schlesinger et al. (2012)
Isochrysis	Calcium hydroxide	90	Schlesinger et al. (2012)
Nannochloris	Sodium hydroxide	66	Schlesinger et al. (2012)
Nannochloris	Potassium hydroxide	66	Schlesinger et al. (2012)
Isochrysis	Ammonium hydroxide	95	Schlesinger et al. (2012)
Isochrysis	Potassium hydroxide	95	Schlesinger et al. (2012)
Nannochloris oculata	Aluminum chloride	97	Garzon-Sanabria et al. (2013)
Chlorella vulgaris	Blast agent (sibfloc)	90	Gorin et al. (2015)
Nannochloropsis salina	Aluminum nitrate Ferric sulfate	84–99	Rwehumbiza et al. (2012)
Botryococcus branuii	Aluminum sulfate	90	Lee et al. (1998)
Chlorella minntissima	Aluminum chloride Ferric sulfate, zinc chloride	>90	Papazi et al. (2010)
Isochrysis galbana	Aluminum sulfate	>97	Sukenik et al. (1988)
Chlorella stigmatophora	Aluminum sulfate	>97	Sukenik et al. (1988)
Chlorella vulgaris	Aluminum sulfate	>97	Sukenik et al. (1988)

2012). Kim et al. (2015) reported 99% biomass recovery of *Chlorells* sp. with ferric chloride and ferric sulfate. Similarly, in a study Gupta et al. (2014) reported 90–95% flocculation efficiency of alum for *Scenedesmus* sp.; however, the biomass recovery with alum was pH-dependent. Guldhe et al. (2016) reported comparatively higher time required for the harvesting of *A. falcatus* by flocculation with alum (flocculation efficiencies 86%) and chitosan (flocculation efficiencies 55%). The major drawback of the harvesting of microalgae with inorganic flocculants is the contamination of the biomass (Grima et al. 2003).

4.3.3 Synthetic Flocculants

The synthetic flocculants are majorly polymeric composite of acrylamide and works on the principle of adsorption bridging of colloidal particles and polymeric chains of the acrylamide. The molecular weight and the charge density of such polymers depend on the monomers and its copolymerization. Therefore, the cationic or anionic potential of such type of synthetic flocculants is being designed for its specific use. Therefore, synthetic flocculants may be polyamines, polyethylene-oxide, polyamides-amines, polyethylene-imines, or sulfonated compounds. For example, the anionic potential increases if the copolymerization of acrylamide is done with anionic monomer such as acrylic acid. Similarly, to prepare cationic flocculant, copolymerization of acrylamide is done with any of the cationic monomer such as polyamine. The synthetic flocculants are polymer or copolymers of various types of monomers; therefore, if the chain length increases, the molecular weight of such polymers/copolymers increases. Various studies have demonstrated that the flocculation potential of such flocculants increases with the molecular weights of these types of the polymers (Wang et al. 2009).

Effect of Synthetic Flocculants on Biomass Recovery

The efforts are being made since long for the development of various types of synthetic flocculants which are primarily used for the water and wastewater treatments (Bolto and Gregory 2007; Choi et al. 2001; Wang et al. 2009). The application of the synthetic polymers for the harvesting of microalgae is comparably new. Various synthetic polymers have been tested for the microalgal harvesting purposes; however, quite a few were found effective (Gupta et al. 2014). A comprehensive evaluation of flocculation efficiency of synthetic flocculant and biomass recovery is shown in Table 6.3.

The designing and synthesis of the new cationic polymers for the harvesting of fresh water microalgae has gained attention and some of the synthetic copolymers have been found economically viable for the harvesting purposes (Gupta et al. 2014). The flocculation efficiency of such synthetic flocculants interlay depends on its chemical composition, i.e., the type of the polymer or copolymer, the ionic potential and molecular weight (Borges et al. 2011). The cationic polymers are

Table 6.3 Comprehensive evaluation of flocculation efficiency of synthetic flocculant and biomass recovery

Microalgae	Natural flocculant	Biomass recovery (%)	Reference
Scenedesmus sp.	Polyamine-based	95–99	Gupta et al. (2016)
Chlorella ellipsoidea	Polyethylenimine	97	Hu et al. (2014)
Chlorella vulgaris	Nano-aminoclays	99	Farooq et al. (2013)
N. salina	Flopam FO 4650 SH	73	Garzon-Sanabria et al. (2013)
N. oculata	Flopam FO 4650 SH	67	Roselet et al. (2015)
C. vulgaris	Flopam FO 4650 SH	100	Roselet et al. (2015)
N. oculata	Zetag 8185	75	Roselet et al. (2015)
C. vulgaris	Zetag 8185	100	Roselet et al. (2015)
C. vulgaris	Flopam FO 4650 SH	99	Roselet et al. (2015)
N. salina	Flopam FO 4800 SH	88	Garzon-Sanabria et al. (2013)
Chlorella sp.	Zetag 8819	98	Udom et al. (2013)
N. salina	Zetag 7570	10	Eldridge et al. (2012)
N. salina	Flopam FO 4990 SH	94	Garzon-Sanabria et al. (2013)
N. oculata	Flopam FO 4990 SH	90	Roselet et al. (2015)
C. vulgaris	Flopam FO 4990 SH	99	Roselet et al. (2015)

effective due to longer chain length which coagulate the microalgal cells by bridging processes. Similarly, polyamine-based cationic flocculants also neutralizes the charges of the algal cells; therefore, in most of the cases, microalgal cells gets agglomerated due to both charge neutralization and bridging (Gupta et al. 2014). In an independent study, Gupta et al. (2014) synthesized a polyamine-based copolymer and tested its effectiveness for the flocculation of *Scenedesmus* sp., a freshwater microalgae. The findings revealed efficient flocculation efficiency (>98%) over very low doses (8 mg/L) of the newly developed polyamine-ethylenediamine copolymer. Harith et al. (2009) reported comparable flocculation efficiency (>80%) of synthetic flocculant Magnafloc on *Chaetoceros calcitrans* and reported that there were no detrimental effects of flocculant over the microalgal cell viabilities.

4.4 Effects of Various Flocculants on Macro-Metabolites of Harvested Biomass

The major macrometabolites of microalgae are fatty acids, proteins, and carbohydrates. All of these metabolites are of economic importance with respect to food and fuel. Numerous studies have shown effective harvesting of both marine and freshwater microalgae with natural, inorganic, and synthetic flocculants; however, the major concern is the contamination of the harvested biomass. Previous studies have showed promising results with natural flocculants such as chitosan, moringa, guar-gum, etc., and found that biomass does not get contaminated (Banerjee et al. 2013; Ma and Hanna 1999; Teixeira et al. 2012). Nevertheless, pilot scale harvesting of microalgae with natural flocculants is not economic (Gupta et al. 2014).

Deleterious effects of chemical coagulants over the biochemical composition of microalgae have been reported earlier (Vandamme et al. 2012b). As some of the chemical coagulants adversely affect the major metabolites qualitatively and quantitatively therefore, the downstream processing of such contaminated biomass gets affected (Vandamme et al. 2012b; Gupta et al. 2014). In a comprehensive evaluation of the harvesting potential of natural, inorganic, and synthetic flocculants, Gupta et al. (2014) reported comparatively lower lipid yield for *Scenedesmus* sp. harvested by alum; however, fatty acid composition was least affected.

The use of harvested biomass for the biofuel production such as algal biodiesel depends on the lipid contents of the biomass. Therefore, harvesting techniques should not affect the lipid yield of the biomass. The influence of the chemical flocculants may be detrimental and can affect the fatty acid composition thus lower the lipid yields (Vandamme et al. 2012b). Gupta et al. (2014) reported the lipid yield of 14.3% DCW in the biomass harvested by chitosan compared to the 14.5% DCW lipid yield obtained in the biomass harvested by polyamine based synthetic flocculant, but substantial decrease, i.e., 10.2% DCW lipid yield was observed in the biomass harvested by alum. The findings clearly showed the deleterious effect of inorganic flocculant over the lipid yield of *Scenedesmus* sp. In a similar study, further analysis of the lipid yield and composition was recommended by Gerde et al. (2013) for the comprehensive evaluation of the effects of flocculants. While comparing the effectiveness of various harvesting techniques, Guldhe et al. (2016) found efficient harvesting, i.e., 91.3% biomass recovery of *A. falcatus* in 30 min by electrochemical techniques compared to the flocculation. It was reported that electrochemical harvesting using nonsacrificial electrodes had negligible effects over the biochemical composition of *A. falcatus* whereas the lipid, carbohydrate, and protein yield were fond comparatively low 24%, 15%, and 43% in the biomass harvested by alum compared to the electrochemical harvesting, i.e., 26%, 16%, and 45% respectively.

5 Evaluation of Harvested Biomass for Biofuels

Harvesting of algal biomass has been a challenge in a cost-effective production of biodiesel. The major constrain in harvesting the algal biomass lies in their small size and high water content (Sharma et al. 2011). Irrespective of the density of the algal biomass, the water content in cultured reactor exceeds 99% which makes the separation process lengthy and energy intensive. Among the common process adopted for separation of algae from its culture in laboratory scale is centrifugation. However, if adopted in large-scale separation, centrifugation process will be highly energy intensive and will have low EROI (energy return on investment)

(Hallenbeck et al. 2016). Hallenbeck et al. (2016) suggests that the low cost alternatives could be natural settling or flocculation-assisted settling which may include application of electrical input, filamentous fungi, etc. A viable option during harvesting of algal biomass includes its pre-concentration using flocculants. Gupta et al. (2014) reported improved microalgal flocculation using polyaniline polymer for harvesting of microalgae.

After the cultured biomass has reached optimum growth, the microalgae are to be harvested. Harvesting of microalgal biomass is achieved through dewatering which is done mostly through centrifugation process. However, centrifugation is considered uneconomical at commercial level. The harvested biomass is then subjected to various techniques to extract the lipids. Among the common method employed in laboratory studies are soxhlet extraction (using solvents like hexane, methanol, etc.), microwave-assisted oil extraction, and sonication. The extracted oil is then used for synthesis of biofuels viz. biodiesel, and bio-oil (Batan et al. 2016).

Among the green fuels derived from the microalgal oil includes biodiesel, bio-oil, bio-methane, and bio-hydrogen. Bio-oil is produced by various methods viz. fast pyrolysis, hydrothermal liquefaction under subcritical and supercritical conditions. The bio-oil derived from microalgae is highly complex containing a variety of constituents ranging from C_8 to C_{22} chain of fatty acids of aliphatic and aromatic hydrocarbons, phenols, and few nitrogen containing compounds (formed due to degradation of protein and chlorophyll present in microalgae). Bio-oil contains a substantial amount of oxygen and nitrogen which needs to be removed through deoxygenation and denitrification respectively for enhancement of their performance as fuel (Toraman et al. 2016). Biodiesel is produced by transesterification using either an acid or a base catalyst. Few authors have also tried doing direct transesterification using microalgal biomass. However, the yield has been less as compared to that obtained from microalgal oil. Gargano et al. (2016) reported alkaline direct transesterification of various species of *Stichococcus* for the production of biodiesel with a maximum yield of 17% at 60 °C and methanol/biomass weight ratio of 79:1.

Xie et al. (2015) reported fast co-pyrolysis of microalgae and scum using microwave technique using a zeolite (HZSM-5) as catalyst. Scum provided hydrogen that increased the effective hydrogen index (EHI) value of the feedstock. The use of two feedstocks to obtain bio-oil provided a synergistic effect at EHI > 7. Co-pyrolysis at a high temperature (500–550 °C) has been reported to favor the deoxygenation and denitrogenation reactions that are essential to enhance the fuel properties of the bio-oil. Chang et al. (2015) have reported catalytic hydropyrolysis of microalgal oil for the synthesis of bio-oil. Temperature has been reported to the most influencing factor in the formation of hydropyrolysis oil. Use of molybdenum carbide (MoC_2) as catalyst led to in situ deoxygenation and desulfurization of the hydropyrolysis. The high heating value of the fuel obtained through hydropyrolysis of microalgal oil has been reported to possess value ranging from 35.3 to 39.3 MJ/kg.

Biodiesel is considered a high value fuel due to feasibility it offers of being used as blend with mineral diesel in the form of blend (up to 20% without any major modification in compression ignition engines). Few studies have been conducted on the utility of microalgal oil in the production of biodiesel. A study of Guldhe et al. (2015) reported conversion of lipids from the microalgae, *Scenedesmus obliquus* using the lipase, *Pseudomonas fluorescens*. Lipid conversion of 66.5% has been reported at 35 °C using methanol to oil ratio (3:1) with 10% enzyme and 2.5% water content (with respect to oil weight). The biodiesel conversion enhanced to 90.8% when methanol was added stepwise. The reusability of the enzyme was checked which showed that it could be reused for 4 cycles with fatty acid alkyl ester content > 95%. Guldhe et al. (2017) reported synthesis of biodiesel from *Scenedesmus obliquus* using tungstated zirconia as a heterogeneous catalyst. A high biodiesel conversion of 94.58% was reported to have obtained at 100 °C, 12:1 methanol to oil molar ratio, and 15% catalyst amount (based on oil weight) has been reported in 3 h. Using heterogeneous catalyst over enzymes has been reported to offer advantages in terms of high conversion. Since microalgae biomass is rich in nitrogen, therefore another important application of these microalgae biomass is for the production of food additives which are much more valuable. Wet algae slurry was successfully converted into an upgradeable biocrude with high levels of carbon conversion to gravity separable biocrude product at 350 °C and 20 MPa in a continuous-flow reactor under subcritical water environment. Conversions up to 35 wt% of dry solids are reported. Catalysts have been used for direct liquefaction of algal biomass in hydrothermal media (Duan and Savage 2010; Kumar 2013)

6 Environmental Sustainability Aspects

It is reported that the microalgae bear a negative surface charge. This characteristic of microalgae is utilized in its harvesting from the culture media. The common flocculants that are used in its harvesting are inorganic (viz. $AlCl_3$, $FeCl_3$, $Fe_2(SO_4)_3$) and organic (polyacrylamide derivatives and polyethylene amine). The chemical flocculants require less energy but contaminates the harvested biomass. This poses questions over their environmental acceptability and sustainability (Farid et al. 2013). Farid et al. (2013) report use of bio-flocculants in harvesting of microalgal biomass. Among the organic bio-flocculants, chitosan that is produced from an abundant and natural polymer material, chitin has been suggested to be highly promising and effective in harvesting of microalgal biomass. Bio-polymers like chitosan does not contaminate the harvested biomass. Hamid et al. (2014) reported using a bio-flocculant derived from *Moringaoleifera* seed derivatives. The bio-flocculant so derived was reported to have a high efficiency (95%) done along with sedimentation for 20 min. The performance of *Moringaoleifera* seed derivatives as bio-flocculant has been reported to have a better performance as compared to the conventional chemical flocculants. Kawaroe et al. (2016) reported that the species, *Tetraselmis suecica* could be used as a flocculating agent that could enhance the harvesting of *Chlorella* sp. and *Nannochloropsis* sp.

Lananan et al. (2016) reported auto-flocculating efficiency of the microalgae, *Ankistrodesmus* sp. that could be utilized as bio-flocculant to harvest *Chlorella* sp. The removal efficiency of *Ankistrodesmus* sp. was reported to be 82% and 55% at pH 7.1 and pH 6.2 respectively. The use of *Ankistrodesmus* sp. as a bio-flocculant has been reported to have a preference over conventional chemical flocculants as the former does not require any change of pH. This has been attributed to the fact that the *Ankistrodesmus* sp. exhibit positive zeta potential value within the isoelectric point range of *Chlorella* sp. Thus, mixing of both microalgae (*Ankistrodesmus* sp. and *Chlorella* sp.) results in spontaneous coagulation and flocculation. Hence, owing to high cost of conventional chemical flocculants, the one that does not leach out and will bear a low cost will be sustainable. Thus, the future prospects of commercialization of microalgal fuel lies in a low cost approach involved in all stages of production of oil from microalgae (viz. cultivation, harvesting, and oil extraction).

7 Economic Evaluation of Various Types of Flocculants

Batan et al. (2016) reported through modeling that the total cost of production of algal raw oil and diesel comes to \$3.46 and \$3.69 per liter respectively. As per the simulation of cost done through Monte Carlo probabilistic analysis by Batan et al. (2016), the microalgal cultivation system, harvesting system, and lipid extraction system account for 30%, 33%, and 32% respectively of the total direct installed capital costs. Whereas, the hydrotreating system needed to convert the oil to biodiesel accounts for just 5% of total capital installed costs. Among the existing methods of microalgal harvesting, use of flocculants is considered to offer a low cost option (Lam et al. 2014). Flocculation augmented with sedimentation using bio-flocculants has been reported to reduce the energy demand of 13.8 MJ kg/dry weight (needed during centrifugation) to 1.34 MJ kg/DW during harvesting of microalgae. Farid et al. (2013) reported use of chitosan, a bio-flocculant in harvesting of microalgal biomass and observed that it would cost about \$0.0246/(kg dry biomass) that includes cost of \$0.0225 and \$0.0021 for chitosan and acetic acid respectively. One of the ways to harvest microalgae includes dissolved air floating without adding flocculant. This will reduce the cost of oil extraction from algae that will make it more suitable for its large-scale production (Zhang et al. 2016). Few mature microalgae when exposed to direct sunlight with minor supply of CO_2 cause auto-flocculation (Milano et al. 2016). This reduces the cost of production of microalgal oil to a substantial extent. Lama et al. (2016) reports that dosage of flocculant and its cost determines the cost of flocculation. The efficiency of one type of flocculant may vary with the type of microalgae harvested. It has been reported that flocculation of *Chlamydomonas* was more cost-efficient when chitosan (65 USD ton^{-1} biomass) was used as a flocculant as compared to using ferric chloride (87 USD ton^{-1} biomass).

8 Conclusions

As demonstrated in previous studies, microalgae harvesting by flocculation has proven to be efficient and comparably cost-effective. Though the inorganic coagulants are efficient and economical to some extent, the contamination of the biomass is of major concern. An efficient harvesting with natural flocculants is possible and there is no risk of biomass contamination. Earlier studies have claimed more than 98% biomass recovery with synthetic flocculants without any detrimental effects on lipid yields and the estimated cost was less than 0.5 USD/kg of harvested biomass (Gupta et al. 2014). Whereas, some of the studies have shown comparatively higher harvesting efficiency with electrochemical floatation than the centrifugation or flocculation (Guldhe et al. 2016). Such findings are promising with respect to economics though further studies are required for the comprehensive evaluation of macro- and micro-biochemical composition of the biomass harvested by synthetic flocculants. Further research is needed to develop more efficient coagulants which should have least or no effect on the harvested biomass. The economic harvesting is the key of sustainable biofuel production from the microalgal biomass.

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Chapter 7
Solar Photovoltaics (PV): A Sustainable Solution to Solve Energy Crisis

A.K. Pandey, N.A. Rahim, Md. Hasanuzzaman, P.C. Pant, and V.V. Tyagi

Abstract Although sun is the source of all forms of energy including the energy contained in fossil fuels, the term "solar energy" is meant the energy obtained directly from sun's radiation. Solar photovoltaic (PV) is the most promising of all the active solar energy technologies. This technology is affordable and the source of this energy is inexhaustible. Moreover, it is the cleanest source of energy developed so far, thereby establishing it as a sustainable solution to solve energy crisis. This chapter presents a succinct picture of the solar PV technology along with classification and application areas. The status of the technology maturity and energy–exergy and economic aspects of PV technology has also been addressed.

1 Introduction

Solar power is regarded as one of the most momentous breakthroughs of the last century. This source of energy is the most susceptible alternative to the dependence on fossil fuels, which are not only pernicious to the environmental but also been consumed over the time. It has been estimated that sunlight falling on the Earth in 1 h, if it could be harvested, is enough to fulfill the world's energy need for 1 year (Green 2012). Solar photovoltaic (PV) is now established as a proven technology. Solar PV devices directly transmute sunlight into electricity without any interposing

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device; it needs neither combustion nor any moving parts for power generation (Roger et al. 2009). Solar PV devices can be employed for power generation anywhere the sun shines.

Photovoltaic cells, also called solar cells, are the basic units of a solar PV system. Solar cells are essentially a type of electric device that can convert light into electrical energy through the photovoltaic effect. The photovoltaic effect was discovered in 1839 by Edmond Becquerel, then in 1954 the scientists of Bell Laboratories discovered that silicon can create an electric charge when exposed to sunlight (Philibert 2011). Photovoltaic energy conversion takes place in two steps: first, generation of electron–hole pair through the absorption of light in semiconductor material; secondly, severance of electron to the negative terminal and hole to the positive terminal by the structure of the device, thereby producing electricity (Markvart and Castafier 2003).

Solar cells should be capable of absorbing light and create electron–hole pairs, separating the charge carrier from opposite types and conducting the extraction of charge carriers through an external circuit connected to it. In order to meet the above requirements, semiconductor materials like silicon (Si) are used to manufacture PV cells. PV cells are basically wafers of semiconductor materials treated with such chemicals as allow formation of electric field. An isolated silicon solar cell has a voltage near about 0.6 V under 25 °C and AM1.5 illumination. Solar cells are arranged mostly in series to increase power and voltage and embedded on a frame to provide a voltage of 12 V (or 24 V and 48 V for greater sizes) forms a solar PV module. Multiple modules may be connected together to build an array in order to produce greater amount of electrical energy. Although the majority of the solar PV systems are made from traditional Si-based solar cells, the use of new thin films is growing day by day. The simplest layout of a solar PV system is shown in Fig. 7.1.

Solar energy is renewable and derived from the inexhaustible source of the sun. One of the most expedient features of solar system is that these systems can be constructed as stand-alone plants providing electricity to practically any extent, from low to high. That is why PV devices are in usage as the power supply for small-scale appliances like calculators to even megawatt-scale power plants.

Another key advantage of solar PV systems is that they do not produce any further pollution while generating power. Solar PV systems are simple in design, have

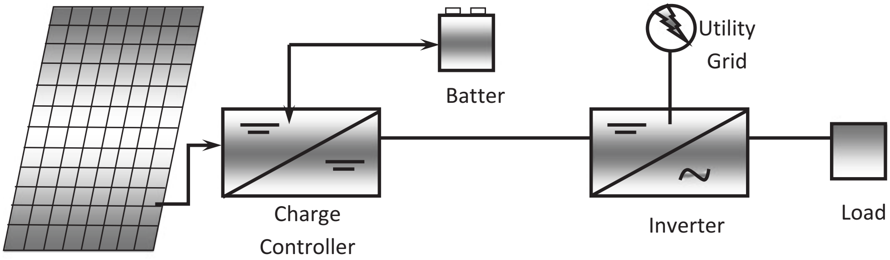

Fig. 7.1 Layout of a solar PV system

long operating life, require very little maintenance and run with high reliability even under harsh weather conditions. However, there are also some short comings of these systems. Solar energy is intermittent in nature and solar electricity needs to be stored in some storage device like batteries. The batteries will require maintenance and replacement which may intervene the smooth operation of the system and will also involve extra cost. Moreover, solar PV systems so far are cost-effective only in the small scale.

2 Classification of PV Systems and Their Applications

The PV systems can be classified based on the operation, components, and their end use. This section presents the classification of PV systems and their applications.

2.1 Classification

The classification of solar PV systems is mainly based on the factors like the functional and operational constraints of the system, configuration of the components involved, and how the system is attached to other power fonts and electrical loads. However, solar PV systems are broadly classified as utility-interactive or grid-tied systems and stand-alone systems. A detailed classification is given in Table 7.1.

2.1.1 Stand-Alone Solar PV Systems

Stand-alone PV systems are designed to operate as autonomous system and supply certain DC and/or AC electrical loads. These systems are basically suitable to provide electricity to isolated users, distant from the electricity grid and that are hard to feed. Energy storage is an important issue at these plants to guarantee energy supply at night or when there is no sun. The small plants only for illumination purpose needs only 12 V DC, however for greater scale 24 V or 48 V stand-alone systems may be designed. In order to have AC power, an inverter may be used that transforms energy from the batteries in DC to AC with 220 V. The key component of a stand-alone solar PV system is a charge controller which regulate charging rates according to the battery's charge level to allow charging near to the battery limit as well as control battery temperature to prevent overheating. There are two types of charge controllers commonly used, viz., *pulse width modulation* (PWM) and *maximum power point tracking* (MPPT), the later one being more expensive but efficient (Esram and Chapman 2007).

The DC output from a stand-alone PV system may directly be fed to the DC load, as shown in Fig. 7.2, without any provision for energy storage. These are called direct-coupled PV system and operate during day time only.

Table 7.1 Classification of solar PV systems

Classification norms	PV class
Materials of PV cell	1. Silicon PV
	I. Crystalline silicon PV
	a. Monosilicon (m-Si) PV b. Polysilicon (p-Si)
	II. Amorphous silicon (a-Si) PV
	a. Single junction
	b. Double junction
	c. Triple junction
	2. Group III–V material-based PV
	3. Thin film solar cell (TFSC)-based PV
	a. a-Si-based PV
	b. CdS/CdTe-based PV
	c. CIS/CIGS-based PV
	4. Dye sensitized solar cell (DSSC)-based PV
	5. Organic/polymer-based PV
Interfacing with load	1. Utility-interactive or grid-connected PV
	2. Off-grid or stand-alone PV
Installation mode	1. Building integrated PV (BIPV)
	2. Rack-mounted PV (RPV)
	a. Roof-top RPV
	b. Ground-mounted RPV
Tracking facility	1. Tracking system PV
	2. Fixed tilt PV
Module geometry	1. Flat plate PV (FPPV)
	2. Concentrator PV (CPV)
System complexity	1. Simple photovoltaic system (PV-only)
	2. Hybrid photovoltaic thermal system (PVT)

Fig. 7.2 Direct-coupled PV system

In some stand-alone PV systems, batteries are used for energy storage. Figure 7.3 shows such a typical stand-alone PV system connected to both DC and AC loads. One of the favorable features of stand-alone PV systems is that no network association is required for these systems. In order to acquire the complete benefit from the stand-alone PV system, low energy and energy-efficient appliances should be used.

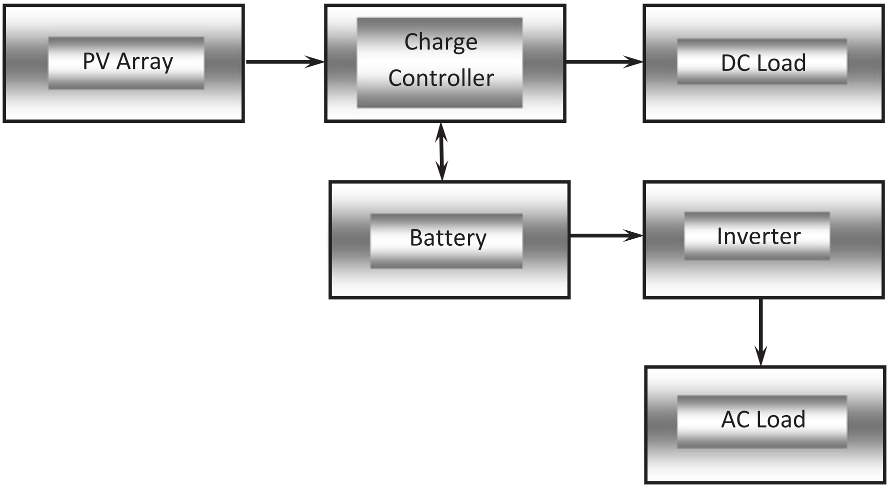

Fig. 7.3 Stand-alone PV system with battery storage powering DC and AC loads

2.1.2 Utility-Interactive Solar PV Systems

Solar PV systems depend on the availability of daylight for power generation. To ensure a connection between the continuing demand and supply of energy, backup is necessary for these systems. Backup for a PV system may be ensured by connecting the system to the utility grid through power conditioning unit (PCU) which may be a high-quality inverter. Utility-interactive PV systems are meant to function in parallel with and interconnected with the electric utility grid (Fig. 7.4). An inverter converts DC output from PV into AC power compatible with the voltage and power quality requirements of the utility grid and automatically stops supplying power to the grid when the utility grid is not energized. A bi-directional interface permits the AC power generated by the PV system to either supply on-site electrical loads or to back-feed the grid when the PV system output is greater than the on-site load demand. On the other hand, when electrical loads are greater than the PV output, the balance of power required by the loads is received from the electric utility. This safety feature ensures the PV system not to operate and feed back into the utility grid when the grid is disconnected for repair and maintenance.

A hybrid scheme of the stand-alone and grid-connected PV system is shown in Fig. 7.5.

2.2 Applications

2.2.1 Stand-Alone Solar PV Systems

As stand-alone PV systems can produce and supply electricity anywhere, the potential applications are increasing day by day. Out of numerous applications of the stand-alone system, the most recurrent ones are discussed below (NCSC 2002).

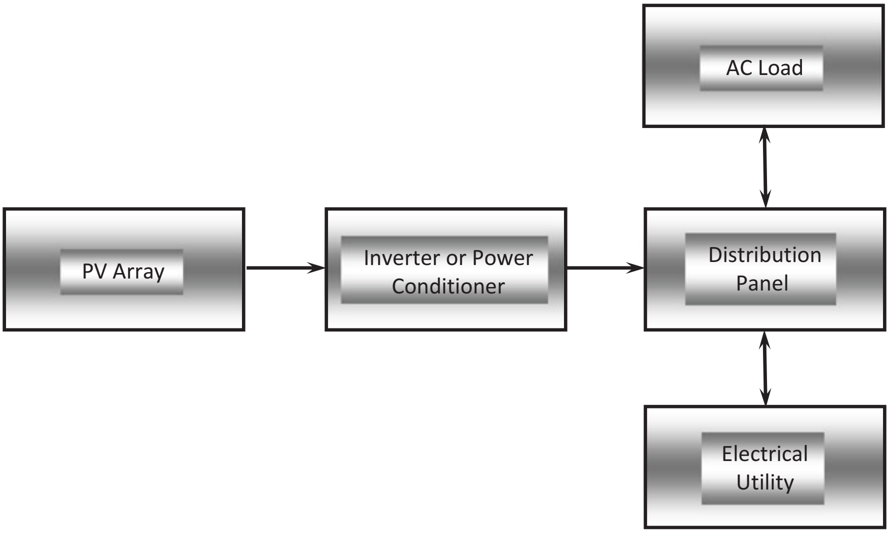

Fig. 7.4 Diagram of grid-connected photovoltaic system

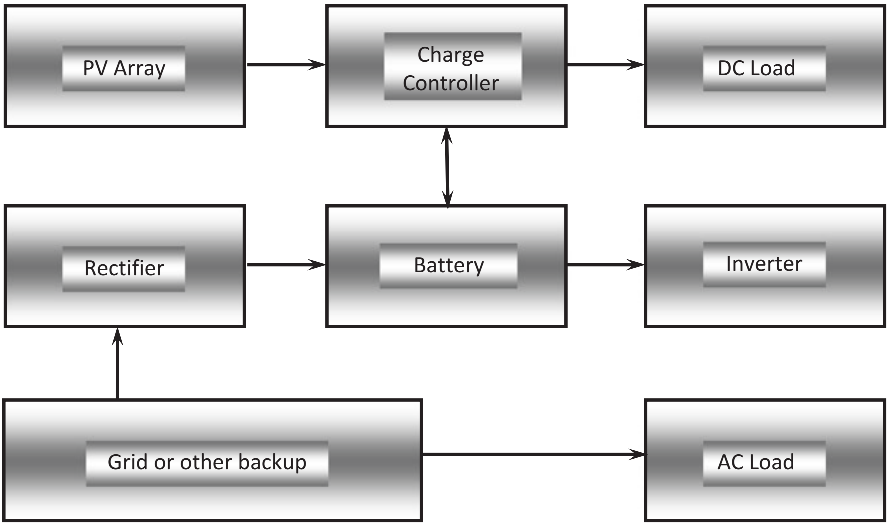

Fig. 7.5 Diagram of photovoltaic hybrid system

1. *Lighting*: Stand-alone solar PV with adequate battery support is ideal source for illuminating the low power DC lighting, such as sodium and fluorescent lights. Also these PV systems may be used to illuminate billboards, highway signs, etc.
2. *Communications*: Stand-alone solar PV system may provide the best possible solution to power the relay towers, installed on the highest possible elevation of remote sites for amplifying the signal of radio, television, and telephone. Small

PV modules may also be used to power portable computers, mobile radio system, and public telephone booths.

3. *Rural electrification*: The electrification of the remote areas that are not yet at the reach of the main electricity grid may be achieved through installation of small-scale diesel generators. Stand-alone PV systems may be the best possible way to provide electricity in remote areas far from utility grid. This may be achieved through installing separate PV systems for individual users or through hybrid generation system wherein diesel generator or wind turbine or micro-hydro generator may be coupled with solar PV system including a storage unit for uninterrupted power supply (Akikur et al. 2013).
4. *Remote monitoring*: Temporary scientific research facilities that are far from utility grid may be run by stand-alone PV systems. Likewise, PV systems can be an effective power source for meteorological information collection, seismic recording, highway traffic situation, structural condition, and irrigation control.
5. *Signs and signals*: Stand-alone PV systems are particularly reliable power source for signal devices as most of these are used at locations that are out of the range of the utility grid. These applications include navigation beacons, sirens, highway warning signs, railway signals, aircraft warning beacons, buoys, and lighthouses.
6. *Irrigation*: Stand-alone solar PV systems are often used for running water pumps for irrigation purpose especially in remote areas. These systems can consistently produce adequate electricity to power livestock and irrigation watering systems. A distinctive advantage solar energy to run agricultural water pumping systems is that increased water requirements for livestock and irrigation tend to match with the seasonal increase of solar insolation. These systems are economic in terms of long-term cost savings with negligible environmental affect compared to conventional power systems (Teresa and Busch 2010).
7. *Vehicle charging*: Stand-alone solar PV system-based charging stations are now providing electric vehicles (EV) with sufficient energy on the way. Electric vehicle needs to be charged at regular interval, but all owners cannot afford to install the charging infrastructure at home. So, installation of solar power EV charging stations at different point of a city may inspire EV ownership. Moreover, these facilities are environmental friendly and low cost. Solar PV-based charging stations will increase the utility of EVs and may contribute to increased adoption of this environmental friendly vehicle (Denholm et al. 2013; NREL 2014).
8. *Natural calamity relief applications*: Natural calamities like hurricanes, floods, tornadoes, and earthquakes often obliterate electricity generation and distribution systems. In such critical state where the power may be cut-off for a long time, portable PV systems are very useful for providing power needs of the affected people including water purification, refrigeration foods and drugs and pumping water as well as operating the communication devices. PV electricity is often a better choice than conventional fuel generators for makeshift shelters or medical clinics, because they are almost noiseless, needs no fuel transport and provide a nonpolluting reliable operation.

9. *Cathodic protection*: Metal structures like pipes, tanks, bridges, and buildings exposed to soil and water undergo corrosion that may reduce their lifetime and reliability. Cathodic protection is an effective technique to prevent corrosion in these cases which is done by applying a small DC voltage to those structures. Utility power in this application suffers from two limitations: first thing is the need for the conversion of AC power to DC and secondly, it will cost extra expense. PV systems can produce low voltage DC power directly, resulting in a much more efficient and cost-effective method to achieve cathodic protection of the metal structures.
10. *Refrigeration*: Mobile storage of medicines and vaccines and medicine storage in remote places can be ensured by supplying DC current from stand-alone PV systems.
11. *Consumer products*: Small-scale PV systems are used to power small DC appliances like watches, radio, television, lanterns, flashlights, calculators, security systems, fans, etc.

2.2.2 Utility-Interactive Solar PV Systems

Not all PV systems may be large enough to meet all the energy needs. Utility-interactive or grid-connected PV systems have got wide and extensive applications in residential or commercial buildings to compensate electricity costs. These systems are particularly good in reducing power bought from the utility during peak hours, which usually coincide with peak sunlight hours. Hence, use of PV systems can significantly reduce electrical bills by curtailing peak demand surcharge charged by power supply companies. Accurately sized PV system with adequate battery backup can be used to supply power during peak hours that may help better savings.

2.3 Other Emerging Applications of PV

The emerging applications of PV such as building integrated solar PV systems (BIPV), concentrated solar PV systems (CPV), photovoltaic thermal (PVT), solar PV desalination systems, and in space was discussed by Pandey et al. in detail (Pandey et al. 2016).

2.3.1 Building Integrated Solar PV Systems

The PV systems can be utilized by integrating them into buildings which is known as building integrated photovoltaic (BIPV) systems. These can be applied to rooftops of the buildings or some other parts viz. window glasses, balcony, or walls. The BIPV systems have several advantages such as utilization of unused space in building, electricity generation, and regulation of indoor environment.

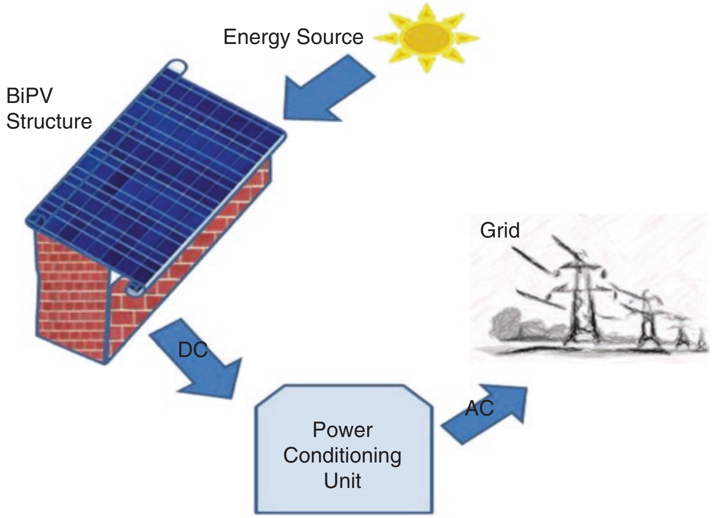

Fig. 7.6 Schematic diagram of the BIPV system (Aaditya et al. 2013)

The application of PV in building has been classified in two ways viz. building applied photovoltaic (BAPV) and building integrated photovoltaic (BIPV) by Peng et al. When the PV is installed on the top of the existing building it is known as BAPV system, while if the PV is part of building material then it is known as BIPV system (Peng et al. 2011).

The performance evaluation of BIPV system in the typical climatic condition of Bangalore, India having capacity of 5.25 kWp was studied by Aaditya et al., the schematic view of the studied system is given in Fig. 7.6. The system was found to be having overall efficiency of 6% (Aaditya et al. 2013).

2.3.2 Photovoltaic Thermal Systems

The solar energy falling on the surface of the PV module is not completely utilized, some part reflected and some transmitted and this increase the temperature of the PV module which ultimately reduces the efficiency of the PV module. Therefore, to reduce the temperature and to utilize the waste thermal energy hybrid photovoltaic thermal (PVT) collector has been developed which can serve both purposes, i.e. electricity and thermal energy at the same time. The temperature rise in silicon PV modules above 25 °C exhibits a power loss with a temperature coefficient of – 0.65%/C (Du et al. 2013).

The PVT collectors are also being encapsulated with phase change materials to enhance the efficiency as well as to store the energy which can be used for later use; these systems are known as photovoltaic-thermal phase change material (PVT-PCM) systems. Browne et al. carried out the indoor performance evaluation of the hybrid PVT-PCM system. As can be seen from Fig. 7.7, PVT-PCM has good potential and performs better than PV alone encapsulated PCM (PV-PCM) systems (Browne et al. 2015).

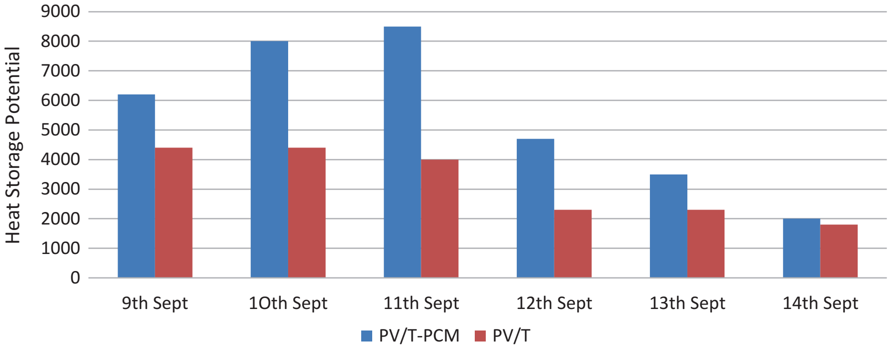

Fig. 7.7 Daily heat storage potential of PVT-PCM and PVT-water systems (Browne et al. 2015)

2.3.3 Space Applications

The PV application in solar is very old; initially, only silicon-based solar cells were employed to harness the energy in space via satellites. But, currently the high-efficiency multi-junction solar cells have come as a solution which has advantage of high power conversion efficiency in compact size. Different aspects of the space solar power have been studied by different authors (Mankins 1997; Jaffe et al. 2012; Jaffe and McSpadden 2013).

2.3.4 Concentrated Solar PV

When the incident solar radiation is concentrated on solar cells, using the mirrors which increases the amount of incident solar radiation many folds is known as concentrated photovoltaic (CPV). This increase in the solar radiation increases the efficiency of the solar cell. However, increasing the amount of solar radiation on the solar cell can cause many problems such as deformation and can damage the solar cell. As the efficiency can be improved drastically by concentrating the solar radiation, therefore the work on CPV is going on Worldwide to overcome the related issues (Looser et al. 2014).

2.3.5 Photovoltaic Desalination Systems

Clean water for drinking is one of the biggest problems in many of the developing and underdeveloped countries. Contaminated water causes lot of health issues especially in rural areas. There are many water cleaning methods available, solar desalination is one of them. There are basically two ways of solar desalination: one is thermal process where phase change occurs and another is using PV modules for producing electricity to support the membrane process in the desalination. The

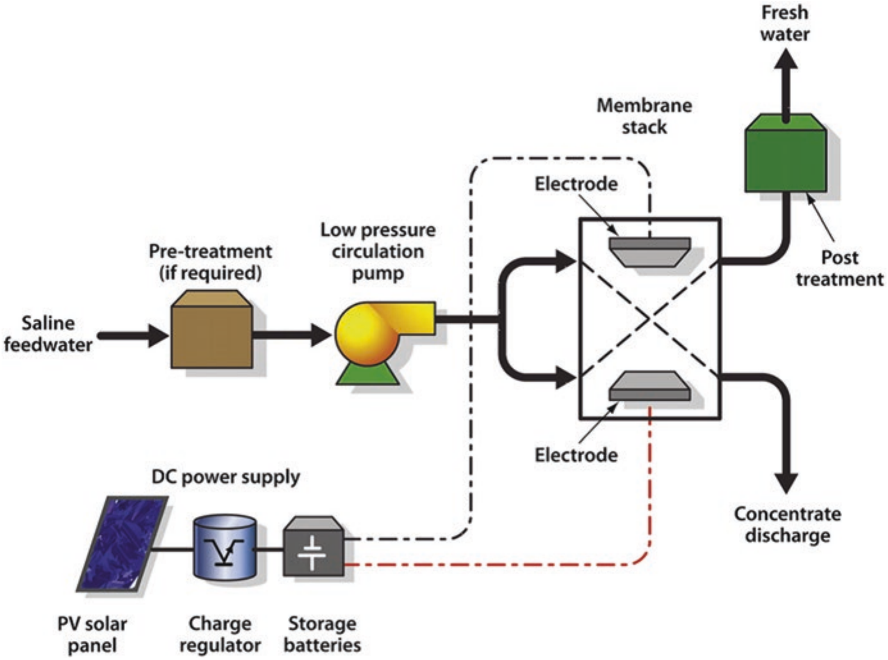

Fig. 7.8 A schematic diagram of a PV-ED system (Al-Karaghouli et al. 2010)

involvement of PV modules and batteries make PV desalination cost-ineffective. However, there are positive changes being made around the World and in future PV desalination will be one of the cost-effective solution (Avlonitis et al. 2003; Susanto 2011).

Al-Karaghouli et al. discussed the technical and economical aspect of PV desalination system which can be seen in Fig. 7.8. They found that small-scale PV desalination systems are technologically as well as economically viable and can be used in the rural areas where there is need of clean water. But, large-scale implementation of such systems is still economically not viable and needs more R&D to overcome technological barriers (Al-Karaghouli et al. 2010).

3 Status of Technology Maturity

Green et al. (2016) continuously publish the latest confirmed highest efficiencies for solar cells and modules. The results from the latest solar efficiency table (version 48) for one cell is shown in Table 7.2 and for module it is shown in Table 7.3 (Green et al. 2016).

Table 7.2 Confirmed terrestrial cell and sub-module efficiencies measured under the global AM1.5 spectrum (1000 W/m^2) at 25 °C (IEC 60904–3:2008, ASTM G. 173.03 global) (Green et al. 2016)

Classification	Efficiency (%)	Area (cm^2)	V_{oc} (V)	I_{sc} (mA/cm^2)	Fill factor (%)	Test centre (date)	Description
Silicon							
Si (crystalline cell)	25.6 ± 0.5	143.7(da)	0.740	41.8^a	82.7	AIST (2/14)	Panasonic HIT, rear junction
Si (multicrystalline cell)	21.3 ± 0.4	242.74(t)	0.6678	39.80^b	80.0	FhG-ISE (11/15)	Trina Solar
Si (thin transfer submodule)	21.2 ± 0.4	239.7(ap)	0.687^c	38.50^c	80.3	NREL (4/14)	Solexel (35 μm thick)
Si (thin film submodule)	10.5 ± 0.3	94.0(ap)	0.492^c	29.7^c	72.1	FhG-ISE^e (8/07)	CGS Solar (<2 μm on glass)
III–V cells							
GaAs (thin film cell)	28.8 ± 0.9	0.9927(ap)	1.122	29.68^f	86.5	NREL (5/12)	Alta Devices
GaAs (multicrystalline)	18.4 ± 0.5	4.011(t)	0.994	23.2	79.7	NREL (11/95)^d	RTI, Ge substrate
InP (crystalline cell)	22.1 ± 0.7	4.02(t)	0.878	29.5	85.4	NREL (4/90)^d	Spire, epitaxial
Thin film chalcogenide							
CIGS (cell)	21.0 ± 0.6	0.9927(ap)	0.757	35.70^g	77.6	FhG-ISE (4/14)	Solibro, on glass
CIGS (minimodule)	18.7 ± 0.6	15.892(da)	0.701^c	35.29^c	75.6	FhG-ISE (9/13)	Solibro, 4 serial cells

CdTe (cell)	21.0 ± 0.4	1.0623(ap)	0.8759	30.25[d]	79.4	Newport (8/14)	First Solar, on glass
CZTSSe (cell)	9.8 ± 0.2	1.115(da)	0.5073	31.95[i]	60.2	Newport (4/16)	IMRA Europe
CZTS (cell)	7.6 ± 0.1	1.067(da)	0.6585	20.43[i]	56.7	NREL (4/16)	UNSW
Amorphous/microcrystalline							
Si (amorphous cell)	10.2 ± 0.3[j]	1.001(da)	0.896	16.36[d]	69.8	AIST (7/14)	AIST
Si (microcrystalline cell)	11.8 ± 0.3[k]	1.044(da)	0.584	29.39[g]	73.1	AIST (10/14)	AIST
Perovskite							
Perovskite cell	19.7 ± 0.6[l]	0.9917(da)	1.104	24.67[i]	72.3	Newport (3/16)	KRICT/UNIST
Dye sensitized							
Dye (cell)	11.9 ± 0.4[m]	1.005(da)	0.744	22.47[n]	71.2	AIST (9/12)	Sharp
Dye (minimodule)	10.7 ± 0.4[m]	26.55(da)	0.754[c]	20.19[c]	69.9	AIST (2/15)	Sharp, 7 serial cells
Dye (submodule)	8.8 ± 0.3[m]	398.8(da)	0.697[c]	18.42[c]	68.7	AIST (9/12)	Sharp, 26 serial cells
Organic							
Organic (cell)	11.2 ± 0.3[o]	0.992(da)	0.780	19.30[i]	74.2	AIST (10/15)	Toshiba
Organic (minimodule)	9.7 ± 0.3[o]	26.14(da)	0.806	16.47[c,g]	73.2	AIST(2/15)	Toshiba (8 series cells)
Multijunction							
Five junction cell (bonded) (2.17/1.68/1.40/1.06/0.73 eV)	38.8 ± 1.2	1.021(ap)	4.767	9.564	85.2	NREL (7/13)	Spectrolab

(continued)

Table 7.2 (continued)

Classification	Efficiency (%)	Area (cm^2)	V_{oc} (V)	I_{sc} (mA/cm^2)	Fill factor (%)	Test centre (date)	Description
InGaP/GaAs/InGaAs	37.9 ± 1.2	1.074(ap)	3.065	14.27[p]	86.7	AIST (2/13)	Sharp
GaInP/GaAs/Ge; Si (minimodule)	34.5 ± 2.0	27.83(ap)	2.66/0.65	13.1/9.3	85.6/79.0	NREL (4/16)	UNSW/Azur/Trina
GaInP/GaAs (monolithic)	31.6 ± 1.5	0.999(ap)	2.538	14.18[i]	87.7	NREL (1/16)	Alta Devices
GaInP/Si (mech.stack)	29.8 ± 1.5[k]	1.006(da)	1.46/0.68	14.1/22.7[b]	87.9/76.2	NREL (10/15)	NREL/CSEM, 4-terminal
a-Si/nc-Si/nc-Si (thin film)	13.6 ± 0.4[j,k]	1.043(da)	1.901	9.92[g]	72.1	AIST (1/15)	AIST
a-Si/nc-Si (thin film cell)	12.7 ± 0.4[j,k]	1.000(da)	1.342	13.45[d]	70.2	AIST (10/14)	AIST

CIGS CuIn1-yGaySe2, *a-Si* amorphous silicon/hydrogen alloy, *nc-Si* nanocrystalline or microcrystalline silicon, *CSTSS* Cu2ZnSnS4-ySey, *CZTS* Cu2ZnSnS4; *(ap)* aperture area, *(t)* total area, *(da)* designated illumination area, *FhG-ISE* Fraunhofer Institutfür Solare Energie systeme, *AIST* Japanese National Institute of Advanced Industrial Science and Technology

[a] Spectral response and current–voltage curve reported in Version 44 of these tables

[b] Spectral response and current–voltage curve reported in Version 47 of these tables

[c] Reported on a ‘per cell’ basis

[d] Spectral responses and current–voltage curve reported in Version 45 of these tables

[e] Recalibrated from original measurement

[f] Spectral response and current–voltage curve reported in Version 40 of these tables

[g] Spectral response and current–voltage curve reported in Version 46 of these tables

[h] Spectral response and current–voltage curve reported in Version 43 of these tables

[i] Spectral response and current–voltage curve reported in the present version of these tables

[j] Stabilised by 1000 h exposure to 1 sunlight at 50 C

[k] Not measured at an external laboratory

[l] Notstabilized, initial efficiency. Reference 19 reviews the stability of similar devices

[m] Initial performance (not stabilized). Reference 62 reviews the stability of similar devices

[n] Spectral response and current–voltage curve reported in Version 41 of these tables

[o] Initial performance (not stabilized). References 63 and 64 review the stability of similar devices

[p] Spectral response and/or current–voltage curve reported in Version 42 of these tables

Table 7.3 Confirmed terrestrial module efficiencies measured under the global AM1.5 spectrum (1000 W/m²) at a cell temperature of 25 °C (IEC 60904–3: 2008, ASTM G-173-03 global) (Green et al. 2016)

Classification	Efficiency (%)	Area (cm²)	V_{oc} (V)	I_{sc} (mA/cm²)	Fill factor (%)	Test centre (date)	Description
Si (crystalline)	23.8 ± 0.5	11,562 (ap)	53.4	6.32[a]	81.6	AIST (1/16)	Panasonic (72 cells)
Si (multicrystalline)	19.5 ± 0.4	15,349 (ap)	41.53	9.299[a]	77.4	FhG-ISE (12/15)	Hanwha Q Cells (120 cells)
GaAs (thin film)	24.1 ± 1.0	858.5 (ap)	10.89	2.255[b]	84.2	NREL (11/12)	Alta Devices
CdTe (thin film)	18.6 ± 0.6	7038.8 (ap)	110.6	1.533[a]	74.2	NREL (4/15)	First Solar, monolithic
CIGS (Cd free)	17.5 ± 0.5	808 (da)	47.6	0.408[c]	72.8	AIST (6/14)	Solar Frontier (70 cells)
CIGS (large)	15.7 ± 0.5	9703 (ap)	28.24	7.254[d]	72.5	NREL (11/10)	Miasole
a-Si/nc-Si (tandem)	12.3 ± 0.3[e]	14,322 (t)	280.1	0.902[f]	69.9	ESTI (9/14)	TEL Solar, Trubbach Labs
Organic	8.7 ± 0.3[g]	802 (da)	17.47	0.569[c]	70.4	AIST (5/14)	Toshiba
Multijunction							
InGaP/GaAs/InGas	31.2 ± 1.2	968 (da)	23.95	1.506	93.6	AIST (2/16)	Sharp (32 cells)

CIGSS CuInGaSSe, *a-Si* amorphous silicon/hydrogen alloy, *a-SiGe* amorphous silicon/germanium/hydrogen alloy, *nc-Si* nanocrystalline or microcrystalline silicon, *(t)* total area, *(ap)* aperture area, *(da)* designated illumination area, *FF* fill factor

[a] Spectral response and/or current–voltage curve reported in the present version of these tables

[b] Spectral response and current–voltage curve reported in Version 41 of these tables

[c] Spectral response and/or current–voltage curve reported in Version 45 of these tables

[d] Spectral response reported in Version 37 of these tables

[e] Stabilized at the manufacturer to the 2% level following IEC procedure of repeated measurements

[f] Spectral response and/or current–voltage curve reported in Version 46 of these tables

[g] Initial performance (not stabilized)

4 Energy and Exergy Analysis

The energy and exergy aspects of solar PV systems are of great importance to clearly define their performance. The energy and exergy analyses of a solar PV as presented by several researchers have been presented in this section (Pandey et al. 2015; Joshi et al. 2009).

4.1 Energy Analysis

The incident solar energy is given by

$$Q_{\mathrm{in}} = I_{\mathrm{s}} A \tag{7.1}$$

where I_{s} is the incident solar radiation in W/m2 and A is the area of the PV module surface in m2.

Actual output of the PV module is given by

$$Q_{\mathrm{o}} = V_{oc} I_{sc} FF \tag{7.2}$$

where V_{oc} is the open circuit voltage, I_{sc} is the short circuit current, and FF is the fill factor which is the ratio of the product of voltage corresponding to maximum power (V_{m}) and the current corresponding to maximum power (I_{m}) to the product of open circuit voltage (V_{oc}) and short circuit current (I_{sc}) and is given by

$$FF = \frac{V_{\mathrm{m}} I_{\mathrm{m}}}{V_{oc} I_{sc}} \tag{7.3}$$

So, combining Eqs. (7.2) and (7.3), the output may be written as

$$Q_{\mathrm{o}} = V_{\mathrm{m}} I_{\mathrm{m}} \tag{7.4}$$

Therefore, the energy efficiency can be found from the following expression as

$$\eta = \frac{Q_o}{Q_{\mathrm{in}}} = \frac{V_{\mathrm{m}} I_{\mathrm{m}}}{I_{\mathrm{s}} A} \tag{7.5}$$

4.2 Exergy Analysis

The input exergy, i.e., exergy of solar radiation is given by

$$Ex_{\mathrm{solar}} = Ex_{\mathrm{in}} = \left(1 - \frac{T_{\mathrm{a}}}{T_{\mathrm{s}}}\right) I_{\mathrm{s}} A \tag{7.6}$$

where T_s is the temperature of the sun which is generally taken as 5777 K.

The exergy output of the solar PV systems can be given by

$$Ex_{\mathrm{out}} = Ex_{\mathrm{elec}} + Ex_{\mathrm{therm}} + Ex_{\mathrm{d}} = Ex_{\mathrm{elec}} + I' \tag{7.7}$$

where I' includes internal as well as external losses. Internal losses are electrical exergy destruction and external losses are heat loss which is equal to the thermal exergy of the PV system.

Electrical exergy of the PV system is expressed as follows on basis of the assumption that exergy content received by the PV surface is fully utilized to generate maximum electrical energy,

$$Ex_{\mathrm{elec}} = E_{\mathrm{elec}} - I' = V_{oc} I_{sc} - \left(V_{oc} I_{sc} - V_{\mathrm{m}} I_{\mathrm{m}}\right) \tag{7.8}$$

where $V_{oc}I_{sc}$ represents the electrical energy and $(V_{oc}I_{sc} - V_m I_m)$ represents the electrical exergy destruction. Therefore,

$$Ex_{\mathrm{elec}} = V_{\mathrm{m}} I_{\mathrm{m}} \tag{7.9}$$

The thermal exergy of the system is the heat loss from the PV surface to the ambient and can be given by

$$Ex_{\mathrm{therm}} = \left(1 - \frac{T_{\mathrm{a}}}{T_{\mathrm{cell}}}\right) Q \tag{7.10}$$

where $Q = h_{ca}A(T_{cell} - T_a)$ and $h_{ca} = 5.7 + 3.8v$ in which h_{ca} is the convective heat transfer coefficient and v is the wind speed.

Combining the above equations, exergy of the solar PV system can be written as

$$Ex_{pv} = V_{\mathrm{m}} I_{\mathrm{m}} - \left(1 - \frac{T_{\mathrm{a}}}{T_{\mathrm{cell}}}\right) h_{ca} A \left(T_{\mathrm{cell}} - T_{\mathrm{a}}\right) \tag{7.11}$$

The solar cell power conversion efficiency is the ratio of actual electrical output to the incident solar radiation energy on the PV surface and can be given by

$$\eta_{pcc} = \frac{V_{\mathrm{m}} I_{\mathrm{m}}}{I_{\mathrm{s}} A} = \frac{FF V_{oc} I_{sc}}{I_{\mathrm{s}} A} \tag{7.12}$$

Therefore, exergy efficiency which is the ratio of output exergy to the input exergy can be expressed as

$$\eta_{ex} = \frac{V_{\mathrm{m}} I_{\mathrm{m}} - \left(1 - \frac{T_{\mathrm{a}}}{T_{\mathrm{cell}}}\right) h_{ca} A \left(T_{\mathrm{cell}} - T_{\mathrm{a}}\right)}{\left(1 - \frac{T_{\mathrm{a}}}{T_{\mathrm{s}}}\right) I_{\mathrm{s}} A} \tag{7.13}$$

5 Economic Discretion of Technology

Photovoltaics cost is one of the most motivation components that influence the share of solar energy in the total power generation in the global. Decreasing cost of photovoltaic systems is very helpful for commercially as well as individually established solar systems and technologies. The global photovoltaic systems installed capacity has been increased very rapidly in last few years due to decreasing PV price, government subsidies and incentives. The U.S. Department of Energy's (DOE) Sun Shot has estimated that the PV systems cost can be decreased up to 75% within 2012–2020 (Feldman et al. 2012). Australian solar pioneer Stuart Wenham forecasted that the solar module price can be fallen 50% within 2020 (CT 2013).

The cost of PV system consists of mainly two components:

1. PV module cost
2. Balance of System (BOS) cost.

Normally, the module costs are in the range of 40–60% of the total PV system costs. The total system installation costs include the cost of site preparation, systems design, and installation labor cost (Dahlan et al. 2014). A commercial PV systems cost about 50–60% of PV modules, 10% of Inverter, 23–32% of BoS, and 7% of procurement (EPIA 2011). According to the global average PV module prices, the PV module cost also depend on the costs of raw materials, silicon prices, silicon processing, cell manufacturing cost, and module organization costs. Generally PV module cost is 40–60% of total PV systems. The cost depends on the project size and PV module types. Table 7.4 shows the module prices from different suppliers for year 2014. Munsell (2016a) mentioned that according to Green Technology Media (GTM) Research's latest edition of the PV Pulse, the tier-1 Chinese produces multicrystalline PV modules fell 10% annually and reached USD0.57/W in Q4, 2015 that is shown in Fig. 7.9. Gallagher (2016) also mentioned that according to

Table 7.4 PV module prices from different suppliers of China for year of 2014 (SHLSC 2014; SP 2014a, b, c)

Type of cell (model number)	Efficiency (%)	Price (USD/W)
Polycrystalline (RS6S-300P)	16	0.50–0.75
Polycrystalline (BCT300–24)	17	0.55–0.70
Monocrystalline (HPSM-300 W)	15	0.73–0.85

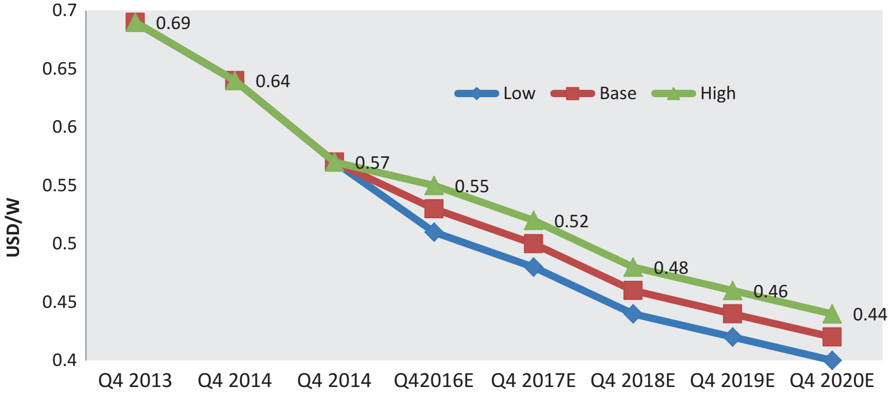

Fig. 7.9 Industry average multi-module Price, Chinese product (Munsell 2016a)

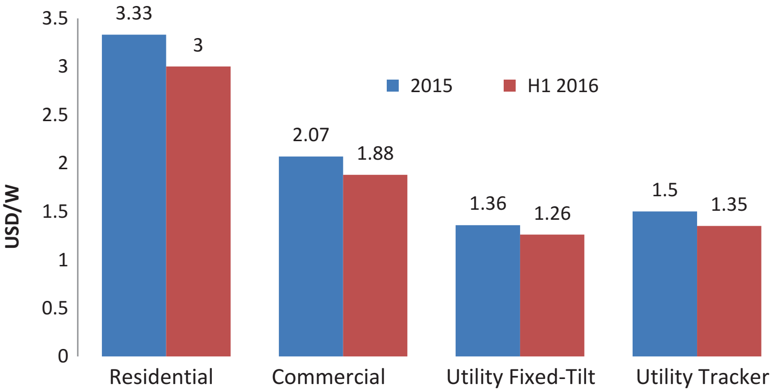

Fig. 7.10 U.S. PV System Pricing $/W(2015 & H1 2016) (Gallagher 2016)

GTM Research's latest report, *"U.S. Solar PV Price Brief H1 2016: Pricing, Breakdowns and Forecasts"*, prices of the fixed-tilt ground-mount PV systems will hit USD 0.99/W by 2020. GTM Research also estimated that modules prices for a utility-scale fixed-tilt ground-mount PV systems about USD1.26/W that is shown in Fig. 7.10 (Gallagher 2016; Munsell 2016b).

6 Contribution of Technology Towards Sustainable Development

Energy generation and use in the current era is dominated by the fossil fuels which is polluting the environment which causes serious health issues in the society. The population is growing at the fast rate on the average of 2%, in the coming time more

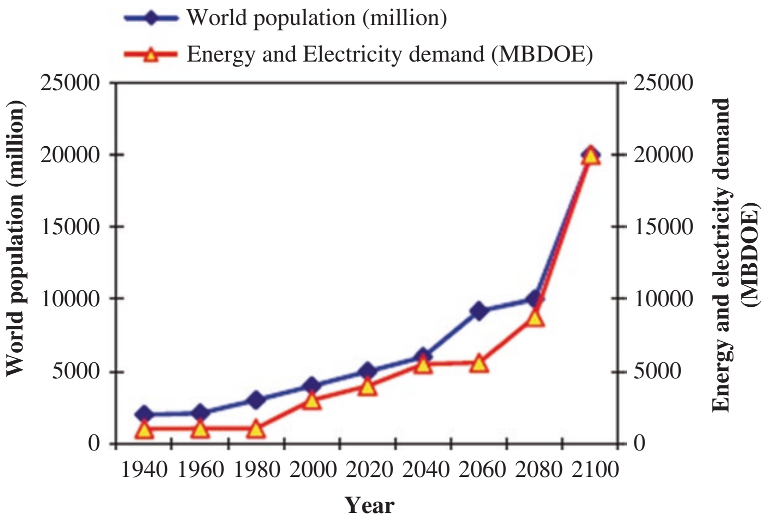

Fig. 7.11 Annual and estimated world population and energy demand. Millions of barrels per day of oil equivalent (MBDOE) (Omer 2008)

energy will be needed for human survival. The lifestyle is increasing and industrialization is growing at fast rate. Increase in population, lifestyle, and growing industrialization increases the use of energy which is currently mostly from fossil fuels. It is estimated that 80% of carbon emission is from the energy fuels and the rate at which fossil fuels are being used may be depleted within few decades such as oil and gas however, coal may be depleted in two centuries. The figure shows prediction of world population and energy and electricity demand by the time. It can be seen from Fig. 7.11 that population and energy and electricity demand is directly proportional to each other. The population and energy demand is continuously increasing and estimated to increase in the coming time, too (Omer 2008).

Therefore, in future, the huge amount of energy will be required and all cannot be fulfilled by fossil fuels. The uses of renewable energies are mandatory for the sustainable development of any country. The sustainable development of any country needs the use of technology which is socially fit, economically viable, pollution free, and technologically sound. The renewable energies such as solar, wind, biomass, and geothermal are clean and green and can fulfill the need of energy in sustainable way (Dincer 2000; Lund 2007). Solar energy being available abundantly in almost all over the world can be harnessed using photovoltaics (PV) or solar thermal. The PV technology is growing and has the potential to fulfill the demand of energy however, the low efficiency is still a cause of concern and the research for enhancing the efficiency and lowering the price is going on worldwide to accommodate the technology for contribution of sustainable development of any country.

7 Conclusions

In this chapter, a layout of the PV technology has been presented for the sustainable development of any country. The types of PV systems, their applications, efficiency status, energy and exergy analysis, economic view, and the contribution of PV technology have also been presented. The scientists around the world are making their effort to enhance the efficiency by discovering new materials or by other method such as concentrating the lights and the introduction of new materials. Solar PV technology has good potential to fulfill all the energy needs by a sustainable way.

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Chapter 8
Biological Limitations on Glyphosate Biodegradation

Juan Francisco Villarreal-Chiu, Alejandra Guadalupe Acosta-Cortés, Shardendu Kumar, and Garima Kaushik

Abstract Glyphosate is currently considered the most important herbicide of the world due to its broad-spectrum activity, effectiveness, and loss of global patent protection. Its ubiquitous presence in the environment due to anthropogenic activities and recalcitrance has the potential to affect animal behavior and interfere with ecological processes. Despite its important role in the protection of crops, it is important to establish strategies to reduce potential human exposure or decrease its presence in the environment. To date, only one microbial enzyme known as C-P lyase is acknowledged to drive complete glyphosate mineralization. AMPA, the common metabolite product of glyphosate biodegradation, still possesses the unique C-P bond of phosphonates and retains it toxic profile and recalcitrance. Thus, it is important to consider glyphosate and AMPA biodegradation altogether. Nevertheless, the potential to develop a bioremediation process is mainly limited by the genetic regulatory system that governs the expression of the C-P lyase, as it strongly depends on low environmental levels of phosphate. Thus, the complete mineralization of this herbicide by the sole use of microorganisms would remain insufficient until (1) more research on additional genetic control mechanisms of C-P lyase expression are explored, (2) alternative enzymes are studied in detailed, or (3) more complex and elaborated processes are considered. This work explores the available information on glyphosate biodegradation over the course of 40 years of study, the different pathways involving the C-P lyase, the genetic and physiological regulatory system that governs these processes, and the factors limiting the development of glyphosate bioremediation technologies.

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1 Introduction

[N-(phosphonomethyl)glycine], known commercially as glyphosate, is a systemic herbicide used widely in agriculture, forestry, and household for the control of more than 160 weed and grass species (International Agency for Research on Cancer 2015; Dill et al. 2010). Due to its broad-spectrum activity, effectiveness and the loss of global patent protection in the year 2000, the use of glyphosate has been rising rapidly around the world. In the US for example, the use of this herbicide was estimated in more than 12.5 million kg over the year 2014 (Benbrook 2016).

From the socio-economic point of view, glyphosate has become a major tool to tackle weed interference. The crop-weed competition is the most important factor for potential loss (34%) of crops around the world (Oerke 2006). In the US and Canada alone, over 142 million tons of corn were lost by weed interference in a period from 2007 to 2013, resulting in an economic loss of US $28 billion annually (Soltani et al. 2016). Therefore, glyphosate and other herbicides are crucial for the reduction of crops lost by weeds. Indeed, it has been calculated that glyphosate alone could help decrease crops lost to only 12% (Pimentel 2005). The reduction of crop lost is imperative for agriculture as the demand for crops for food and fuel is increasing at a rapid pace (Edgerton 2009) considering that world's population has more than doubled since 1960 (Gilland 2002).

Despite the success of pesticides in helping agriculture coping with global population growth, these xenobiotic chemicals are designed to be recalcitrant to biodegradation. Consequently, pesticides can migrate and accumulate through different parts of the environment after their release (Arias-Estévez et al. 2008). For instance, glyphosate has been detected in Argentinian cultivated soils at concentrations ranging from 35 to 1502 $\mu g\ kg^{-1}$ after agricultural activities (Aparicio et al. 2013). Similarly, glyphosate concentrations of 54 $\mu g\ L^{-1}$ and 41 $\mu g\ L^{-1}$ have been reported in field studies of Australia and Canada (Struger et al. 2008; Davis et al. 2013). Strong evidence suggests that glyphosate can easily migrate from soil into aquatic ecosystems. In the United States, for example, glyphosate was detected in eight of nine Midwestern states streams at concentrations of 0.10–8.7 $\mu g\ L^{-1}$ (Battaglin et al. 2005), possibly due to runoff events following herbicide application (Battaglin and Goolsby 1999). It has also been detected in riparian groundwater in urban catchments in Ontario, Canada (Van Stempvoort et al. 2014), rivers Ruhr in Germany (Skark et al. 1998), and Jauron in France (Pesce et al. 2008). Glyphosate recalcitrance could be responsible for its constant presence in coastal waters, as detected in the French coast Marennes-Oléron Bay (Samain and McCombie 2008).

The ubiquitous presence of glyphosate in the environment due to anthropogenic activities has the potential to affect animal behavior and interfere with natural interactions between species present in an ecosystem. For instance, it has been reported that glyphosate affects directly the morphology, behavior, and reproduction of several species of earthworms (Springett and Gray 1992; Morowati 2000; Verrell and Van Buskirk 2004; Casabé et al. 2007; Yasmin and D'Souza 2007; Correia and Moreira 2010; García-Pérez et al. 2014), including their interactions with arbuscular mycorrhizal fungi, which help plants to capture nutrients from soil (Zaller et al.

2014). Exposure to glyphosate has also been linked to decreases in the number of surviving amphibians in nature (Relyea 2005b; Relyea 2005a), as well as changes in the behavior and long-term survival of arthropods (Evans et al. 2010). For example, significant shifts in the dynamics between predators and potential prey were observed in wolf spiders *Tigrosa helluo* and *Pardosa milvina* (Rittman et al. 2013). In higher animals present in agroecosystems routinely exposed to glyphosate such as rats, glyphosate has been linked to a variety of functional abnormalities during pregnancy (Daruich et al. 2001), as well as low toxicity on the male rat's reproductive system (Dai et al. 2016). All these complex and multifactorial biological interactions may produce a certain grade of bioaccumulation in exposed flora and fauna. For instance, it has been demonstrated that in black worm *Lumbriculus variegatus*, a bioconcentration factor of 1.4 and 5.9 occurred after 4 days of exposure to 14C labeled glyphosate (Contardo-Jara et al. 2009).

Despite all the evidence of behavioral changes on fauna present in agroecosystems, the toxicity of glyphosate in humans has not been conclusive. While it has been proposed that glyphosate may be responsible for the increased hemolysis and methemoglobin on erythrocytes (Kwiatkowska et al. 2014), or DNA damages that derivate on cancer and apoptosis in human cell lines (Richard et al. 2005; Benachour et al. 2007; Benachour and Séralini 2009; Gasnier et al. 2009; Mink et al. 2012; Thongprakaisang et al. 2013; Chaufan et al. 2014; Guyton et al. 2015), some authors have not found a direct relationship between glyphosate and health risk to humans (Williams et al. 2000; Portier et al. 2016; FAO/WHO 2016). Nevertheless, the International Agency for Research on Cancer has classified glyphosate as a possible carcinogen (IARC groups 2A), as a preventive measure (International Agency for Research on Cancer 2015).

Regardless of its toxicity, glyphosate has been detected in urine (Acquavella et al. 2004; Brändli and Reinacher 2012; Krüger et al. 2014; Niemann et al. 2015), blood (Zouaoui et al. 2013), and breast milk (Honeycutt and Rowlands 2014) on farming communities and urban populations across Europe and America. Considering that larger volumes of this herbicide will be applied as a direct consequence of the appearance of glyphosate-resistant weeds (Myers et al. 2016), it will be important to assess health and safety hazards from potential exposure to glyphosate, especially in vulnerable populations. In consequence, the development of procedures for its control and elimination from the environment should be considered a priority.

2 Chemistry of Glyphosate and the C-P Bond

2.1 Chemical Structure and Properties

Glyphosate (*N*-(phosphono-methyl) glycine; *CAS No.* 1071-83-6) belongs to a chemical family of compounds called phosphonates. This family of molecules is characterized by the presence of an unusual carbon-phosphorus (C-P) bond. As

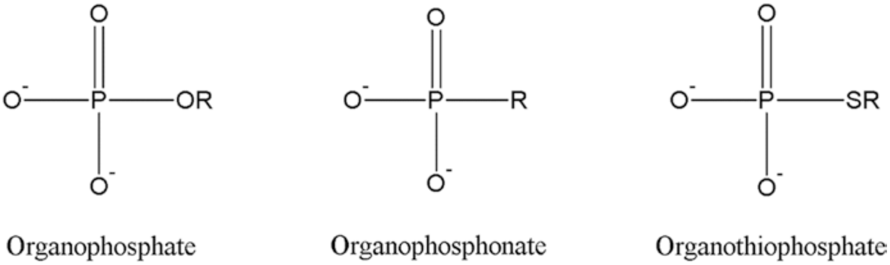


Fig. 8.1 General chemical structure of organophosphorus compounds

shown in Fig. 8.1, this C-P bond is chemically different from the more common carbon-oxygen-phosphorus (C-O-P) or carbon-sulfur-phosphorus (C-S-P) bonds found in other organophosphorus pesticides (Villarreal-Chiu et al. 2012).

The direct covalent bond between the carbon and phosphorus atoms provides these molecules unique physical and chemical properties, such as high solubility in water, strong adsorption, ability to chelate metal ions, compatibility with other chemicals and detergents; all of these, observed in glyphosate formulations (Mamy and Barriuso 2005; Zhang et al. 2015). These formulations consist of an ammonium, potassium, isopropylamine, or sodium salt of glyphosate, supplemented with a polar surfactant, such as polyethoxylated tallow amine (POEA) to increase its solubility in water and absorption in plants. Such commercial formulations are commonly accompanied by a small portion of impurities, such as formaldehyde and N-nitrosoglyphosate (FAO 2000).

The C-P bond confers a strong stability to phosphonates, making them chemically and thermally inert (Quinn et al. 2007). Thus, glyphosate and other phosphonates are more resistant to chemical, physical, and biological degradation compared with the more reactive nitrogen-phosphorus (N-P), sulfur-phosphorus (S-P), or oxygen-phosphorus (O-P) bonds (Ternan and Quinn 1998). Glyphosate, as other phosphonates, is resistant to hydrolysis in a pH range between 3 and 9. As a result, the breakdown of this class of compounds requires high amounts of energy and severe chemical conditions (Nowack 2003).

2.2 Glyphosate Mechanism of Action

Glyphosate is an herbicide intended for foliar absorption. It must contact the weed canopy and diffuse through different absorption barriers to reach its subcellular target site. Foliar absorption of glyphosate occurs only in liquid phase, thus, once a water droplet has dried on the leaf surface and the compound has crystallized, no additional absorption occurs, and there are no further biological effects on the plant (DuPont Pioneer 2016).

Once glyphosate has entered the plant, it acts as an inhibitor of the 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS). This enzyme is a key player of the chloroplast-localized shikimate pathway that drives the biosynthesis of aromatic amino acids and other essential metabolites (Hetherington et al. 1999). Normally, EPSPS catalyzes the transference of the enolpyruvyl moiety of the substrate to shikimate-3-phosphate (Funke et al. 2009). However, structural similarities with the C-P bond enable glyphosate to compete with phosphoenolpyruvate for the active site of the enzyme, denying any further catalytic reactions (Fig. 8.2). Calculated Ki values of EPSPS reflects the high affinity of this enzyme binding glyphosate rather than phosphoenolpyruvate, the natural substrate of EPSPS (Cao et al. 2012).

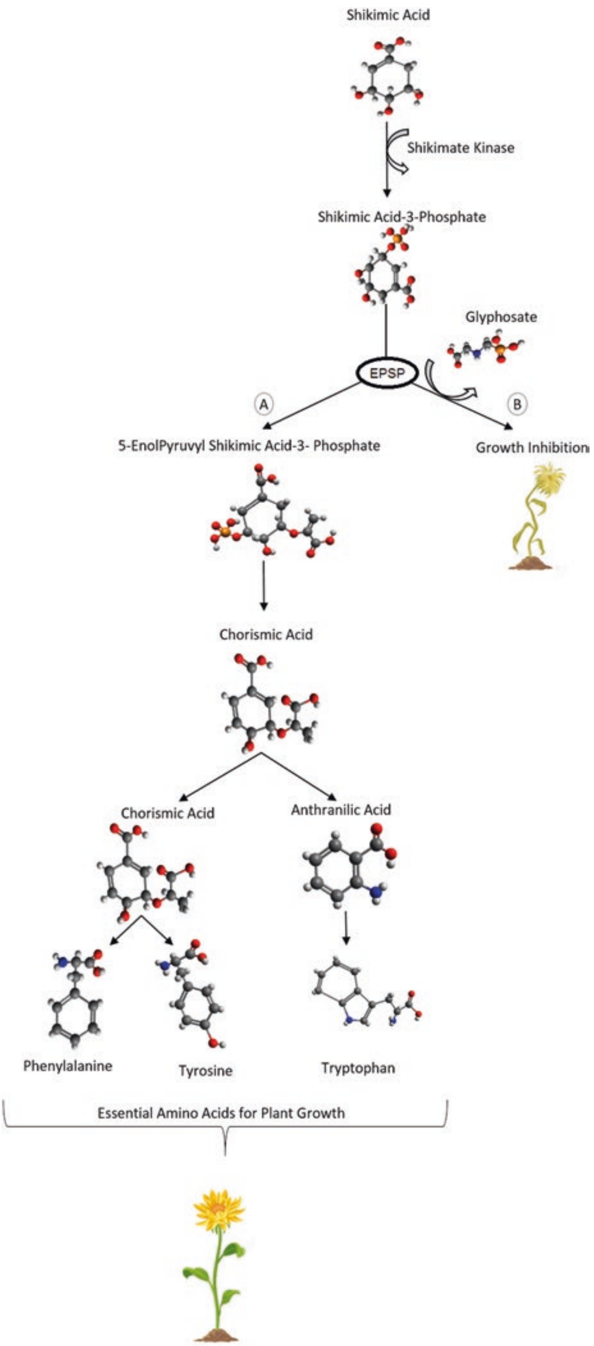
Since the EPSPS enzyme is highly consistent in higher plants, glyphosate may affect a wide range of grass and weeds, which makes it ideal for pre-plant, in fallow fields, or directed use to control them (Powles et al. 1998). However, the intensive use of this herbicide in agriculture has led to the occurrence of glyphosate-tolerant and glyphosate-resistant weed species. Thus, alteration of EPSPS has been an attractive way to obtain glyphosate-tolerant plants which have been used successfully in commercial transgenic crops (Cao et al. 2012).

2.3 Environmental Fate and Degradation Patterns

To date, more than 750 glyphosate products exist on the market, all with different formulations and trade names (NPIC 2010). In 2014 alone, the total use of glyphosate was estimated at 826 million kg over 130 countries, being China, India, and countries in the Asia-Pacific region the primary consumers (Transparency Market Research 2014). Considering that nearly 6.1 billion kg of glyphosate have been applied over the world in the last decade (Benbrook 2016), it can be considered that glyphosate is nowadays ubiquitous in the environment (Hénault-Ethier 2016).

Glyphosate levels on the environment depend directly on the type of soil and the time of application (Peruzzo et al. 2008; Tang et al. 2012). Phosphonates have a strong interaction with surfaces. Thus, after released into the environment, glyphosate adsorption is strongly dependent on pH, iron concentration, and phosphate contents of soil (McBride and Kung 1989; Mamy and Barriuso 2005). Therefore, glyphosate is immediately inactivated upon adsorption into soil components, such as clay and humic substances (Piccolo and Celano 1994) and its efficiency is reduced considerably (Zhou et al. 2006). As shown in Fig. 8.3, the time glyphosate remains adsorbed is dependent on how strongly soil components bind it, and how readily it is degraded by microorganisms or photolysis (Arias-Estévez et al. 2008). Under laboratory conditions, it has been estimated that 14C-glyphosate degradation half-life ranged from 7 to 28 days (Cassigneul et al. 2016). However, under natural conditions, glyphosate degradation half-life can be prolonged due to the formation of metal complexes with highly chelating cations (e.g., Cu^{2+} and Fe^{2+}), which significantly reduce the bioavailability of glyphosate for microbial decomposition (Tsui et al. 2005; Dollinger et al. 2015). For instance, it has been observed that after 80 days

Fig. 8.2 Aromatic amino acid biosynthesis by the shikimate pathway in plants and its interference by glyphosate



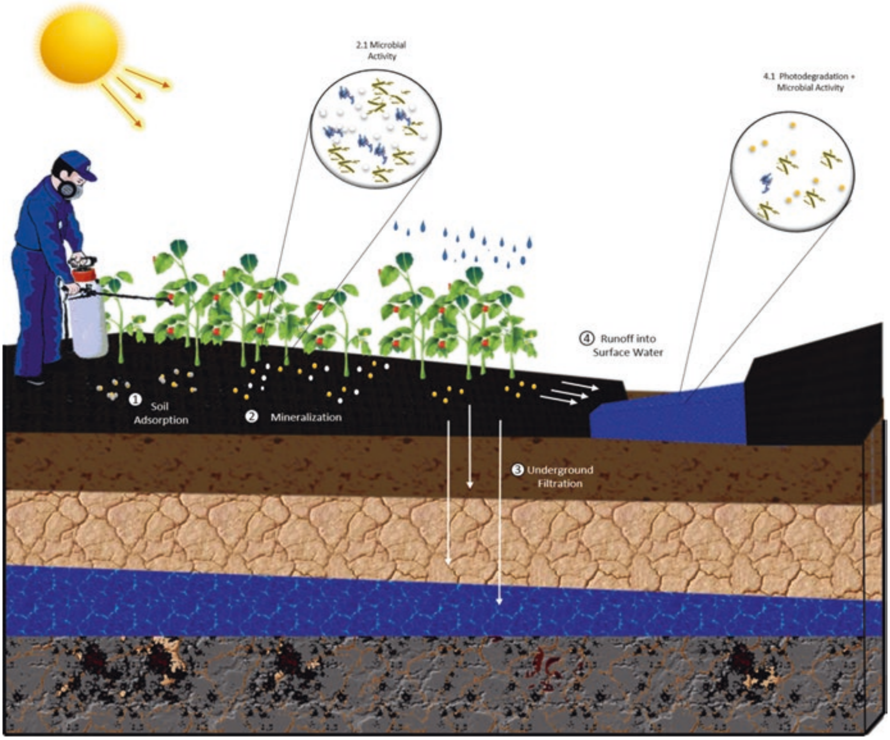


Fig. 8.3 Glyphosate environmental fate and degradation patterns

of soil incubation, extractable 14C-residues mostly contained the main metabolite of glyphosate: aminomethylphosphonic acid (AMPA) (Mamy et al. 2016).

AMPA is more persistent in the environment than glyphosate, with half-lives ranging from 76 to 240 days (Grunewald et al. 2001; Mamy et al. 2008). It has a larger vertical mobility and concentrations in cultivated soils can range from 299 to 2256 μg kg^{-1}, while glyphosate concentrations only range from 35 and 1502 μg kg^{-1} (Aparicio et al. 2013). In fact, AMPA was detected in 97.7% of 4750 samples collected between 2001 and 2010 from Midwestern streams of the USA (Battaglin et al. 2014). Due to its high recalcitrance and its strong correlation with glyphosate (suggesting that AMPA is derived mainly from glyphosate degradation rather than alternative sources; Battaglin et al. 2005; Van Stempvoort et al. 2014; Struger et al. 2015), AMPA can be used to monitor the environmental fate of glyphosate (Battaglin et al. 2005; Aparicio et al. 2013).

Due to their high solubility (Veiga et al. 2001; Fu et al. 2010), glyphosate and AMPA can migrate through lower soil depths and access by rapid transport processes ground and surface waters (Arias-Estévez et al. 2008; Tang et al. 2012). Once in limnological environments, glyphosate, as other members of the phosphonate family, can undergo UV light conversion producing AMPA as the main by-product of the photodegradation (Jaworska et al. 2002; Lesueur et al. 2005). This conversion process has been calculated under laboratory conditions to last 4 days under UV light (Lund-Høie and Friestad 1986). However, under natural conditions, it has been demonstrated that photodegradation plays a minor role in the environmental decomposition of glyphosate (Rueppel et al. 1977).

Both glyphosate and AMPA are known to produce shifts in the rhizosphere microbial community following long-term applications. These shifts are potentially dangerous as each of these phosphonates may produce certain toxicity to nitrogen-fixing bacteria, resulting in adverse effects on soil functions. For example, Bradyrhizobium and Mesorhizobium, which are important legume-root nodulating, micro-symbiotic nitrogen-fixing bacteria added to legume seed to improve crop yields, are particularly vulnerable to this herbicide as they possess the same EPSPS enzyme that is affected by glyphosate in plants (Ahemad and Khan 2012; Duke et al. 2012). On the other hand, a significant decrease of Acidobacteria population was reported following glyphosate exposure in greenhouse studies, being substituted by Xanthomonadaceae and other members of the gamma-proteobacteria (Newman et al. 2016). This dominant class of soil bacteria is known to possess enzymes involved in the catabolism of phosphonates (Villarreal-Chiu et al. 2012). Thus, it can be inferred that glyphosate application generates an artificial selection that stimulates existing bacteria capable of degrading this herbicide, in a process called biostimulation (Kryuchkova et al. 2014; Zhang et al. 2015).

3 Metabolism and Biodegradation of Glyphosate

3.1 Enzymes of Glyphosate Metabolism

Biogenic phosphonates were commonly considered highly recalcitrant and for hence, with no biogeochemical implications (McGrath et al. 2013). However, new evidence suggests that phosphonates may be crucial for the adaptation and survivability of microbes in variable phosphorus environments (Dyhrman et al. 2007), playing a major role in population dynamics. In the ocean, for example, biogenic phosphonates may account for up to 25% of dissolved organic phosphorus (Kolowith et al. 2001), which can potentially rival phosphate in oligotrophic areas (Van Mooy et al. 2015). This originates significant seasonal shifts in microbial populations that are adapted for phosphonate consumption, such as the cyanobacterium Synechococcus (Ilikchyan et al. 2009; Ilikchyan et al. 2010), resulting in important changes in the biogeochemistry of the ocean and the planet as a whole (Karl et al. 2008).

The study of such biogenic phosphonate's degradation has led to the identification of four major catabolic enzymes and their pathways: (1) carbon-phosphorus lyase (C-P lyase), (2) phosphonoacetaldehyde hydrolase (phosphonatase), (3) phosphonoacetate hydrolase, and (4) phosphonopyruvate hydrolase; in addition to several other less distributed C–P bond cleavage enzymes (Villarreal-Chiu et al. 2012). From this catabolic repertoire, glyphosate can only be degraded by the C-P lyase, due to its ability to cleave the C–P bond of a broad range of substituted and unsubstituted phosphonates (Wanner 1994). In contrast, all other reported C–P hydrolases have a high specificity to individual substrates and do not recognize glyphosate for biotransformation (Kononova and Nesmeyanova 2002).

The enzyme denoted with the general name “C-P lyase” represents a small family of multi-enzyme systems that catalyzes the difficult dephosphonation reaction that cleaves the C-P bond present in glyphosate and other phosphonates, producing the corresponding hydrocarbon and inorganic phosphate (Quinn et al. 2007). The great variation of the C-P lyase gene clusters observed in bioinformatics analyses suggests a significant operon evolution (Huang et al. 2005; Villarreal-Chiu et al. 2012) that has led C-P lyase to present different substrate specificities and biological activities (Kertesz et al. 1991; White and Metcalf 2004). These genetic variations may be directly related to the fact that glyphosate degradation proceeds by either of two different degradation routes that involve a C-P lyase (Fig. 8.4). One includes the cleavage of glyphosate’s carboxymethylene–nitrogen bond in a reaction catalyzed by an oxidase (Hove-Jensen et al. 2014), producing AMPA and

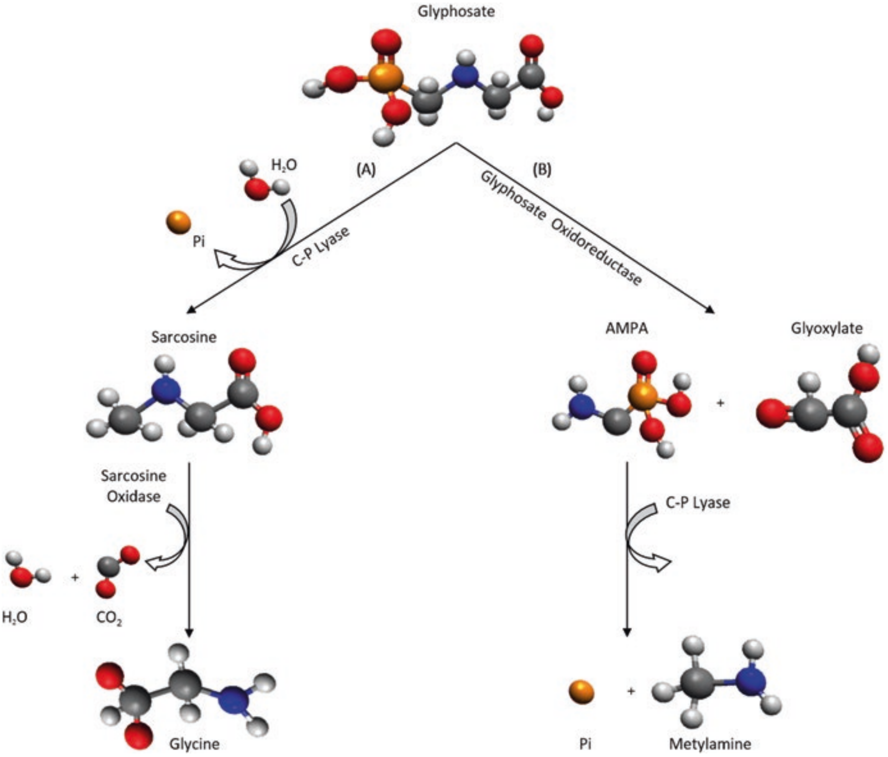


Fig. 8.4 Microbial catabolism of glyphosate by C-P lyase activity

Fig. 8.5 C-P lyase operon structure in *E. coli* K12

glyoxylate (Rueppel et al. 1977). In this reaction, AMPA, which is toxic for bacteria (Bonnet et al. 2007), can be released extracellularly to the environment (Jacob et al. 1988), or it can be furtherly catabolized by the C-P lyase being entirely converted to CO_2 (Pipke and Amrhein 1988). A second less distributed biochemical reaction that comprises the direct cleavage of glyphosate C-P bond results in the formation of sarcosine, which is furtherly transformed into amino acids methionine and serine (Shinabarger and Braymer 1986; Kishore and Jacob 1987; Kryuchkova et al. 2014).

The molecular analysis of the genes that transcribe for C-P lyase was initially completed in *Escherichia coli* (Chen et al. 1990). It was demonstrated that the catalytic machinery for the C-P lyase consists of an operon of 10.9 kilobases now called phn, which as shown in Fig. 8.5 is composed of 14 genes denoted as phnCDEFGHIJKLMNOP (Makino et al. 1991). Mutational analysis demonstrated that membrane-associated components phnGHIJKLM are essential for the biochemical transformation of phosphonates (Metcalf and Wanner 1993). Indeed, it has been demonstrated by X-ray crystallography that the protein complex PhnGHIJ contains two potential active sites that enable the coupling of phosphonate compounds to ATP and subsequently hydrolyze the C–P bond (Seweryn et al. 2015), being PhnJ the main responsible for the catalytic cleavage of the C-P bond (Kamat et al. 2013). Components phnCDE are known to encode for an ATP-binding cassette (ABC) transport system with high affinity to phosphonates (Metcalf and Wanner 1991; Rizk et al. 2006), while phnF has been annotated in databases as a gntR regulatory gene (Metcalf and Wanner 1993). Deletion experiments demonstrated that PhnF acts as a repressor of the phnDCE operon in *Mycobacterium smegmatis* (Gebhard and Cook 2008). phnNOP participate in the metabolism of derivatives ribosyl intermediates formed on this pathway for further metabolism in the C-P lyase pathway (Hove-Jensen et al. 2010; Hove-Jensen et al. 2011).

The significant genetic variation and distribution of C-P lyase in bacterial genomes and metagenomic databases (Villarreal-Chiu et al. 2012) denotes that this multi-enzyme system has ancient origins and pre-existed glyphosate (Kertesz et al. 1994). Experiments carried out with Arthrobacter atrocyaneus ATCC 13752, a bacterial strain which was deposited in a culture collection before it was exposed to glyphosate, demonstrated that it was able to utilize this herbicide as a phosphorus source (Pipke and Amrhein 1988).

3.2 Pho Regulon. Genetic Regulatory System Governing C-P Lyase Expression and Its Consequences on Glyphosate Biodegradation

Despite that C-P lyase is the second most abundant pathway for phosphonate degradation amongst sequenced bacteria, and that it is widely distributed in the environment (Villarreal-Chiu et al. 2012), it is known that glyphosate biodegradation by this enzyme is directly related to nutritional stress (Liu et al. 1991). This is important, as complete mineralization of the herbicide may be limited by the repression performed by pre-existent nutrients in the environment or its breakdown products, such as inorganic phosphate (Quinn et al. 2007).

The nutritional stress by which the C-P lyase responds is slightly different among microorganisms, resulting in direct differences in how glyphosate may be uptake. To date, every study has demonstrated that glyphosate serves mainly as phosphorus source (Hove-Jensen et al. 2014). However, there have been reports that glyphosate may also be used as a carbon source (McAuliffe et al. 1990; Krzyśko-Łupicka and Orlik 1997; Nourouzi et al. 2011). These differences respond to particular genetic changes in the two-component regulatory system that governs the expression of C-P lyase and other genes of the Pho regulon (known as PhoR–PhoB in *E. coli*; Tommassen et al. 1982, PhoR-PhoP in *Bacillus subtillis*; Hulett et al. 1994). The main reason why glyphosate is used as phosphorus source is that the genetic expression of the C–P lyase strongly depends on the exogenous and endogenous concentrations of inorganic phosphate (Dick and Quinn 1995). As shown in Fig. 8.6, during inorganic phosphate limitation (<4 μM in most cases, McGrath et al. 2013), PhoB, the transcription regulator of the two-component regulatory system PhoR-PhoB, activates the expression of nearly 400 Pho-regulated genes destined to the scavenging of inorganic phosphorus, which include phosphatases, phosphate- and phosphonate-specific transporters, and the C-P lyase (Makino et al. 1989; Wanner 1996; Santos-Beneit 2015). Consequently, glyphosate uptake occurs only under conditions of phosphorus deficiency. Nevertheless, this rarely takes place in natural environments (Sviridov et al. 2015). In fact, the excess of inorganic phosphate released by the C-P lyase activity itself serves to repress and/or inhibit any further breakdown (McGrath et al. 1997). This may help explain the recalcitrance and distribution of glyphosate and AMPA in the environment, and the need for a coupled adsorption process to fully mineralize these compounds in natural environments.

Reports on glyphosate utilization as a carbon source is currently limited and needs to be explored in full detail (Ermakova et al. 2010; Nourouzi et al. 2011; Sabullah et al. 2016). However, it is known that the Pho regulon may be cross-regulated by the transcriptional regulator CreC (carbon source responsive), which can transphosphorylate PhoB in response to carbon sources (Amemura et al. 1990; Wanner and Wilmes-Riesenberg 1992), unaffected by phosphate limitation (Makino et al. 1984; Ludtke et al. 1984). The cross-regulation of CreC would provide an

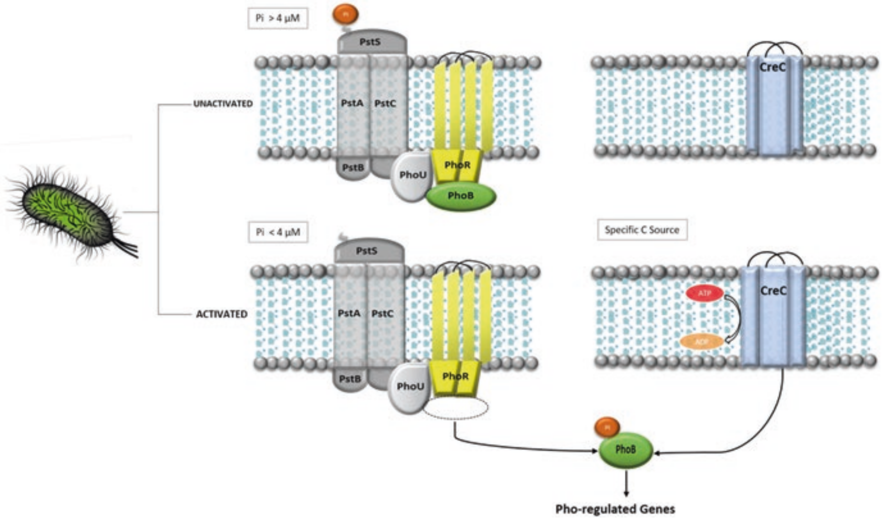


Fig. 8.6 The Pho regulatory system governing C-P lyase expression in *E. coli* K12

efficient system for the complete mineralization of glyphosate and AMPA in natural nutrient-rich environments and the development of successful bioremediation processes. As mentioned previously, the needs to explore this scenario is important and necessary, as the cellular growth attributed to the degradation activity (commonly measured as changes in optical density) is often mistaken by the production of polyhydroxyalkanoate (PHA) or other stress-induced responses due to nutrient limitations (Jacquel et al. 2008), which change the size and morphology of bacterial cells (Tay et al. 2010).

4 Strategies for Glyphosate Biodegradation

Since the beginning of glyphosate biodegradation studies in 1975 (Sprankle et al. 1975), it has been demonstrated that glyphosate microbial uptake strongly depends on low environmental levels of phosphate (Sviridov et al. 2015). Despite the metabolic pathway by which glyphosate is degraded by environmental strains, this metabolic process was regularly inhibited by the presence of inorganic phosphate. As phosphorus depletion rarely occurs in soil, and natural environments, the complete mineralization of this herbicide by the sole use of microorganisms would remain insufficient until (1) more research on additional genetic control mechanisms of C-P lyase expression are explored, (2) alternative enzymes for glyphosate degradation are studied in detail, or (3) more complex and elaborated processes are considered.

4.1 Practical and Economic Feasibility

To date, in-situ and in-vitro glyphosate biodegradation remains to a certain extent unviable due to its low efficiency. For example, studies performed with sediment extract media, the compositions of which resemble a natural soil sample, demonstrated that the strain *Pseudomonas* 4ASW was unable to degrade glyphosate, even it had been previously shown that this strain was able to mineralize it within 72 h in a phosphate-free synthetic medium (Bazot and Lebeau 2008).

The generation of AMPA as the main metabolite of the biodegradation process remains a major issue, as this compound still contains the C-P bond and retains it toxic profile and recalcitrance. This metabolite has been detected as the main glyphosate degradation product in diverse field studies carried out in glyphosate-treated and untreated soils from Brazil (Araújo et al. 2003), Denmark (Gimsing et al. 2004), Argentina (Arregui et al. 2004), and Canada (Cessna et al. 2002). As discussed earlier, the presence of AMPA in crop fields may reduce the number of viable bacteria in soil (Araújo et al. 2003) and produce a certain degree of phytotoxicity (Duke 2011). For hence, the development of a coupled glyphosate-AMPA biodegradation process would represent the best practical approach to eliminate these compounds from the environment.

4.2 Field Scale Demonstration

Only a few studies have demonstrated the biodegradation potential shown by microorganisms in soil samples. The *Bacillus subtilis* strain Bs-15 was able to degrade 67% of 5000 mg L^{-1} glyphosate in sterile soil experiments after 96 h, and showed an improvement up to 72% in unsterilized soil probably due to the stimulation of endogenous microorganisms (Yu et al. 2015). It has been demonstrated that glyphosate mineralization in soil samples occurs in two phases, an initial rapid phase attributed to a direct microbial action followed by a slow phase describing a plateau that may be due to the following microbial metabolism on the adsorbed herbicide (Alexa et al. 2009). This behavior has been confirmed in cultures of *Achromobacter* sp. Kg 16 and *Ochrobactrum anthropi* GPK 3, in which the glyphosate biodegradation rate of the soluble fraction was maximal during the first phase and soil-adsorbed molecules were utilized at a slower rate after 14 days of experimentation (Ermakova et al. 2010). However, glyphosate biodegradation efficiency remains low and depends strongly on the soil type, phosphate and herbicide concentrations (Carlisle and Trevors 1988), as this phosphonate also inhibit soil microorganisms metabolism due to its own toxicity to the cell (Moneke et al. 2010).

Field scale demonstrations using a pilot plant with immobilized bacteria on a diatomaceous earth support have been shown to remove glyphosate effectively from wastewater effluents. Authors reported a low-maintenance operating mode in which glyphosate concentration dropped from 50 to < mg L^{-1} in a period of 21 days (Hallas et al. 1992). Their preliminary studies demonstrated a biotransformation efficiency of 98% at flows of hydraulic residence time of 23 min using either a biostimulated bacterial consortium or a pure strain of *Pseudomonas* sp. LBr (Heitkamp et al. 1992). Yet, AMPA remained as the main by-product of the bioprocess. Both studies demonstrated that immobilized bacteria might be effective in removing low levels of glyphosate in waste streams. However, no further studies on the metabolism of glyphosate in such complex matrix nor the effect of diatomaceous earth on glyphosate adsorption were presented.

4.3 Technology Transfer

Based on the immobilized-bacteria reactor mentioned previously, Monsanto Agricultural Company developed in 1994 the US patent 5,288,635 in which a mixed culture of microorganisms (ATCC 55050) was attached to an inert, immobile support for the biodegradation of a glyphosate in waste streams (Adams et al. 1990). On the other hand, significant efforts have been focused on the development of optimal storage conditions for cultures of *Ochrobactrum anthropi* GPK 3 and *Achromobacter* sp. Kg 16 intended for glyphosate bioremediation. These promising bacterial strains possess high-degradation capabilities and do not accumulate or release AMPA as a by-product. These bacteria did not exhibit any toxicological hazard for human health and were demonstrated to be suitable for the reduction of glyphosate in contaminated soils to an ecologically safe level (Shushkova et al. 2012).

5 Concluding Remarks

Glyphosate is and will remain to be the main herbicide used in agriculture, forestry, and household for the control of interfering weed and grass species. Larger volumes of this herbicide will be applied in the near future as a direct consequence to the widespread emergence of glyphosate-resistant weeds. Consequently, controversial issues regarding changes in animal behavior, interference with natural ecological processes, and human toxicity should be expected. Therefore, despite its important role in the protection of crops, it is important to establish strategies to reduce its presence in the environment.

From the rich catabolic repertoire for phosphonate degradation, glyphosate can only be degraded by the nonspecific enzyme C-P lyase, due to its ability to cleave the distinctive C–P bond. However, the expression of C-P lyase in microorganisms is governed by the Pho regulon, which is activated by the restriction of inorganic phosphate. This factor has limited the development of glyphosate bioremediation technologies, as phosphorus present in soil and wastewater usually overcomes the concentration required for the activation of pho-regulated genes. Therefore, to develop a complete glyphosate mineralization process the following issues should be considered: (1) more research on additional genetic control mechanisms of C-P lyase expression, (2) screening fot alternative enzymes for glyphosate biotransformation, or (3) consider more complex and elaborated processes.

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Chapter 9
Bioindication-Based Approaches for Sustainable Management of Urban Ecosystems

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Abstract Urbanized areas are covering less than 3% of the land, but the majority of Earth's population and industry is concentrated at these territories. There is an urgent need for development of a comprehensive approach to the assessment of environmental quality in these areas. Bioindication allows estimating the entire complex of negative factors simultaneously. However, there are still large gaps in our knowledge of the urban ecosystem functioning. This chapter aimed to review the existing approaches to the bioindication of urban areas, i.e., microbial and plant bioindicators, as well as complexity of urban ecosystem, soil and its types, anthropogenic impacts, pollutants, effect on microbial community, other existing problems in this field and suggest the possible ways to solve them. The development of reliable bioindicators used on the basis of systematic approach would contribute greatly to rational land use and sustainable functioning of the urban environment.

1 Introduction

Although the cities cover a very small share of the world territory, they are home to great numbers of people. According to modern estimations, more than 60% of world population will inhabit urbanized areas by the year 2030 (Alberti et al., 2003). In industrialized countries, the percent of urban population is already very high. By 2015 over 90% of Belgium, Uruguay, Argentina, Brazil, Japan, South Korea, and Israel population inhabited cities and suburban areas. Thus, the importance of studies dedicated to urban ecology and sustainability of urban ecosystems is rapidly growing. Our knowledge of the urban ecosystems and the laws of their functioning and development remains insufficient. This leads to difficulties in urban planning and low sustainability of the urban environment.

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The starting point of any development is the assessment of the existing state of the system. In a complex and highly heterogeneous environment of a city too many variables have to be measured, and it is hardly possible to estimate the results of the interaction of many different factors. Bioindication allows an approach to assess the entire complex of negative factors simultaneously. Moreover, the use of living organisms for the estimation of the environmental quality can make it easier to predict the possible negative effects for the inhabitants of the city.

2 Urban Ecosystems

It can be stated that urban ecosystems have become the main habitat for humans. The rapid urbanization and migration from rural areas to cities can be recognized as the most significant human ecological event of the past 100 years (Rees 1997). This process, along with industrialization and technical development has led to formation of a principally new type of environment, with specific traits in biogeochemistry, energy consumption and even climate. Presumably, it will also have a strong impact on evolution of many species in urbanized areas, first of all on humans, and the long-term consequences can hardly be determined.

There is a set of principal factors that determine the formation and evolution of any ecosystem (Chapin et al., 2011). These are:

1. The climate of the territory, where the ecosystem is located
2. The substrate of ecosystem (soils and sediments)
3. Biota
4. Relief
5. The time (age of ecosystem from its formation, succession phase, etc.)

Urban ecosystems are affected by the same set of factors, which are transformed due to anthropogenic impact. The most prominent trait of urban climate is the formation of heat islands. The temperature in most cities is usually 1–2 °C higher than in surrounding rural areas.

The heat island phenomenon is due to several factors, including the lower vegetation cover and darker surface materials in the urban landscape (Akbari et al. 2001). Urban surface and air temperatures also increase due to soil sealing and decreases in evapotranspiration (Alcoforado and Andrade 2008). Urban areas also usually have decreased albedo, which contributes largely to the temperature rise. The differences in climate between city and countryside have biological and human implications (Pickett et al. 2011). Regarding the plants, increased temperature leads to later leaf drop time, earlier leaf emergence and flowering in the regions with moderate climate. Some migratory bird species can stay in the cities instead of using traditional migration roots and feed on landfill sites (Gilbert et al. 2016). There is also a distinct impact on soil. The increase of soil temperature alters soil microbial activity leading to enhanced nitrogen mineralization (Pouyat et al. 2003).

Large cities can also alter the atmospheric precipitation though it is largely dependent from world region (Mishra et al. 2015). It has been shown that urban heat island effects influence the rainfall largely during the early urbanization stage. However, at the stage of agglomeration formation, the regional moisture depression induced by the soil sealing has an effect on atmospheric instability energy, which might negate the city's positive impact on regional rainfall (Wang et al. 2015).

The hydrology of cities is completely different from rural areas. The main cause is the sealing of soils with asphalt, concrete, and other impermeable materials. Sealing of soil leads to drastically decreased evapotranspiration, while increasing the surface runoff. There are also major changes in groundwater flow, due to basement construction and reduced income from rainfall.

The soil is a very important part of urban ecosystem as it is the source of nutrients for urban vegetation, the basis for biogeochemical cycling and also the medium that accumulates and transforms various contaminants. Urban soils are considered as a separate soil type in many modern soil classifications (Prokof'eva et al. 2014), because of their deep transformation in urban environment. Taking into account the importance of soil for urban ecosystem sustainability and their convenience for bioindication will be discussed below in more detail.

In the urban ecosystems studies two approaches are usually considered. One defines the city as one single ecosystem; the other sees it as a composition of more or less separate ecosystems, such as parks, ponds, streets, city gardens, sewerage system, etc. (Bolund and Hunhammar 1999). We consider both approaches applicable, depending on the scale of study. When studying the so-called urban metabolism, including the inflow of resources, energy consumption and waste management, the city should be considered as an integrated heterogeneous system. Oppositely, most of the biodiversity studies face too much heterogeneity, when dealing with a whole city, because the type of land use differs sharply in different parts of the city, and it has a strong impact on animal, plant and microbial communities. This heterogeneity has led to attempts of classification of different parts of urban territories into several types, linked to descriptive studies of flora and fauna. For example, Dorney (1977) has classified urban landscapes into six groups creating a gradient from urban to rural ecosystems – business central part, old quarters, new quarters, construction sites, suburban areas, and rural areas.

The biodiversity of plants in the urban areas is quite high, and that species richness is mostly artificial—floral complexes of cities include large percentages of exotic species that were intentionally introduced. At the same time, the number of native species tends to decrease (Rapoport 1993). Thus, the urban ecosystems tend to harbor new plant biocenosis that are not characteristic of the surrounding rural area.

The biodiversity of most animal taxa is decreased in urban areas due to the loss of strictly specialized species. However, some species adapt to the conditions changed by urbanization process and colonize the available habitats rapidly. Therefore, diversity depends on the balance between extinction and colonization, which differ regionally and taxonomically. At moderate levels of urbanization, species richness may actually be higher than in nearby wild lands (Pickett et al. 2011).

We can conclude that urban ecosystems comprise a set of biotic and abiotic conditions that are substantially different from those occurring in natural environment. These systems are subject to the general environmental laws, but apparently have their own unique characteristics that require attention and investigation.

3 Urban Soils: The Basis of Ecosystem Infrastructure

Soils emerging in the urban environment act as a base component of the ecosystem. They are the central part of biogeochemical cycles, mediate the biochemical conversion of the cultural layer, the transformation of surface water into the ground water. They also act as a nutritious substrate for plants. The soil is a bank of seeds, the regulator of the gas exchange, etc. (Dobrovolsky and Nikitin 1990). Nevertheless, urban soils have a number of specific features, which leads to classifying them as a new type of soil.

Urban soils research began a long time ago, in the 60s of twentieth century (Zemlyanitsky 1963; Zelikow and Popkov 1962). However, at that time they were considered as a special case of natural soils that have been subjected to the influence of the urban environment. For the first time the definition of a new type of soil was proposed by Bockheim in 1974. According to this definition, the urban soil is a soil material with an artificial surface layer which does not possess agricultural value, which thickness exceeds 50 cm, formed by mixing, filling or pollution of the soil surface in the city or surrounding areas (Bockheim 1974). Sometimes it is also stated that a sign of urban soils, in addition to the above, is the presence of synthetic or toxic substances in quantities greater than those in the natural soils (Craul 1985a, b; Blume 1986; Burghardt 1996).

There is no common classification of urban soils at the moment. One of the reasons for this is the lack of a common approach to nomenclature and taxonomy of urban soils.

The most widely accepted system is “World reference base for soil resources”—WRB, adopted at the 1992 Congress in Montpellier in France. During the period 1998–2006, WRB acquired the status of a formal system of nomenclature and classification of soils in the European countries and the Central African Soil Science Association.

The main diagnostic horizon of urban soils is the horizon termed in Russian soil classification as “urbic” (UR)—sinlithogenic diagnostic horizon: gradually formed by bringing a variety of substrates to the surface in urban and rural settlements. It has often brownish-gray color tone, expressed on a scale of Munsell as follows: value (lightness) of less than 6, chroma (color) 1–4. The horizon contains more than 10% of artifacts (mainly construction and household waste), often sandy and/or rocky. The chemical properties are highly variable and evaluated in relation to the natural counterparts; usually the soil has a neutral to alkaline reaction, often calcareous. The content of individual chemical pollutants does not exceed 2 MPC. The content of available phosphorus (extractants 0.2 M HCl, 1% $(NH_4)_2CO_3$, 0.5 M $NaHCO_3$) is increased (on average no more than 0.1–0.2% (100–200 mg/kg). The humus content is highly variable, and its composition reflects zonal conditions (Prokof'eva et al. 2014).

The WRB classification system defines most urban soils as Technosols, while anthropogenically transformed agricultural soils fall into Anthrosols group. Technosols should contain artifacts, can contain pieces of rocks, and often contain toxic substances. Technosols include soils from wastes such as landfills or mine spoils, pavements and underlying materials, soils with geomembranes and constructed soils in human-made materials (Nachtergaele 2005 in Anne Naeth et al. 2012).

Urban factors directly and indirectly affect soil chemical, physical, and biological characteristics (Pickett et al. 2011). The direct impact on soil includes physical disturbances, i.e., mixing of the upper soil layer with various natural and artificial materials, burial and sealing of soils, creation of artificially constructed soil layers. The changes in the environment have various side effects on soils, which can affect pedogenic process and have long-term consequences. These changes include heat island effects, changes in moisture level, higher levels of nitrates in atmospheric precipitation, changes in biotic environment including new species of plants and animals.

4 The Sources and Types of Anthropogenic Impact on the Soils in Urban Areas

Same to the natural ecosystems, the urban ecosystems are shaped by a complex of environmental factors, but the anthropogenic influence becomes dominant in the city. While the water and the atmosphere are mobile parts of the environment, the soil is accumulating all the impacts, including those originating from atmospheric and water sources and can be used as a reliable object for the bioindication of the whole urban environment. Therefore, we focus on the impacts on soil. Anthropogenic impacts on the soil of a city can be divided into three types:

1. Chemical impact—the income of various pollutants linked to human activities into the soil.
2. Physical and mechanical—change of soil structure by direct mechanical action, exposure to sound and different types of electromagnetic radiation, including overheating.
3. Biological—the income of foreign soil microorganisms, reduction of organic matter income from leaf litter, introduction of exotic plant species and others. The following is a brief overview of these types of anthropogenic impacts on the soil in urban ecosystems.

4.1 Chemical Pollution in Urban Environment

4.1.1 Contamination of Soil with Heavy Metals

Since the sixties of twentieth century until now the environmentalists, urban planners, and soil scientists are interested in the problem of pollution of urban soils with heavy metals. It should be noted that this type of soil contamination is the most extensively studied, since almost every publication on urban soils contains information about the trace elements pollution. Many urban ecologists believe that all urban soils are contaminated with heavy metals.

Industrial emissions enter the soil with rainfall, the precipitating dust and aerosols, or by direct absorption of soil gaseous compounds. They can also be absorbed from the atmosphere by the plants, accumulate therein and be transmitted into the soil with the litter. Accumulating in the soil in large quantities, the heavy metals become quite mobile and can leach into the groundwater, causing the pollution to spread to remote areas from the primary source (Steinmann and Stille 1997; Wilcke et al. 1998).

Heavy metals involved in the biological cycle are transmitted by food chains and cause a number of negative consequences. At the maximum levels of chemical contamination the soil loses its ability to support the growth of plants and lacks biological self-cleaning. This can lead to a loss of ecological functions and death of the ecosystem. In addition, such heavy metals as Hg, Cd, Ni, Cr, Cu, Co, etc., cause carcinogenic, mutagenic, or teratogenic effects on humans. There is also evidence for neurotoxicity for some elements (Jooste et al. 2015). The key contaminating elements vary in different cities around the world, but most frequently the urban soils are polluted with lead, zinc and cadmium. Among the key heavy metal contaminants one can also mention Cu, Cr, Ni, Co, and Hg (Alloway 2013). The impact of different metals on the ecosystem is highly variable. For some metals, no distinct biological role is determined up to date. These are Sn, Ga, Zr, and members of lanthanoid element series. The contamination of urban environment with these metals is uncommon, and their toxicity is quite moderate. Fe, Mn, and Mo are important micronutrients with low toxicity level. However, many essential elements show high level of toxicity when their concentration is elevated. These are Zn, Cu, Ni, V, and Co. There is also a group of highly toxic elements with no significant biological role, i.e., Cd, Ag, Hg, Pb, Sb, and other elements (Wyszkowska et al. 2013).

4.1.2 Contamination of Soil with Non-metallic Elements

In addition to increased concentration of heavy metals, urban soils are also vulnerable to the adverse effects of a number of non-metallic elements. Especially dangerous is the pollution of urban soils with arsenic. Arsenic in urban environment is often preserved from the times when it was actively used as wood-protecting agent in construction materials. Also, arsenic compounds were used as pesticides and can be found in soils of old gardens, later included into the city borders (Elless et al. 2008). High content of arsenic was also observed near the metallurgical industries and enterprises, where the coal is burned (Lambert and Lane 2004). Arsenic contamination significantly affects the microbial activity and the composition of the microbial community. Its effects include sharp decline in urease, protease, and phosphatase activities in soil (Lorenz et al. 2006).

Another frequent problem is the chloride contamination. In many cities technical salt containing up to 99% of sodium chloride is used on the roads to combat the icing. Though chloride ions are not very toxic for humans, they can cause dangerous effects to the urban ecosystems. In the soil, increased concentration of chloride and sodium ions affects its structure, water and air permeability, osmotic potential and leads to the loss of stability of the soil, as well as osmotic stress for all the organisms inhabiting it (Černohlávková et al. 2008). Furthermore, the use of defrosting agents results in mobilization of heavy metals in the soil, improving their bioavailability and toxicity (Bäckström et al. 2004). In addition to sodium chloride, calcium chloride is often used as a de-icing salt. Excessive intake of calcium significantly increases the pH of urban soils, which affects the properties of the soil and its microbial community (Puskás and Farsang 2009). Salinization affects the proportions and species composition of microbial communities in the soil, while significantly decreasing their metabolic activity (Yuan et al. 2007).

4.1.3 Contamination of Soil with Organic Compounds

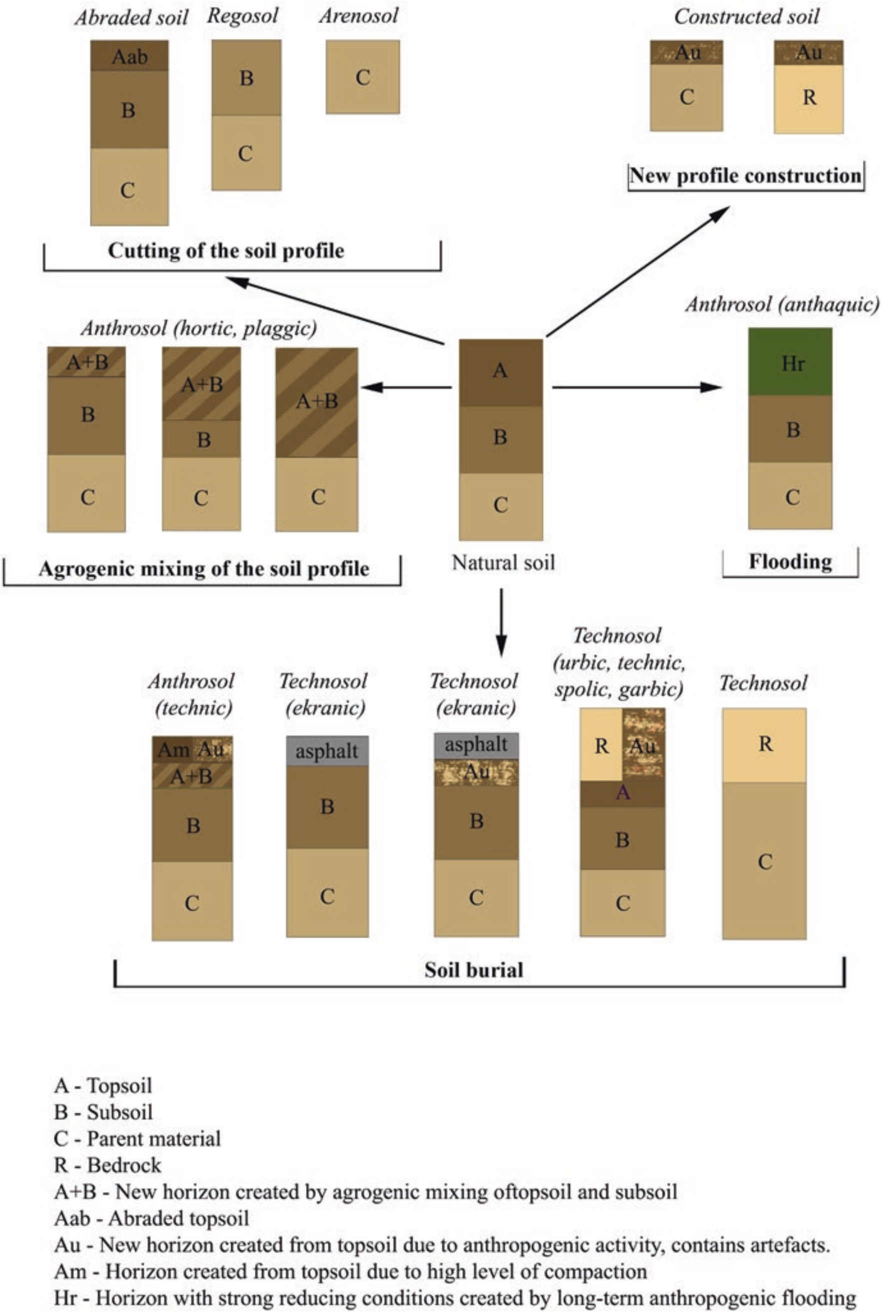
The global chemical pollution of the biosphere with synthetic organic compounds is one of the most acute problems of our time, causing justified concern about a possible violation of ecological processes and ecological balance in certain areas of the biosphere. With reference to urban soils, the following groups of organic contaminants are often considered in the literature: polychlorinated biphenyls, polyaromatic hydrocarbons, phthalates, dioxins and dibenzofurans, as well as aliphatic and alicyclic hydrocarbons, contained in oil products. The sources of polychlorinated biphenyls and naphthalenes in urban soils may be vehicles, burning of household waste, as well as industrial enterprises. In addition, these substances were for a long time included in the composition of technical greases, were used as wood-protecting agents and dielectrics (Krauss and Wilcke 2003). Polycyclic aromatic hydrocarbons are products of incomplete combustion of fuel in the engines of cars and industrial plants that use coal. These substances are almost exclusively anthropogenic accumulate particularly intense in urban soils. As well as polychlorinated biphenyls, polycyclic aromatic hydrocarbons have been shown to have mutagenic, carcinogenic and teratogenic effects, so their accumulation in urban soils is a serious threat (Aichner et al. 2007). The concentration of polycyclic aromatic hydrocarbons in urban soils often more than ten times exceeds their concentration in the natural soil. At the same time, the accumulation of them in urban soils with different land-use types also varies greatly. It reaches a maximum at the sides of the busy streets, significantly decreasing in residential and recreational areas. The decrease in the concentration of organic contaminants is also observed when moving from the center to the outskirts of cities (Liu et al. 2010). Another important class of organic pollutants are phthalic acid esters, which are widely used as plasticizers in a variety of types of plastics. Every year nearly six million tons of these materials are produced in the world. Phthalic acid esters have a hormone-like effect, and therefore can cause serious disruptions to the reproductive system of many organisms. Urban soils are considered as one of the main reservoirs of this type of pollutants, from where they can leach into the ground water or sublime to the atmosphere (Zeng et al. 2009).

4.2 Physical Disturbance of Soils in Urban Environment

4.2.1 Urban Pedogenic Process

The soils in urban environment can undergo radical transformation of profile under the influence of anthropogenic effects or direct construction of new soil profiles. Acquiring a new horizons system, which is unique in comparison to the natural soils leads to grouping such soils in special "man-made" departments with new names. Several types of restructuring of soil profile are possible (Fig. 9.1):

- As a result of deep and long homogenization of the upper part of the profile due to agricultural use of soils. At the same time the soil, depending on the thickness of the whole profile can lose the signs, allowing to identify its original typical identity. The mixed agrogenic horizon occurs on the remains of the median horizon or on soil-forming rock. Such soils in urban landscapes are identified as anthrosols (hortic, irragic, plaggic, anthaquic subtypes are determined based on the type of land use). These soils usually occur at the outskirts of the cities that had been involved into urbanization process in the past few decades.
- As a result of any mechanical abrasion or cutting the top natural horizons. On the surface there are median horizons, and the remaining soils are classified as regosols and arenosols.
- As a result of deliberate or provoked deposition of mineral (often containing humus) material on the natural or cutted surface of the soil. Such soils can be classified both as anthrosols and technosols, depending on the thickness and artifact content of the newly formed layer. Technosols usually contain 20% or more artifacts, while technic anthrosols contain less artifacts. The soil surface can be also sealed with impermeable layers (asphalt, concrete) forming an ecranic technosol profile.
- As a result of prolonged flooding, coupled with partial mechanical disturbance of the natural profile (usual suffixes "aquic" "anthraqic").
- As a result of creation of a new soil profile during remediation or construction of the urban environment. In some cases, the new soil profile is formed over an impermeable geomembrane to avoid the transport of hazardous substances from the underlying layers.



A - Topsoil
B - Subsoil
C - Parent material
R - Bedrock
A+B - New horizon created by agrogenic mixing oftopsoil and subsoil
Aab - Abraded topsoil
Au - New horizon created from topsoil due to anthropogenic activity, contains artefacts.
Am - Horizon created from topsoil due to high level of compaction
Hr - Horizon with strong reducing conditions created by long-term anthropogenic flooding

Fig. 9.1 Main types of soil profile formation in urban environment

4.2.2 Compaction of Soils

Most of the disturbing impacts on soil in urban areas are considered to be inherent to industrial areas or business center of the city with intense traffic. However, some types of anthropogenic impact are more significant in recreational areas, i.e., parks or city forests. The recreational use of these areas leads to compaction of soil surface layer because of intensive trampling. It has been shown that the level of soil compaction is much higher in neighborhoods with low socio-economical level, due to higher population density and lack of open spaces for walking and rest (Zhevelev and Bar 2016). There is also evidence for a positive correlation between soil pH level and soil compaction (Andres-Abellan et al. 2005). However, it is not clear whether this correlation is due to changes in the soil properties, or it is related to the positive correlation of higher pH values and the level and time of the urbanization process.

The level and the consequences of soil compaction are different in various microenvironments in the recreational areas. The paths and picnic areas are the most compacted, which leads to impairment of vegetation development and decrease in soil organic matter content (Sarah et al. 2016).

The negative effects of soil compaction include the decrease of soil arthropods biodiversity (Moriyama and Numata 2015), which leads to the further compaction of soil, because the soil animals play an important role in formation of soil structure. There is also some recent evidence for the loss of plant biodiversity in urban forests due to soil compaction (Vakhlamova et al. 2016).

4.2.3 Microclimate Changes Impact

One of the most important impacts on the urban ecosystem as a whole and particularly on urban soil is the local climate changes observed in urban areas. The most studied phenomenon is the urban heat island effect. This effect is mainly due to increased heat production by industry, lack of natural heat sinks, decrease of evapotranspiration-related cooling due to soil sealing, decrease in airflow because of dense building, increased solar radiation absorption by asphalt and roofs of the buildings, etc. The influence of increased temperature on urban environment has been at part discussed above in this chapter, and it is widely described in the literature.

The other microclimate parameters have been studied to a much less extent. The ventilation in the urban areas plays an important role for the nutrient cycling, pollution control and can have long-term effects on the whole urban ecosystem. There are specifically urban atmospheric phenomena, affecting the city environment, i.e., street canyons, which cause significant changes in the air circulation in urban areas. The airflow is usually significantly lower in urban areas than in the city surroundings. It has been shown that the wind speed in the city center can be as low as $\frac{1}{3}-\frac{1}{4}$ of the speed outside the city (Dimoudi et al. 2013). The decreased ventilation can lead to deposition of larger amounts of dust, which plays an important role in soil pollution and pH shifts in urban areas. The use of street vegetation strongly affects the rate of deposition and dispersion of dust in urban areas (Janhäll 2015). The vegetation filters the air, improving its quality, but at a cost of increased deposition of pollutants to the soil surface. It should be noted that the pollutants incorporated into the soil can be resuspended to the atmosphere with the soil-borne dust. A recent study has shown a correlation between lead concentration in soil, in the air dust, and in the blood of children, inhabiting the city, which was dependent from season (Zahran et al. 2013). Therefore, while the vegetation-based air filtration is important for the quality of the environment, the corresponding side effects should be taken into account. The proper design of the city environment can mitigate these effects. The urban planning should allow the clean air income, and the high trees should not block the airflow, especially in the streets with heavy traffic. This can improve the wind transportation of pollutants outside the city and decrease of their concentration below the harmful levels.

Some changes also occur in the hydrology of the urban ecosystem. These changes are highly dependent from the increased temperature, because of the growing transpiration rates which can lead to the depletion of the moisture in the surface soil layer by the grass (Arden et al. 2014). The other effects include the major changes in the water income due to the soil sealing. The large territories, covered with impermeable layers lead to uneven distribution of atmospheric precipitation, entering the soil. The areas that remain unsealed can be flooded by the rainwater from the adjacent sealed areas. This can lead to water erosion and the degradation of the topsoil layer, which is washed out to the roads. After drying, the washed soil can be turned into the dust and pollute the city atmosphere.

4.3 Biological Impacts in Urban Environment

The organisms inhabiting the urban areas influence the environment in many ways. Most of these impacts are positive and even necessary for the proper functioning of the urban ecosystem. These positive impacts are same to the role of the biota in natural ecosystems. The plants produce the organic matter and play an environment-forming role for many animal species. The animals feed on plants and transfer the organic matter. Soil dwelling animals form the normal soil structure and fertility of soil, fungi and bacteria decompose the litter and drive the main nutrient cycles. However, the anthropogenic activity in the urban environment can lead to significant changes in these natural processes. Many exotic plant species are intentionally introduced (Sjöman et al. 2016), but not all of them are suitable for the existing ecosystem. In example, the introduction of spruce (*Pīcea pūngens*) in the cities of steppe area in Russia leads to the degradation of grasses under the canopy, not only because of shading, but mainly because of acidification of soil. Landfills, then migrate to urban forests and recreational areas for an overnight stay and the soil surface at these areas becomes heavily polluted with bird droppings, including undigested pieces of plastic. The low pH also alters the solubility of heavy metals that become mobile and can enter the groundwater and the food chains. The animal species in urban areas can become a significant source of pollution. Large colonies of rooks (*Corvus frugilegus*) tend to feed on. Both examples represent a normal functioning of natural biota in unusual conditions. There is also another thing to be noted: the people, inhabiting the city and their domestic animals are also an important source of biological impact on the urban environment. The fecal pollution of urban water sources and in some cases of urban soils (due to drainage from the sewage system or due to the free-range domestic animals) is not uncommon in urban areas, especially in developing countries. An important issue is the epidemiological safety of the urban environment for the city inhabitants. Urban areas are the most densely populated and the polluted urban environment can be considered as a bioreactor with intensive horizontal gene transfer (Rizzo et al. 2013; Riber et al. 2014). Most plasmids, bearing the genes of antibiotic resistance have emerged in urban areas. Given the growing population density and the speed of spreading of the multiresistant strains of bacteria, it could lead to devastating outbreaks of diseases. Therefore, the biological impacts, while being largely ignored in urban studies, should be considered as a major threat to the sustainability of the urban ecosystem.

5 Monitoring and Bioindication as the Key Point for Developing the Sustainable Land Use Strategy

The assessment of the environmental quality in urban ecosystem is the key step to find the ways to overcome the existing problems and develop a sustainable land use strategy. The major hindrance is the complexity of the urban environment and a set of simultaneously acting negative factors that may affect the inhabitants of the city. Some of these factors are obvious, and can be measured directly. The others can remain unknown to the researcher. Furthermore, the interaction of different factors represents a new form of an impact, which often cannot be predicted.

The use of bioindication approach allows estimating the entire complex of negative influences, regardless of our knowledge about them. Bioindicators can also tell about the history of the problem, because the changes in communities can develop slowly, and reflect the time of the negative impact. The same is also true for long-living bioindicators, such as trees.

While the estimation of each factor via bioindication is usually inferior in accuracy to the direct measurement (i.e., chemical analysis), it may be much more informative for the final goal – the estimation of environmental quality in each particular case.

The principle of bioindication is the use of some biotic parameters to deliver the information on the changes in the environment. These parameters can scale from cellular level to the whole communities. On the cellular level, the changes of ultrastructure can be observed, with the genetic material being especially sensitive. In example, the anaphase analysis has been used to estimate the level of genotoxicity of urban soils (Gorbov et al. 2015). On the organism level, the changes can include the shifts in morphology (size of the organisms, teratogenicity), physiology and metabolism or behavior. The community-level bioindicators include the general biodiversity, species composition, the presence or absence of sensitive or tolerant species, which can help to indicate the nature of the negative impact.

Bioindication-based approaches can be divided into passive and active methods. Passive methods include the observation of existing communities and sampling the organisms from the environment to study them in the laboratory. The microbial and microinvertebrate bioindication is usually based on passive methods (Amossé et al. 2016; Pedrini-Martha et al. 2012). Active methods are based on placing the test organisms into the studied environment and detecting their response to the conditions. Active bioindication approach is usually based on plants that are planted in particular areas of the city.

Both approaches have strong and weak sides. The active bioindication is usually more accurate, because an appropriate control is always available. The bioindicators used share the same origin, and in case of plants, the same genotypes. However, it can only be used to estimate the "acute" environmental toxicity, present at the moment of introduction of the bioindicators. Nothing can be known about the time of the negative impact present. Passive bioindication can reflect the long-term effects of the negative factors; it is also more useful for the estimation of the local ecosystem stability, because the autochthonous organisms are studied.

The main problem emerging while using this approach is the proper control selection. It is particularly difficult for the urban studies due to high level of heterogeneity that is inherent to urban environment. For the urban soil studies it is even more difficult, because the areas in the city and outside its borders have different soil types. For the comprehensive estimation of the urban environment, we recommend to use both approaches to overcome the weaknesses of each method.

6 The Use of Plants as Bioindicators

Urban plants live in a highly unnatural environment. One of the most important environmental threats to the urban ecosystem is the atmospheric pollution created by anthropogenic activity. It is necessary to understand the ecosystem responses to the influence of urbanization in order to ensure that urban areas are well managed.

Plants used for monitoring environmental conditions are called bioindicators or biomonitors. Bioindicators can demonstrate the presence of air and soil pollutants and facilitate the estimate of the frequency of the occurrence of damaging levels. A bioindicator is any biological species (an "indicator species") or group of species whose function and population can reveal the qualitative status of the environmental conditions. Good biomonitor will indicate the presence of the pollutants and also attempt to provide additional information about the amount and intensity of the pollutants exposure.

A bioindicator can be defined as a vascular or nonvascular organism (in this case, plants) exhibiting a typical and verifiable response when exposed to a specific stressor, such as excessive pollution. These sensitive plants can be used to detect the presence of pollution at a specific location or region, which provides unique information regarding changes in air quality.

6.1 Characteristics of Bioindicators

Criteria for a suitable bioindicator are as follows:

- Plant should be easily found across wide geographic range
- It can grow in diverse habitats
- Easily recognized and has smaller-sized plants within its population
- Should have specific proven symptoms appear when exposed with pollutant
- It should display a consistent, increasing response

Several species have been extensively researched and are now available for planting as a bioindicator. Many highly pollutant-sensitive plant species have been evaluated for their potential use as a bioindicator species capable of detecting the presence of ozone air pollution through the development of very specific and distinctive foliar symptoms. Among the various categories, air pollution by automobiles is the most insidious one, which exerts highly detrimental effects on living organisms, vehicles releasing large quantities of pollutants such as oxides of nitrogen, sulfur, carbon, heavy metals, dust, and particulate matter.

Hijano et al. (2005) research shows that the coniferous species such as *Pinus pinea*, were more sensitive to SO_2 atmospheric concentration than leafy species as *Quercus ilex* subspecies *ballota* and, in the same way, bush species, such as *Pyracantha coccinea* and *Nerium oleander*, and were more sensitive than woody species, such as *Cedrus deodara* and *Pinus pinea* respectively.

6.2 Potential Bioindicator Plants

Forbs: *Centaurea nigra* and *Impatiens parviflora*.

Shrubs and herbaceous plants: *Alnus incana*, *Corylus avellana*, and *Sambucus racemosa*, *Rubus* sp., *Apocynum cannabinum, Aster macrophyllus*, *Apocynum cannabinum*, *Rumex patientia* L., *Viburnum tinus,* and *Sambucus nigra.*

Trees: *Prunus serotina*, *Liriodendron tulipifera*, *Fraxinus americana*, *Sassafras albidum, Tilia americana*, *Platanus occidentalis*, *Salix herbacea*, *Fagus* sp., *Prunus pensylvanica.*

Roadside plants: *Bougainvillea spectabilis*, *Ageratum conyzoides, Ficus religiosa*, *Cynodon dactylon*, *Peltophorum pterocarpum*, *Portulaca oleracea*, *Ricinus communis*, *Bambusa bambos*, and *Terminalia catappa*.

Roadside plants in urban areas demonstrate wide responses when exposed to atmospheric pollutants in the form of respiration, photosynthesis, enzymatic reactions, stomatal behavior, membrane disruption, senescence, and ultimately death (Rai and Panda 2015). Hydrangeas are a good example for bioindicator plant, however Al is necessary for blue flowers, in alkaline soils, there may be Al deficiency, which result in pink *Hydrangea* flowers. Algae can also be a good indicator of water quality because they react rapidly to changes in levels of N and P. Lichens live on surfaces such as trees or rocks or soil and are very sensitive to toxins in the air. Black poplar (*Populus nigra* L.) is a good indicator for ground level ozone and high sulfur dioxide in air damages coniferous trees.

7 Microbial Bioindication in Urban Areas

The sustainability of the entire urban ecosystem functioning is based on the properties of the soils, which are influenced by the resident microbial communities. These communities can be used to extend the number of bioindicators applicable for the monitoring of urban environment.

The assessment of microbial communities has a number of potential advantages. First, the microbial populations can react rapidly on the changes in the environment. Secondly, the microbial communities are quite sensitive and even small doses of contaminants can lead to sharp population decrease or even eradication of some species. The reaction of microbial communities to the environmental stress can include shifts in numbers of not only particular species, but even whole functional groups within the population, the overall loss of biodiversity or the changes in the biochemical activity of microbial community.

The main challenges associated with microbial indicators root from the same properties of bacteria that lead to the advantages. The ability of fast reproduction that is inherent to the microbes can lead to high temporal and spatial variability of microbial populations, which is particularly notable in heterogeneous urban environments. The accurate identification of the microbes can also be quite difficult and time-consuming. The possible ways to overcome these problems include the search for stable microbial indicators and reference microorganisms that are identified easily and react to specific types of impact. Another possible solution is the determination of microbial communities' characteristics for different land-use types within the city. Therefore, the development of new approaches to microbial bioindication in urban environment is a challenge that needs joint efforts from microbiologists and urban ecologists. The next subsections are aimed to summarize the experience that exists to date in that field together with presenting some observations made by the authors.

7.1 Microbial Biomass

Microbial biomass and biomass-related indicators are widely used in the assessment of the community reaction to the stress. Microbial biomass reflects the total amount of the microbial community, and is usually reduced if the soil is contaminated with hydrocarbons (Lorenz and Kandeler 2005; Megharaj et al. 2000). Nevertheless, the microbial biomass should be used with caution, since conflicting data exist on its dynamics at pollution. In particular, the microbial biomass is not a reliable indicator of soil pollution with heavy metals, as different studies have demonstrated an increase in microbial biomass upon the addition of a mixture of metals to the soil and decrease upon the use of silt contaminated with heavy metals as a fertilizer (Gil-Sotres et al. 2005). Some indicators related to the microbial biomass have proven to be reliable in urban studies. It has been shown recently that microbial biomass nitrogen can serve as important soil health indicator to predict soil quality and productivity in highly disturbed soils in urban areas (Knight et al. 2013). The existing controversy in the direction of microbial biomass changes under stressful conditions may be due to the lack of unified method of its determination. The methods include various modifications of chloroform fumigation-extraction method (Brookes et al. 1985), microwave-irradiation (Islam and Weil 1998), indirect estimation based on basal respiration measurements (Anderson and Domsch 1978), and direct estimations based on cell counts and recalculation. Given that the soil is an extremely complex environment, even slight modifications of each of these methods can lead to achievement of results that are hardly comparable to those obtained by other researchers. The results obtained by the methods that rely on different principles are even more difficult to compare. The actual microbial community includes active, potentially active, dormant and dead cells (Blagodatskaya and Kuzyakov 2013). The degree of dormancy can also vary greatly. Some cells are still living, but metabolically inactive—such microorganisms would contribute to biomass, determined via microscopic analysis, but would not to respiration-based methods. The physiological state of the microorganisms is also very important: the starving cells can have lower protein content, thus affecting the microbial biomass C and N parameters. To conclude, for accurate estimation of microbial biomass in soil, at least two different methods should be used simultaneously.

7.2 Changes in Soil Respiration, Carbon Cycling, and CO_2 Emission

The most important function of both natural and urban soil is the biogeochemical cycling of elements. The global carbon cycle includes the processes of carbon release into the atmosphere via natural biochemical processes (fermentation, respiration) and anthropogenic activities (fuel combustion). The opposite part of the cycle is CO_2 fixation by autotrophic organisms (photosynthesis and chemosynthesis) and subsequent carbon deposition in the form of organic matter or sedimentation as calcareous rocks. Soil organic matter is a major pool of biogenic carbon, which is estimated to be over 1500 billion tons (Lal 2004). The soil organic matter plays an essential role in soil structure formation, its functioning and productivity. The soil organic carbon pool exists as an equilibrium between gains and losses and the increased rates of decomposition of soil organic matter can lead both to soil degradation and extensive emission of CO_2. Urban areas have been characterized both by higher SOC densities in comparison to rural areas (Pouyat et al. 2002) and by lower (Jo 2002) depending on the climate in the studied cities. However, the difference is rather not quantitative, but qualitative. Urban soils are subjected to specific pedogenic processes with a leading anthropogenic factor, such as mixing, burying, sealing, etc. Such processes modify the quantity, quality, and depth distribution of substrates for decomposition; the microbial communities of decomposers are also altered, as well as the diffusive transport through soil profile (Lorenz and Lal 2009). It is clear that a better understanding of the urban soil properties is urgently needed in the context of their role in the global carbon cycle as well as their local scale ecosystem-maintaining role.

The studies of soil respiration are most frequently used to assess the soil quality and the state of the microbial community dwelling in it. The basal respiration has been shown to be sensitive to heavy metal pollution (Gülser and Erdoğan 2008), decreasing with the growing level of pollution. However, the metabolic quotient, qCO_2 is generally considered a more reliable indicator. This quotient serves as an estimation of metabolically active portion of microbial community and is calculated as basal respiration to microbial biomass. It has been shown that higher qCO_2 values are associated with younger urban landscapes gradually decreasing to the older areas of the city. This suggests that the gradual decrease of the metabolic quotient value reflects the succession in urban landscape with older areas of the city closer to steady-state conditions (Scharenbroch et al. 2005). The highest qCO_2 values have been found in urban lawns, which are considered to be caused by land management and disturbing impacts on soil microbial community (Vasenev et al. 2015). Recent studies have shown that in a complex urban environment edge effect should be taken into account when considering soil respiration and carbon cycling. The respiration rates have been shown to be highest at the edge of sealed areas, gradually decreasing in the direction of the green area interior (Wu et al. 2016).

Another valuable tool in respiration-based bioindication studies is the method of substrate-induced respiration (SIR). The method allows to estimate both general microbial activity in soil, by using glucose as a substrate, and to study more specific changes in microbial community response to environmental stress by using a set of different substrates and obtaining catabolic profiles. It has been shown that the tolerance of SIR to Pb contamination was concomitant with deep changes in catabolic profiles in these soils (Bérard et al. 2016). It may be possible to establish a set of indicative patterns of catabolic profile changes for different soil and land-use types which makes SIR a valuable instrument for microbial bioindication in urban environment.

7.3 Changes in Nitrogen Cycling and N_2O Emission

The global nitrogen cycle is the second largest biochemical cycle after the carbon cycle. The natural nitrogen cycling processes are greatly affected by anthropogenic activities. These impacts include inputs of reactive nitrogen species derived from fossil fuel combustion, application of the chemical fertilizers and transformation of the environment (Pierre et al. 2016). The nitrogen cycling includes the process of nitrogen fixation, leading to incorporation of chemically inert dinitrogen gas into bioavailable compounds entering the food chains. The opposite process is nitrogen mineralization, where organic N is converted to ammonium (NH_4^+) and then to nitrate (NO_3^-). The latter is reduced to N_2O via microbial denitrification process, and finally to N_2. Nitrous oxide is an important greenhouse gas, and the urban land-use has been shown to alter N cycling rates and N_2O fluxes to the atmosphere.

Nitrogen cycling has been poorly characterized in urban areas (Zhu et al. 2005). Still, there is some evidence that nitrogen-cycling parameters are affected by urban impacts and therefore can be used in bioindication. Various activities have been studied in relation to nitrogen cycling in soils. The proteolytic activity has been shown to have no coherence with numbers of proteolytic bacteria of site-specific properties (Bach and Munch 2000). This is possibly due to the fact that proteolytic activity changes are caused by differences in expression rates and not by the changes in microbial population. Moreover, the exoenzymes exist in soil independently from the bacteria that have produced those (Schloter et al. 2003). This leads to a slower response of this parameter to changing conditions. However, a deep decrease of proteolytic activity may indicate a long-lasting negative impact on the microbial community.

One of the most sensitive stages of the nitrogen cycle is nitrification. It has been shown that nitrification is altered by several soil properties, such as pH values, soil organic matter, and heavy metal content. The sensitivity of nitrification to a set of environmental properties makes it quite difficult to interpret the results of testing. Thus this process should not be considered as a straightforward bioindicator (Sauvé et al. 1999).

Nitrogen fixation has been shown to be also very sensitive to anthropogenic impacts (Filip 2002). In urban soils, the total nitrogen income is higher than in natural environments due to abundance of artificial sources of reactive nitrogen species. This can possibly play a role in repression of biological nitrogen fixation, as the presence of nitrate is well known to inhibit the nitrogenase activity (Cejudo and Paneque 1986). Therefore, the presence of reactive nitrogen species in soil samples should be taken into account when using direct measuring of nitrogen-fixing activity (i.e., via acetylene reduction technique) as a bioindicator.

The process of denitrification has been widely discussed in literature due to the role of this process in the emission of N_2O, an important greenhouse gas (Raciti et al. 2011). However, little attention has been paid so far to the use of this process for bioindication purposes, especially in urban environment. The quantity of denitrifying bacteria has been placed among most sensitive parameters (Filip 2002) indicating the anthropogenic impact. Our findings (Gorovtsov et al. 2013) have shown that the numbers of denitrifying bacteria, determined by MPN method varied greatly between the sampling sites from as high as 10^7 cells/g to as low as 10^2 cells/g of soil. The highest numbers were found in the sampling site close to the market entrance, where the soil was amended with food waste, and the lowest at the street side with degraded grass cover (bare soil), indicating the importance of available carbon sources for denitrification.

The nitrogen cycling features of urban soil remain to be poorly studied from the point of bioindication, and further research is needed to establish reliable parameters for practical use.

7.4 *Changes in Microbial Abundance and Community Structure*

The microbial communities react to the anthropogenic impacts by changes in their quantitative and qualitative characteristics. The decline in numbers of microorganisms, caused by the initial impact can be restored after some time by the adaptation of the community and growth of tolerant strains of bacteria which occupy the vacant ecological niches. Thus, the estimation of the numbers of bacteria in soils is insufficient to estimate the level of the environmental stress.

The changes in species composition and proportions of different groups of bacteria can be obvious even at the initial stages of the study. In our studies, the simple plating of soil dilutions on the nutrient agar revealed the deep differences in soil microbial communities in the city of Rostov-on-Don (Fig. 9.2).

The soils of the urban areas in Rostov-on-Don were rich in culturable bacteria forming pigmented colonies of yellow, orange, pink, and red color, which were absent in relatively undisturbed soil of the old fallow field adjacent to the city. The pigmented microorganisms were later identified with MALDI-TOF Biotyper, Bruker Daltonics, as members of *Microbacterium*, *Arthrobacter,* and *Rhodococcus* genera. The strains belonging to *Arthrobacter* sp. were dominant in the soils of the central parts of the city.

The proportion of *Bacillus* sp. gradually decreased in the urban–rural gradient toward the city center. The proportion of *Bacillus* spores in the culturable part of the

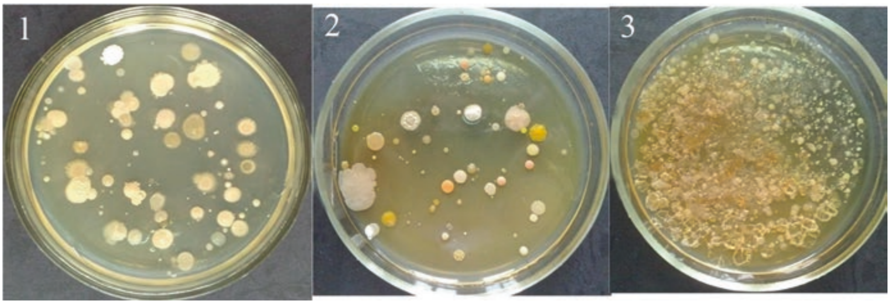


Fig. 9.2 The colonies of culturable bacteria on nutrient agar obtained from undisturbed soil (*1*), soil from city outskirts (*2*), soil from city center (*3*)

microbial community was determined by plating the same soil suspension before and after pasteurization. In undisturbed soils, the *Bacillus* spores comprised at average 4–5% of the bacteria, growing on nutrient agar, reaching a maximum of 25% at the plot of virgin steppe outside the city. In soils of city outskirts the share never exceeded 3–4%. In the soils of the city center, a sharp and statistically significant decline was observed with the proportion of *Bacillus* sp. spores as low as 0.1–0.01%. The proportion was greatly dependent from the state of grass cover, being the lowest at the plots with bare soil. It was also higher in autumn than in summer and spring. These findings indicate that the leading factor for the decrease in proportion of *Bacillus* sp. in urban soils is the availability of fresh organic matter from plant residues. The prevalence of *Arthrobacter* and *Rhodococcus* species in the soils of city center may be indicative of soil pollution with organic compounds, such as PAHs and petroleum hydrocarbons. Based on our observations, we therefore recommend to use the proportion of *Bacillus* sp. in the microbial community as a bioindicator of urban soil quality.

The reaction of various groups in the microbial community to specific pollutants has been reported in literature. It has been shown that cultivable *Pseudomonas* spp. increased its abundance upon the treatment of soil with PAHs (Niepceron et al. 2013).

There are also studies indicating that the contamination with heavy metals have various adverse effects on the microbial community (Lenart-Boroń et al. 2014). It has been shown that bacterial community was more sensitive to contamination with Zn and Cu than fungal community, which led to an increase in fungal/bacterial ratio with increasing level of contamination. However, the lower pH values enhanced the negative effect for bacteria, but not for fungi (Rajapaksha et al. 2004). Juwarkar et al. (2007) have shown the decrease in abundance of several groups within the microbial community for cadmium and lead contaminated soils. The decrease was most significant for actinomycetes and diazotrophic bacteria of the genus *Azotobacter*, indicating the possible use of these groups in bioindication of contaminated soils. Lenart and Wolny-Koładka (2013) have shown that in the urban soils of Cracow with polymetallic contamination all groups of soil microorganisms reduced their numbers compared to uncontaminated soil.

Thus, the quantity of certain groups of bacteria and the taxonomic structure of the microbial community is a valuable tool in bioindication. The development of molecular methods and culture-independent approaches will contribute greatly to the search for bioindicator organisms by the inclusion of unculturable groups of bacteria that are abundant in soil environment.

7.5 Changes in Soil Enzymatic Activity

Though the quantity of particular groups of bacteria may be a useful parameter in soil studies, it is not always directly linked to the soil quality. This is due to the fact that the proper functioning of the soil is dependent on the biochemical processes that are mediated by different enzymes. The enzymatic activities in soil are highly dependent on the physiological state of the microorganisms that produce these enzymes, which can alter the levels of expression of these proteins. For instance, if the majority of the cells are in dormant state due to unfavorable conditions, the enzymatic activity can be very low, while the direct counts, plate counts or qPCR-based estimations can still show large numbers of bacteria present in the sample. Another important factor is the influence of soil conditions. The activity of certain soil enzymes, especially exoenzymes, can be altered by soil pH, the heavy metal ions disrupting the disulfide bonds, nonspecific influence of soil organic matter, etc. Thus, even the sufficient level of expression in soil bacteria does not guarantee the high level of soil performance in the ecosystem. Still, the study of the soil enzymatic activity is irreplaceable for understanding of the soil functioning and the mechanisms of negative anthropogenic impacts.

There is a plenty of enzymes that can be studied for their activity in soil and many of them have a potential to be used in bioindication. These enzymes can be grouped according to the biogeochemical cycles they are involved into.

The enzymes involved in the carbon cycle include mostly hydrolases that are used to decompose polysaccharides of the plant residues. This group include amylases, α-glucosidase, β-glucosidase, 1,4-β-cellobiosidase, β-xylosidase, polyphenol oxidase, and invertase.

Among these, β-glucosidase is used most widely, as it is considered to indicate the soil quality and is linked to the SOM quantity and quality (de Almeida et al. 2015).

The nitrogen cycle is represented by urease, N-acetyl-β-D-glucosaminidase, total protease activity or more specifically, arginine aminopeptidase and tyrosine aminopeptidase activities. These enzymes play a crucial role in nitrogen mineralization, decomposing proteins, chitin and urea. The most commonly studied enzyme in this group is urease, due to the simplicity of the determination and high sensitivity to various negative impacts. Li et al. (2015) observed a significant decrease in urease activity in roadside soils in Beijing.

Phosphorous cycling enzymes are represented mainly by phosphatases with different optimal pH levels. Most commonly, the activity of alkaline (Wang et al. 2007) and acid phosphatases (Wieczorek et al. 2014) are measured, but some studies also include the phosphatase activity at neutral pH values (Cui et al. 2013). Due to high sensitivity of these enzymes, phosphatase activity is one of the most popular parameters in soil ecotoxicological studies, second only to dehydrogenase activity.

Among the sulfur cycle enzymes, the arylsulfatase activity is studied most extensively. This enzyme has been shown to be particularly sensitive to zinc, copper, and nickel contamination (Wyszkowska et al. 2016).

There is also a group of enzymes that are not directly linked to any biogeochemical cycle but play a crucial role in microbial metabolism. This group of parameters includes, for instance, catalase and dehydrogenase activities. The latter is the most widely used parameter in soil enzymology. It should be stressed that the enzymes of this group can be used for the estimation of the general state of the soil microbial community, while the enzymes involved in biogeochemical cycles of elements can reveal its more specific traits.

It is also notable that the vast majority of the element-cycling related enzymes belong to the hydrolase class. They are considered mainly from the point of mineralization of the corresponding elements for plant nutrition. This approach roots from the early studies, the majority of which was dedicated to agricultural soils.

Far too less attention is paid to the synthesis of the soil organic matter, which is a critical step for sustaining the soil quality in human-affected environments. Thus, while the up mentioned enzymes have proven to be useful tools for bioindication, there is still an urgent need to develop additional parameters for soil quality assessment.

8 Conclusions

Bioindication is a valuable instrument for the environmental quality assessment. It allows estimating the entire complex of negative factors and predicting their impact on the living organisms. However, there is no method that can allow making an accurate estimation, taken separately. We recommend using both active and passive bioindication to benefit from the strong sides of each approach.

We also recommend choosing carefully among the great number of parameters employed previously in bioindication studies. In general, all the parameters can be divided into two groups: those that characterize the whole community (i.e., microbial biomass, soil respiration, total dehydrogenase activity), and those that measure some specific features or processes (numbers of particular groups of bacteria, activity of enzymes with high substrate specificity, catabolic patterns). For the impacts that apparently influence a wide range of organisms, the choice of the parameters from the first group is more preferable. The parameters from the second group may be very sensitive, but they characterize only some part of the microbial community and should be used in the studies of particular soil functions or properties. In other words, a set of the parameters for bioindication should be consistent with the aim and scope of each study—there are no universal solutions.

The methods of bioindication of the environmental quality are developing rapidly, but there is still much to do in this field. Some parameters (like microbial biomass) remain controversial. Further research is needed to establish reliable parameters to study the element cycling, especially in urban environment. There is also a need for generally accepted list of microbial and plant bioindicators, which would contribute to our knowledge by comparative studies in different conditions.

In our view, the main future prospective in the field of bioindication is the transition from random selection to a common system of indicators, with the use of standard methods, based on a comprehensive approach to the studies of environmental quality.

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Chapter 10
An Overview of Sustainable Dimensions and Indicators for Smart City

Swatantra Kumar Dubey and Devesh Sharma

Abstract This paper provides an overview and literature review to understand the concept of smart cities with sustainability dimensions and indicators. Based on the outcomes of various studies, it is realized that several dimensions are identified with related factors for smart cities, i.e., economics, environmental, social, and governmental. These dimensions are further divided into indicators which are linked with various aspects like management and organization, technology, policy decision, public participation, socio-economic, infrastructure availability, and clean environment. It is very important to use these indicators to examine the participation and role of government for various development activities for smart city. These different definitions and indicators propose agendas for smart city development and provide an outline for government and other relevant organizations to take necessary steps in the formation of policy and development plans considering the future scenarios.

1 Introduction

It is mentioned that about 54% of the total global population is living in the urban areas in 2014 compared to about 30% in 1950, and it is expected to increase up to 66% by 2050 (United Nations 2014). Due to rapid urbanization and population growth, there are several environmental challenges like environmental pollution, waste management, safe drinkable water, lack of infrastructure, etc. (Kim and Han 2012; Marceau 2008). Toppeta (2010) emphasizes the sustainability of the smart city and how to improve the livability in the city. Washburn et al. (Washburn et al. 2010) explained the critical infrastructure and assessed the different types of technologies and services of a smart city. The urbanization is self-motivated and people shift to cities for searching the good life for their family (Owen 2009). In Europe, approximate 75% of total population already live in an urban society, and it is expected to increase up to 80% by 2020 (United Nations 2008). Nam and Pardo (2012) explained the different concept behind the smart city and other related terms

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in three categories, i.e., technology, people, and community. According to Komninos et al. (2013), innovation, technology, economics, social, and urban development will play significant role in the smart cities in the future prospective. Smart City acquires the different indicators for development, i.e., culture, education, training, industrial development, social, cultural, and economic dimensions for urban population benefits (Winters 2011). According to him, education is an important indicator of smart city, i.e., higher education has direct link with smart people, smart environment, and smart living. Due to rapid changes in urban areas, major key sectors for improvement are management and allocation of resources, availability of basic facilities and infrastructure, societal health. There is an urgent need of expertise from various fields like architecture, environmental, urban planner, water manager, transportation, social science, NGOs, information technology, etc. This paper provides the general overview of various dimensions and indicators related to smart cities. It also highlighted the various key challenges for smart cities. It also provides the literature review of various development theories, dimensions, and indicators of smart city.

2 Definition of Smart City: Overview

Before going to various details related to dimensions and indicators, there is need to understand the concept and essential elements about smart city. The smart city concept is emerging, and there are various concepts and definitions. Various authors described the concept of smart and digital city as summarized in Table 10.1.

3 Dimensions and Sustainability Indicators

There are many dimensions of smart city that are used by the various authors. Broadly, there are dimensions related to Economic, Social, Environmental, and Institute/Governance as given in Table 10.2. From the literature analysis, it shows that different types of dimensions are used for smart city.

The term “smart city” has been defined in the various scientific literatures. On the other side, the idea of a “smart city” refers to the urban places which are extant and composed as “every ware” (Greenfield 2006); where the digital devices built according to the urban environments. Smart city has to perform the different dimensions and long-term perspective of integrated approach of various sectors. These sectors are divided into different dimensions and shared the definition of smart city in various aspects and these are well-established in various scientific literatures (Giffinger et al. 2007). Six different types of dimensions of smart city are shown in Fig. 10.1.

Caragliu et al. (2011) developed different dimension and identified different levels of a smart city, i.e., the city layer, the green city layer, the interconnection layer, the instrumentation layer, the open integration layer, the application layer, and the innovation layer. It is realized that there is no exact definition of a smart city and

Table 10.1 The working definitions of smart and digital city

Working definitions	References
"A city that monitors and integrates conditions of all of its critical infrastructure, including roads, bridges, tunnels, rails, subways, airports, seaports, communications, water, power, even major buildings, can better optimize its resources, plan its preventive maintenance activities, and monitor security aspects while maximizing services to its citizens"	Hall (2000a)
"A city that monitors and integrates conditions of all of its critical infrastructures"	Hall (2000b)
"A smart community is a community that has made a conscious effort to use information technology to transform life and work within its region in significant and fundamental rather than incremental ways"	California Institute (2001)
"A Digital City has at least two plausible meanings: (1) a city that is being transformed or re-oriented through digital technology and (2) a digital representation or reflection of some aspects of an actual or imagined city"	Schuler (2002)
"The areas which have the ability to support learning, technological development, and innovation procedures on the one hand, with digital spaces, information processing, and knowledge transfer on the other hand"	Komninos (2002)
The "smart community" where "public administration, enterprises and residents have understood the opportunities offered by IT and attempt to use those technologies to improve their day-to-day life and work in a significant and efficient manner"	Morse (2004)
"One emerging thought is that for a city to function well as a healthy system it needs an appropriate level of complexity at all levels in its operations. This complexity is needed not just in its physical networks – its roads, its buildings and its communications, but also culturally, and economically. There are perhaps pockets in a city that are 'mono-culture', which makes them very difficult places in which to thrive when the external conditions are changing. This applies both at the poor end of the spectrum in areas with a high index of multiple deprivation and rich societies"	Diamond (2005)
"The places where ICT is incorporated into the living and work environments"	Steventon and Wright (2006)
"A Smart City is a city well performing built on the 'smart' combination of endowments and activities of self-decisive, independent and aware citizens"	Caragliu et al. (2011)
"Digital city denotes an area that combines broadband communication infrastructure with flexible, service-oriented computing systems. These new digital infrastructures seek to ensure better services for citizens, consumers and business in a specific area"	Komninos (2008a, b)
"The term Digital City (a.k.a., digital community, information city and e-city) refers to: a connected community that combines broadband communications infrastructure; a flexible, service oriented computing infrastructure based on open industry standards; and, innovative services to meet the needs of governments and their employees, citizens and businesses. The goal of a Digital City is to create an environment for information sharing, collaboration, interoperability & seamless experience for all its inhabitants anywhere in the city"	Yovanof and Hazapis (2009)

(continued)

Table 10.1 (continued)

Working definitions	References
"Smart city is defined by IBM as the use of information and communication technology to sense, analyze, and integrate the key information of core systems in running cities"	IBM (2010)
"Concept of a Smart City where citizens, objects, utilities, etc., connect in a seamless manner using ubiquitous technologies, so as to significantly enhance the living experience in twenty-first century urban environments"	Northstream (2010)
"Green City follows the Green Growth which is a new paradigm that promotes economic development while reducing greenhouse gas emissions and pollution, minimizing waste and inefficient use of natural resources and maintaining biodiversity"	OECD (2010)
"A city to be smart when investments in human and social capital and traditional (transport) and modern (ICT) communication, infrastructure, fuel, sustainable economic growth and a high quality of life, with a wise management of natural resources, through participatory governance"	Caragliu et al. (2011)
"Smart City is the product of Digital City combined with the Internet of Things"	Su et al. (2011)
"Smart City is a city in which it can combine technologies as diverse as water recycling, advanced energy grids and mobile communications in order to reduce environmental impact and to offer its citizens better lives"	Setis-Eu (2012)
"A smart city is a well-defined geographical area, in which high technologies such as ICT, logistic, energy production, and so on, cooperate to create benefits for citizens in terms of well-being, inclusion and participation, environmental quality, intelligent development; it is governed by a well-defined pool of subjects, able to state the rules and policy for the city government and development"	Dameri (2013)
"The concept of Digital City is to build an arena in which people in regional communities can interact and share knowledge, experiences, and mutual interests. Digital City integrates urban information (both achievable and real time) and create public spaces in the Internet for people living/visiting the city"	Sorrentino and Simonetta (2013)

there are many dimensions identified which include economy, mobility, environment, people, living, and governance (Giffinger et al. 2007).

Table 10.3 presents the dimensions used in different literature and these are based on the economic aspects, environmental aspects, and governance aspects. These dimensions are divided into different other indicators like technology, education, skills, management, urban vision, policy, citizens, and private partners.

The idea of a "smart city" is perceived to refer the development of the area with respect to the mobility, economy, environment, and knowledge of the city-region (Kourtit and Nijkamp 2012a, b). The smart city perception of economy and governance are involved in innovation, creativity, and entrepreneurship; the indicators are passed by smart people. In the smart city city, there is importance of information and communication technology to provide platform for sharing and implenting the ideas and innovation for various sectors like education, governance, transport, water and elctricity, etc. (Hollands 2008). In this scenario, the technologies are used for innovation and create facilities for social, environmental, economic, and cultural development (Allwinkle and Cruickshank 2011).

Table 10.2 Dimensions and categories of Sustainability indicators

Dimensions of sustainability	Categories of sustainability
Economic	Income
	Growth/development
	Consumption
	Infrastructure services
	Tourism
	Transportation
Social	Demographics
	Education
	Health
	Equity
	Poverty
	Housing quality
	Safety
	Access to basic needs (energy, water, sanitation)
Environmental	Climate change
	Air pollution
	Soil pollution
	Water pollution
	Urban green spaces
	Land use pattern
	Energy
	Water consumption
Institutional/Governance	Government
	Industry

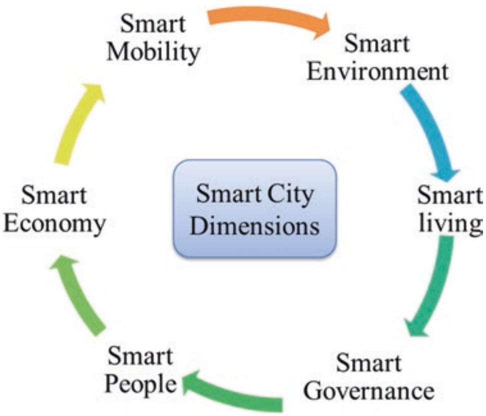

Fig. 10.1 Dimensions of Smart City

Table 10.3 The different dimensions of the Smart City

Dimension of Smart City	References
(a) IT infrastructure and education (b) IT economy (c) Quality of life	Mahizhnan (1999)
(a) Smart economy (b) Smart mobility (c) Smart environment (d) Smart people (e) Smart living (f) Smart governance	Giffinger et al. (2007)
(a) Skills (b) Knowledge (c) Digital spaces (d) Innovation performance	Komninos (2008a, b)
(a) Technological development (b) Economic development (c) Job prospectus (d) Quality of life	Eger (2009)
(a) Sustainable economic development (b) Management of natural resources through participatory approach (c) Integration of economic, social, and environmental goals	Thuzar (2011)
(a) Economic, socio-political issues of the city (b) Economo-techno-social issues of the environment (c) Instrumentation (d) Integration (e) Applications (f) Innovations	Nam and Pardo (2011)
(a) Technology (b) Citizens (c) Public private partnerships (d) Urban vision	Pardo and Taewoo (2011)
(a) Mobility management (b) Resource management (c) Quality of life	Fondazione Ambrosetti (2012)
(a) Human capital (skilled labor force) (b) Infra-structural capital (high-tech communication facilities) (c) Social capital (intense and open network linkages) (d) Entrepreneurial capital (creative and risk-taking business activities)	Kourtit and Nijkamp (2012a, b)
(a) Policy (b) Citizens (c) Research (d) Private partners	Baccarne et al. (2014)

Table 10.4 Sustainability indicators used for urban area development and transportation

Topics	Author (Year)	Sustainable development indicator	Sustainable transportation indicator
"Practical appraisal of sustainable development, methodologies for sustainability measurement at settlement level"	Moles et al. (2008)	40	11
"Sustainable transportation indicators, Subcommittee of the Transportation Research Board"	Litman (2009)	30	12
"Intelligent Cities and Globalisation of Innovation Networks"	Komninos (2008a, b)	35	–
"Measurement indicators and an evaluation approach for assessing urban sustainable development: (China's Jining City)"	Li et al. (2009)	52	3
"ELASTIC – a methodological framework for identifying and selecting sustainable transport indicators"	Castillo and Pitfield (2010)	–	20
"Evaluation of the Q-method as a method of public participation in the selection of sustainable development indicators"	Doody et al. (2009)	37	5
"Measuring the sustainability of cities: an analysis of the use of local indicators"	Tanguay et al. (2010)	233	63
"The role of common local indicators in regional sustainability assessment"	Mascarenhas et al. (2010)	55	5
"Smart Cities in Italy: An Opportunity in the Spirit of the Renaissance for a New Quality of Life"	Fondazione Ambrosetti (2012)	27	–

Table 10.4 shows the sustainability indicators for urban area development and transportation, because in the smart city development and transportation play the important role in the growth and facilitated by the different basic needs by these developments. In the literatures, different indicators were used for development and transportation; these indicators are varied in numbers because of different locations and methodologies.

4 Key Pillars of Sustainable Smart City

For the smart city perspective, different types of dimensions play a major role and that dimensions are divided into different indicators to develop the city for smarter to the various aspects like environmental, social, economic, and policy. In this study, some important dimensions are mentioned as pillars of smart cities.

4.1 Smart Environment

Smart Environment represents a quality of living in the urban areas. The area covered under green space is an important dimension of city development; good environment can create many types of social and economic benefits for smart people (del Saz-Salazar and Menéndez 2007; Jim 2013). Many of the cities are polluted by using the excess resources and resulting in more pollution (point as well as non-point). It is very necessary to adopt the smart and innovative solution to reduce the further degradation of environment. There is urgent need to develop and implement plans mainly focused on transportation sector, energy and water conservation, urban planning and green building, and waste management. Efforts are required by the policy-makers to initiate the process and management to mitigate pollution and their relevance in public domain (Glaeser 2011). According to Giffinger et al. (2007), the smart environment is based on conservation of natural resource, and different types of environmental pollution indicators are used; the characteristics of different indicator are as follows:

(a) Natural conditions of resource
(b) Environmental pollution (air, water, soil, waste, etc.)
(c) Environmental protection
(d) Sustainable resource management

4.2 Smart Society

Smart society is a strong component of smart city as the people are more exposed to the technology, policy, and environmental aspects. The large cities attract human capital (Elvery 2010) because of the resource production, i.e., infrastructure, telecommunication, water resource, and IT structure in the cities. The smart city concept is used for different techniques to increase the sustainability of resources related to the different dimensions of cities. Some characteristics of the smart society are as follows:

(a) Level of qualification
(b) Affinity for lifelong learning
(c) Social and ethnic plurality
(d) Creativity
(e) Participation and coordination in development process

4.3 Smart Economy

Smart Economy includes the factors related to the market and development of the urban areas including the economic competitiveness, entrepreneurship, efficiency, and flexibility of the labor market at various levels. In the city, the GDP and growth rate is influenced by several reasons. High GDP growth rate go through a higher economic development, which influences the investment in the financial sector, transportation sector, utility and telecommunications sectors, infrastructure, and education sectors (Al-Hader and Rodzi 2009). People are mainly attracted by cities with high economic growth and good living standard (Cheshire and Magrini 2006; Lambiri et al. 2007). According to Giffinger et al. (2007), the smart economy is based on the competitiveness of the indicators and that indicate the particular feature of smart city. Some of them are as follows:

(a) Innovative spirit
(b) New opportunity (entrepreneurship)
(c) Economic image & trademarks
(d) Economic growth (productivity)
(e) Flexibility of labor market
(f) Collaboration at national and international level
(g) Capability to transform
(h) Development of ICT facilities
(i) Safe and reliable transport systems

4.4 Education and Capacity Building

Education, training, and capacity building programs are effective tools for smart city as there is direct linkage between education and society behavior. Education can play important role in the making and planning of smart city to inhabit socially a particular kind of a smart citizen (Foucault 2007, Ben Williamson 2015). Ben Williamson (2015) coupled the smart city and smart education, considering the future education in the various aspects like the level of qualification, activities for lifelong learning, flexibility, and creativity. Some indicators that correspond to education and capacity building are as follows:

(a) Students per inhabitant
(b) Satisfaction with accessibility and quality of educational system
(c) Awareness of citizens to use the latest green and innovative technologies
(d) Participatory approach of community and society for overall national development

5 Conclusion

This chapter is compilation of various concepts related to smart city with various approaches, types of dimensions, and indicators. The smartness of any city should be measured with the help of various dimensions focused on sustainability approach based on input from different indicators. A strong driver for a smart city implementation is the city dimension, to improve the technological progress, economic development, education, and awareness. There is also need of contributions and supports from various national or international organizations, institutes, non-government organizations, and funding agencies so that further improvements can be considered to develop key aspects of smart city like healthy lifestyle, clean and green areas, infrastructure facilities, social development, government policies, sustainability, economic progress, education development, and environment. A city is able to achieve the vision of smart city by connecting the different dimensions and different indicators and make them operational with key participation of the society.

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Chapter 11
Need for an Integrated Approach Towards Environmental Quality Control in Developing Countries

Virendra Misra, Ritu Singh, and Sanjeev Kumar

Abstract Rapid industrialization has led to pollution of air and water and generation of hazardous wastes leading to significant environmental problems. The interrelations among population growth, lack of resources, general ill health and socio-economic factors with environmental pollutions have to be accounted for. Therefore eco-restructuring of economic development is necessary which addresses the role of globalization on growth and development. Regulatory aspects in chemical safety for environmental control in developing countries have to be integrated in the overall perspective of the local situations. Conceptual considerations along with challenges for sustainable development, role of research and development in solving environmental problems, anticipatory actions, environmental quality control and approaches to solutions are presented here.

1 Introduction

There is a fundamental interdependence between the world's environment—the physical, biological and social surroundings and their interactions that sustain life—and the objectives of human development. Many environmental problems at the global level mean that circumstances and situations indispensable to sustain development and quality of life in developing and industrialized nations are meticulously linked to each other. The continuously increasing depletion of natural resources is

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restraining the development potential of both developing and industrialized nations, threatening the global environment with unsustainable forms of development. Therefore, it has become imperative that all the nations work collectively in order to resolve threats to world's environment, and specifically those, which undermines sustainability at local levels.

Due to the twin needs of providing a better quality of life to the masses through developmental activities and of assuring balanced use of the natural resource base, a strategy for sustained development without environmental degradation is essential for developing countries. Large-scale industrial and agricultural expansion to meet the day-to-day demands and to create employment has led to hitherto unfamiliar levels of pollution (Jaffery et al. 1992; Schoneveld et al. 2015). Although industrial development has made significant contribution in the economic growth of India, it has been done at cost of environment. Increasing industrial pollution is not only posing threat to public health and environment but a significant portion of India's gross domestic product (GDP) gets consumed over the strategies and efforts which are made for pollution abatement. This coupled with inadequate technology for pollution control and lack of awareness on safe use of chemicals enhances the toxicity risk due to occupational and environmental chemicals. Superimposed conditions locally prevalent, such as special high-risk groups, protein and calorie malnutrition, parasitic infestation and pathogenic diseases, make effective environmental management more difficult in Third World countries (Viswanathan and Misra 1989). Geoclimatic factors could also influence effects of environmental stress (Viswanathan and Krishna Murti 1989). In long term, for reaching to a "safe" level from present state of global climate change, a strong linkage between climate change policies and sustainable development strategies has become a necessity. From a "developing country perspective", exploration of connections between climate change and sustainable development must be initiated by considering local and regional circumstances and policy contexts (Beg et al. 2002; La Rovere 2002; Halsnaes and Shukla 2008). Studies show that some of the most adverse effects of climatic change will be in developing countries, where populations are more vulnerable and least likely to easily adapt to climatic change. Changes in temperature, water supply and quality will impact on agricultural production, human settlement and health, biodiversity and animal migration patterns (Intergovernmental Panel on Climatic Change 2001a, b; Tokimatsu et al. 2012). In a detailed study of India, Kumar and Parikh (1997, 1998) examined the impact of temperature rise on agricultural yields, output, income and prices. An increase in temperature with an accompanying precipitation increase results in a fall in the farm level total net revenue. Apart from industrial effluents and emanations and agricultural run-offs, many other factors such as domestic fuel burning, biological refuse and decay, natural dusts, etc. create additional problems.

As we are facing environmental problems of unprecedented complexity, an integrated approach to our nation's environmental research and development activities has to be undertaken. Now, the traditional practices that are based on reviewing individual problems of environment and formulating narrowly focused remediation/mitigation strategies to tackle them will no longer be suffice. Developing a deeper scientific understanding of ecosystems is the need of present time. The study of the sociological and economic aspects of human interactions with the environment also required to be assessed. Therefore in developing countries, environmental planning, management and risk assessment (Misra et al. 1991), including research opportunities, priorities and strategies have to be pursued not in isolation but as a part of the entire scenario of development to more effectually address current as well as future environmental problems. The essential components of such an approach, the rationale behind the concept of sustained development without destruction and possible solutions to achieve this are critically appraised later. A quantitative conceptual treatment of the interrelations is also attempted.

2 Improving Quality of Life for the Present and the Future

An imperative goal of the mankind is to enhance the quality of our environment for the present and the future generations. Economic and social development is indispensable for ensuring favourable environment for man and so is the natural resource base. As per UNPD (1998a), the world population will most likely reach 7.6 billion in 2020, an increase of 31% over the mid-1996 population of 5.8 billion and over this period ~ 98% of the projected population growth will take place in developing countries. It has also been estimated that the developing world's urban population will get double, reaching 3.4 billion between the years 1995 and 2020 (United Nations Population Division 1998b). The impact of human on the environment will be in terms of (a) total population numbers, (b) population growth, (c) population density and population distribution determined by migration and urbanization and (d) population composition in respect of population age and household size. Each of these pose great challenges to food systems and consumption levels trends. This, along with general ill health lower living standards, continued depletion of resources and environmental pollution forms segments of a vicious circle threatening the progress of many Third World countries. How these factors are interrelated and how each of these can be controlled, so that ultimately the goal of overall development could be achieved is conceptually depicted in Fig. 11.1.

Food systems in developing countries are not as organized as in the industrialized countries. Furthermore, problems of growing population, increasing urbanization, lack of resources to handle pre- and post-harvest food losses, problems of environmental and food hygiene, all these indicate that food system will remain to be stressed in developing countries. Hence, the people in developing countries are exposed to a widespread range of potential food quality and safety risks (Turi et al. 2014; Bazga 2015). Apart from this, rapid urbanization has headed urban services to be extended beyond their boundaries resulting in inadequate supplies of necessary services like potable water, sewage disposal, etc.

Standard of living is a relative term subject to dynamic changes within the availability of resources and traditional way of life. Considering the fact that the minimum essential requirements for individuals and societies vary, even then a certain level of dignified living condition has to be maintained which, with more and more progress, should automatically rise with time. The absence of even these lead to a situation of poverty caused by generations of substandard living, indiscriminate

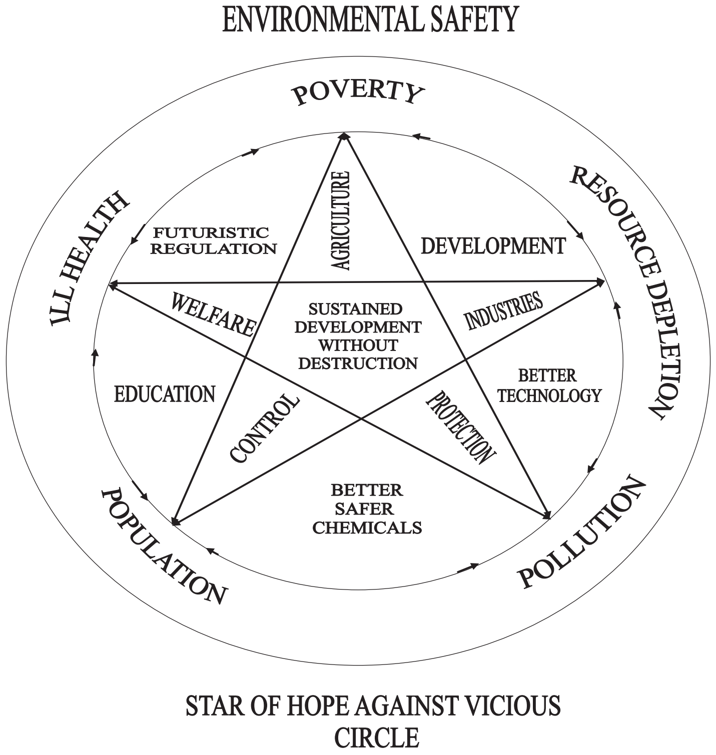

Fig. 11.1 Steps towards sustainable development

depletion of natural resources (both renewable and non-renewable), perennial shortage of food, drugs, shelter, etc. This could gradually be improved by intensive developmental activities in agriculture, industries, consumer goods production, cheaper medicines, adequate quality and quantity of food, etc. But poverty per se is directly related to population along with the traditional family structure and way of life with the characteristic phrase, “more numbers mean more working hands” especially in rural agrarian sectors. Some 90% of this greatly expanded population is in the developing countries, most of which is hard pressed and the implications are alarming. This is due largely to underdevelopment and poverty, which, in turn, contribute to more resource wastage and depletion. These burgeoning numbers translate into increased human needs and intensify pressure on the available resources that have been dangerously diminishing. Resources may be a free gift to mankind but this gift is of incalculable value, which is being damaged, threatening millions of world’s people, rich and poor alike. Population, environment, natural resources and development are interlinked. Poor communities and the disadvantaged—the poor, women and children, and indigenous peoples are more vulnerable to environmental problems. Thus, to address the socio-economic prerequisites of these groups, improved efforts must be made in rural and urban communities.

More of industries, power generation, agrochemical use, transport systems, construction activity all lead to pollution. A thousand hutments clustered together using cheap inferior quality fuels could be highly polluting (Centre for Science and Environment [CSE] 1985). The scenario of pollution in rural area is bleak in spite of the absence of major industries (Viswanathan 1981). The trend of migration of rural folk to urban slums further aggravates environmental and socio-economic problems, which if unattended, build up ecological pressures. In today's Third world, it is the means that are sparse and demographic pressures are significant limitations on efforts to increase these means. Lack of adequate amounts of quality food and water, economic constraints coming in the way of adequate health protection and many other factors, the interrelation between poverty and ill health are traditionally well established.

Non-communicable diseases are slowly occupying the whole globe. An increasing trend in developing countries has been observed, where the demographic and socio-economic transition enforces more restraints on handling double burden of infectious and non-infectious diseases, characterized by ill health systems (Mathers et al. 2003; WHO 2003). If the present trend continues in future, it is predicted that by 2020, non-communicable diseases will account for about 80% of global burden of disease, causing 7 out of every 10 deaths in developing countries (WHO 2002). In parallel, infectious diseases remain to be the main cause of mortality in developing nations.

Malnutrition and under-nutrition not only cause health problems reducing productivity and welfare but also enhance health care costs. Further, these predisposing conditions lead to anaemia, diminished body defences against infections and toxic chemicals, and also decreased capacity for detoxifying and clearing off of xenobiotics (Calabrese 1984; McMichael 2000; Smith et al. 1999). Economic necessities and lack of more suitable alternative sources of earning make even such high-risk populations take up hazardous occupations. Socio-economic traditions greatly influence the earlier scenario. Lack of education leads to absence of awareness regarding safe use of newer technologies and chemicals.

In the last two decades, developing countries have marked a remarkable increase in food production but at the cost of environment. Systematic large-scale uses of pesticides have developed in enhancing crop production and storage facilities; however, undesirable side effects of environmental residue build-up due to indiscriminate and often improper use, exposure risk to workers and even accidental or homicidal exposure have shown an upward trend. Lack of environmental consciousness also adds to improper usage and disposal aggravates health and environmental risks (Cairns 1982). Continuous increase in the levels of pollutants contaminating every sphere of environment, degrading soil quality, deteriorating air and water qualities are increasing concerns as the populations continue to grow.

Until recently, it was generally thought that economic development is an indispensible necessity for enhancing the health status of a population and health was categorized as a non-productive sector. Impact of environment causes measurable changes in the production of a specific good or service, and thereby affects human welfare and health. But recent evidences revealed that improved health is far more than a consequence of development. It is a principal contribution into socio-economic development as well as poverty reduction (Freedman et al. 2005; Ismail et al. 2012). Population health becomes more than either a determinant or an incidental consequence of economic development (McMichael 2000; McMichael and Kjellstrom 2002; Ismail et al. 2012). In developing countries, health innovations are supposed to be the best solutions for tackling diseases of the poor (Mashelkar 2005).

Traditional domestic fuels used in a sizeable proportion of our over ten million households add to smoke and related pollutants. Provision of cheaper, efficient, non-smoky fuels could be the answer, but it involves massive resources and distribution machinery. If the available Indian figures for population, annual death rate, the percentage practicing traditional cremation, the amount of wood burnt and the composition of the products of biomass burning are computed, a correct picture of the declining tree cover and pollution can be obtained. The contribution of human carcasses to river pollution is another instance. Centuries-old practices cannot be drastically changed and alternatives have to evolve gradually.

3 Promising Signs

Now governments and industries, both are making efforts to reduce their environmental "footprint" on the world. The three 'R' strategy—'Reduce, Reuse and Recyle is one of the steps in this direction. Other inputs include dropping down the quantity of materials and energy which was used in providing their services. There is an increasing trend of reducing the impacts of industry and agriculture owing to pollution laws and regulations, public pressure and growing awareness about environmental performance and benefits. However, the initial cost of installing new technologies and inadequate conservation learning put a setback to better efficiency gains. The majority of farmers still irrigate their field with age-old inefficient practices. India has made noteworthy efforts in the area of environmental protection, developing environmental standards for products and processes, necessitating environmental impact assessment and introducing environmental audits. The Government of India has undertaken few initiatives for environment benefits such as introduction of emission norms, alternative fuels and mass transit system to control air pollution. Recently, State governments have taken the initiative of using bio-diesel as an alternate fuel for transportation sector in Gujarat, Karnataka, Maharashtra and Haryana. Similarly, the commissioning of hydrogen-compressed natural gas (HCNG)-dispensing station marks a fresh breakthrough in India's expedition for alternative fuels (Bhattacharya 2005).

4 Challenges for Sustainable Development

The concept of sustainable development encompasses environmental sustainability integrated with economic, social, cultural and political sustainability. To achieve sustainable development, free from adverse effects realistic assessment of the present situation, identification of priorities and approaches for action and long-term planning for anticipatory action based on futuristic models are vital (Klauer 1999; Ross 2009). Since the causative factor culminating into a deteriorating situation of environmental degradation and further drop in the quality of day-to-day life is intermingled deeply, the solution also has to be evolved in a coordinated manner. Some of the issues that work in contradiction to the environmental interest are (i) inadequate financial and human resources, (ii) unfledged institutional and technological capabilities, (iii) insufficient prospects for people to contribute significantly in the development process, (iv) constricted economic and social policies and (v) poor incentives for environmentally sound behaviour (Azapagic et al. 2016; Husted and Filho 2016).

Challenges for sustainable development require social, political and economic considerations. In fact, there are five allied facets of the sustainability concept which are as follows:

(a) **Economic Sustainability** which deals with pertinent economic policies, effective resource apportionment and use and more equitable sharing of resources,
(b) **Social Sustainability** means ensuring more rightful income distribution among the participating beneficiaries and those who may get affected in the judgements which are related to their lives,
(c) **Cultural Sustainability** means sensitivity towards cultural factors and cultural diversity,
(d) **Political Sustainability** requires good governance, reassurance of human rights and advancement of democratic development and
(e) **Environmental Sustainability** requires managing the ecosystem for the maintenance of its structure and functions and economic productivity, conserving the diversity of life in natural as well as human-managed systems and protecting the environment from contamination and pollution to maintain the quality of environmental matrices (Azapagic et al. 2016; Husted and Filho 2016).

In this context, pollution control and environmental conservation cannot be tackled in isolation. These have to be linked with other developmental activities such as modern agricultural technology, efficient use of raw materials, recycling of wastes, including chemicals and water, and social forestry for resource generation and conservation. Community health care programmes could be linked up with occupational health control, accident prevention, safe consumer goods, food and water quality and environmental clean-up; general procedures for improved hygiene including nutritional improvements and immunological protection. Protection of wildlife through "reserves" and clean-up of rivers and lakes could also boost recreational activities.

Creation of awareness regarding chemical safety and environmental conservation cannot be separated from the totality of bringing the benefits of education to all. By better education, health and necessities for existence, overall welfare and progress can be achieved. Thus, an integrated "area development" is the need of the hour wherein all the independent approaches towards progress have to be harmoniously interlinked. For this, it is also equally essential to strengthen the efforts for preventing population explosion.

5 Conceptual Model

For any meaningful and sustainable reform of the environment, the society in total has to be considered, which consists of:

1. The ecological system: represents the physical environment, i.e. an ecosystems stability, potential and limits
2. The social or political system: consists of those formal and informal groupings through which groups and individuals interact—the relationship among population, poverty, and natural resource consumption and degradation
3. The cultural system: collection of activities and attitudes through which we exercise self-expression and creativity—many of which are spiritual and aesthetic
4. The economic system: caters to the material well-being of man. Also, recognizing environmental values in economic decision-making.
5. The security system: protects the integrity of the entire set of subsystems.

Human health can be regarded as a function of a variety of elements. The relation between these exogenous and endogenous elements illuminates why the response to environmental exposures may vary considerably from one individual to another (Doll 1992; Ozonoff 1994; Cesario 2016; Deniz 2016). Basically, four main groups of determinants can be distinguished; lifestyle, the physical environment, the social environment and endogenous individual attributes, either genetic or acquired during life (Fig. 11.2). The most appealing example of a genetic factor affecting a person's state of health of course is gender: regardless of the prevailing health system, all over the world women live several years longer than men do (Manton et al. 1991).

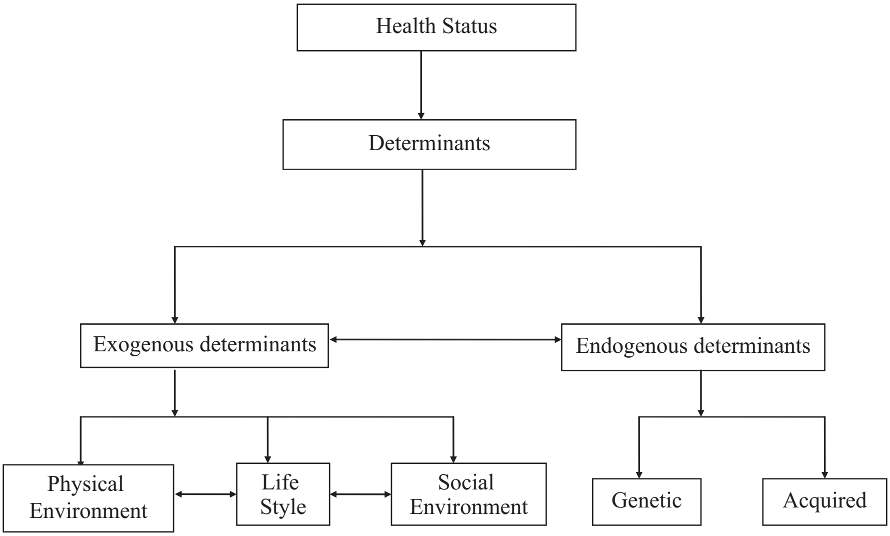

Fig. 11.2 Determinants in health and environment

Most endogenous determinants develop through interactions between genes and environmental factors and thus have both a genetic and an acquired component, for example body weight, blood pressure, blood lipoprotein composition (familial risk factors) and personal (psychological) attributes (Cambien 1996; Romieu and Trenga 2001; Doevendans et al. 2001). The physical and social environments, as well as lifestyle are regarded as exogenous determinants. The physical environment includes factors such as radiation, noise and heat, hazardous substances in the outdoor and indoor environment, including the working environment (e.g. chemicals, bacteria, viruses and other micro-organisms which may have both positive as well as negative effects on health status). Lifestyle factors include diet, smoking, drug abuse, sexual habits, etc. The social environment encompasses socio-economic status, the pattern of social networks and cultural factors (Ruwaard and Kramers 1998). There is a complex relationship of cause and effect between poverty, population, natural resource consumption and environmental degradation.

The chief elements of hazard and risk assessments are stress, human activities, resource and environment, which show a cause-and-effect relationship. If H represents human activity, R resource and E environment, then the overall complexity for a given stress is simply the sum of the system complexity index Ci:

$$Ci = CiE + CiH + CiR$$

Like all other living beings, man has to rely on the environment for his survival and welfare as he cannot live without his natural resources (food, energy, shelter and clothing) or the inevitable disposal of waste material. This is best known of the interaction between the environment, development and health. An appropriate development of this sort places an undue burden on the natural resources of the environment, and thus damages human health. The living standard of any country Q is defined as:

$$\mathrm{Q} = \frac{\text{Total resources available}}{\text{Population density} \times \textit{per}\ \text{capita consumption}}$$

Low values of Q suggest poor living conditions due to excessive pressure on available resources, and high values of Q indicate a good situation for present as well as future. For environmental management, efforts should be directed to attain high Q.

In the case of developing countries, better and safer technology can play a very important role. Technology can increase productivity; it can also lift the limits of growth but to sustain growth indefinitely the following points are needed:

(a) Technological solutions cause new problems such as pollution abatement devices expend resources, resource recycling and substitution require new energy, and increased agricultural productivity yields aggravated pollution from fertilizer run-off and pesticide use.
(b) Population stability, which can only be achieved by massive educational effort.

In economic sense, technology is considered as a multiplier on the production function

$$Q = f\left(L,C\right) \times T$$

Where Q = production, a function of labour L. and Capital C, multiplied by a technology factor T. Technology then represents getting more goods for given inputs of labour and capital. Appropriate technology is to be developed/adopted for each scenario.

6 Role of Research and Development to Solve Environmental Problems

Research is prerequisite to enhance our understanding of the physical–chemical and biological processes of environment in association with the social and economic processes that control our interactions with those systems. Development of more efficient environmental research tools through exploitation of advances in computational, communication and biological technologies; more sophisticated environmental models; and improved laboratory, data analysis, and assessment methods are needed.

Many of the environmental problems that have been understood and managed as isolated/individual phenomena are, in fact, closely entangled. For instance, a single pollutant species such as nitric oxide (NO), produced from the combustion engines of automobiles/aircrafts can (i) modify the rate of ozone depletion, (ii) contribute to global warming by producing ozone, (iii) trigger health problems particularly among asthmatics, (iv) be oxidized to nitric acid contributing to acid rain, (v) get deposited as nitrate contaminating a drinking water body or (v) adding to the eutrophication. However, deposited nitrate ions can also act as fertilizer for crops and plants. Thus, the strategies formulated to mitigate one problem may aggravate another. Therefore, the application and sustenance of meaningful environmental R&D programmes will be critical if a holistic view is undertaken by environmental science and engineering community for management of current and future environmental problems.

Safer substitutes for hazardous chemicals and processes used in all walks of life will also help in reducing the problems. Environmental problems such as global warming (due to greenhouse gases), ozone layer depletion (because of CFCs), toxic waste movement across international borders and destruction resulting from acid rain are increasingly becoming a global concern. As a result, development of new environmental technologies to address these problems to ensure sustainable social and economic development is now regarded as a major international issue. As CFCs have a number of excellent properties, developing their substitutes that do not harm the ozone layer or cause global warming could be an R&D objective (Porrit 2005). To prioritize research, mechanisms are to be developed to recognize emerging environmental issues and apply risk-based assessment methods/techniques. R&D partnerships with industry, universities, government and non-government organizations involved in environmental research should result in simpler, faster and less costly techniques to help local officials make wiser and fairer decisions.

Manpower and managing human resources is the most important and precious of all resources in the world (Sors 1982). It is people that drive social progress, advances science and technology and continuously transform the human environment. If the large pool of available talent is channelized towards solving environmental problems through indigenous research and development capabilities, only then will sustainable development be possible. The objective is to create knowledge that would identify problems as accurately as possible and help in resolving them. Each problem should be assessed and a rational method developed for placing them in proper order of priority. Once problems have been identified and rated according to their priority, a proper technology assessment of each should be made, which will determine various types of research that are required to provide the knowledge necessary to solve the problem. The work done at various places must be managed in coordinated fashion so that all elements contribute to the overall objective. The last step is the final application of the results and solving of problems.

7 Integrated Strategy for Anticipatory Action

As stated earlier, overpopulation in developing countries leads to a life of poverty and misery, ill health, poor education, unemployment, malnutrition and starvation. Therefore, population growth control is of utmost importance that has already stretched the ecological and economical limits in many Third World countries. Land is a key resource and a vital component of the life support system. The growing scarcity of arable land pushes people to cultivate marginal lands, steep slopes, drought-prone lands, resulting in severe soil erosion and productivity loss. Expansion of agricultural populations has led to encroachments on grazing lands, while growth of pastoral populations accompanies increase in the number of cattle, sheep and goats. The end result is overgrazing and ultimately desertification.

The forest resources of the Third World are amongst the principal victims of man’s onslaught. Most of the people are dependent on wood as the chief source of domestic fuel. Under the pressure of escalating human and animal population, forest cover is being rapidly stripped and land productivity is being diminished, increasing the severity of droughts and floods. Most countries of the Hindukush Himalayas—Afghanistan, Pakistan, Bangladesh, Bhutan, Burma, China and India are confronted by these problems to a greater or lesser extent. There is an acute shortage of firewood. Hillside erosion, rapid population growth, floods and diminishing wildlife and genetic resources threaten each country. Restoration of a balance between man and land is to be established to halt desertification. In all seriousness and earnestness, population cannot be isolated from resources, development and the environment. Ecological disasters are no longer predicted they are here. The depletion of ground water resource in India through deforestation and other consequences of large irrigation schemes has left 23,000 villages without drinking water. Within two decades, a soil and water crisis, unless checked by anticipatory action, could bring famine of Ethiopian dimensions.

Thus, a comprehensive land use policy should be made to create a balance between agriculture, industry and urbanization. Land, water and natural resource management has to be in line with environmental preservation and sustainable development. Data-based information should be made available for land classification for crops, forests, permanent vegetation, grasslands, water regimes, industrial uses and human settlements. Well-developed countries like Japan and France have already had land use plan and land preservation programme for quite some time now. An environmentally sound and economically efficient water conservation and management policy is the need of the hour. Resource policies must be coordinated among neighbouring Third World countries if impending crises are to be averted; river control watershed management, use of scarce land, soil erosion, energy needs and deforestation all have to be considered.

The extent of degradation into wastelands is astounding. Due to topsoil loss, infertility and barrenness of the land has resulted. Besides, unabated devastation of forestland has contributed its own share of land and soil degradation. The proliferation of restored and such reclaimed land should be used for forestry or agriculture. This gigantic task has to be accomplished on a crash basis. The drift of the people from rural areas into cities adds to the number of urban unemployed. This has become a continuous process now in most big cities, thus swelling the already bursting populations. The number of young and old relentlessly wages a daily battle for survival to earn same money or beg for the next meal. If better incentives and conditions are provided to the rural sectors, this efflux could be checked to a certain extent.

8 Environmental Quality Control

Among the developing countries, India has emerged as an industrial nation and a major producer of manufactured and agricultural products within the last two decades. Because the population is large and industrial activities are intense, large volumes of gaseous, liquid and solid wastes are continuously released into the environment. Surface waters and ground waters too have been polluted in urban and rural areas (Pye and Kelly 1988; Industrial Toxicology Research Centre 1990; Siva et al. 2016). Exploitation of renewable and non-renewable resources supports economic utilization and an increase in economic utilization will enhance waste generation Brown et al. (1991). Although the waste is returned back to renewable resources through the degradation process (Woldeyohannes et al. 2016) on the other side, more waste causes more pollution, which reduces life-supporting systems, consequently reducing ecological biodiversity and carrying capacity of ecosystem (Tzilivakis et al. 2016). The higher the ecosystem carrying capacity, larger will be the degradation and purification, which ultimately helps in waste reduction and enhance life-supporting systems (Fig. 11.3).

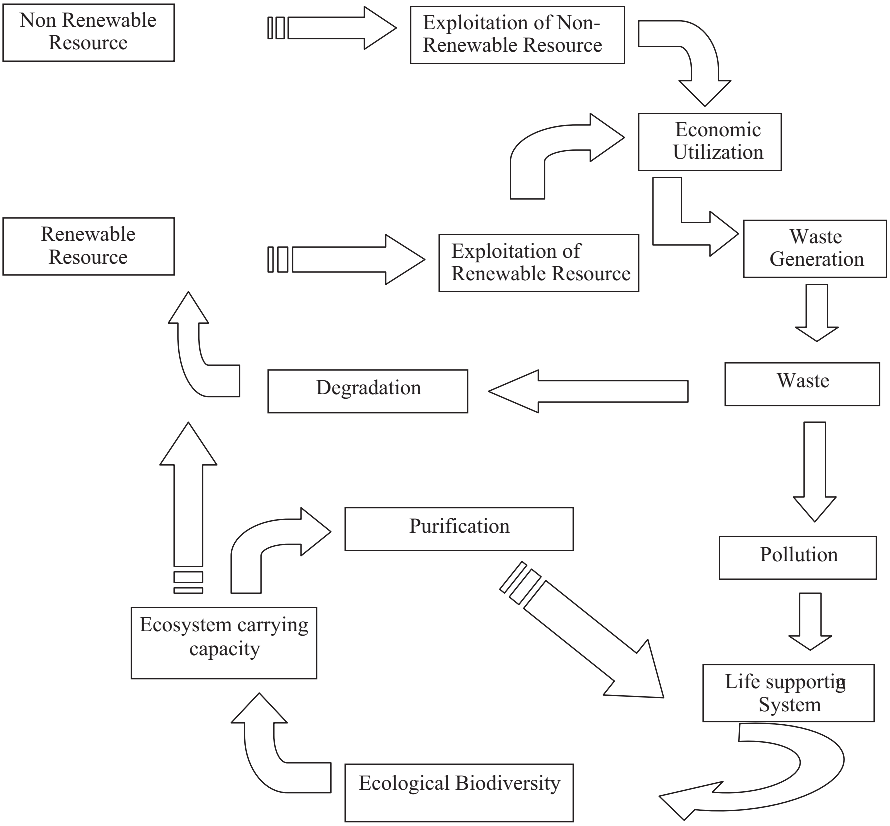

Fig. 11.3 Role of renewable and non-renewable resources in environmental and ecological processes

Problems of developing countries are not always in the forefront of ecological or environmental sciences. While abatement of pollution and ecosystem stresses are concerns of the entire world, a lack of attenuating responses is particularly distressful to people in the Third World countries. The rapid aggregation of population in major urban centres, polarization of industries and the major reliance on hazardous chemical products are leading towards severe deterioration of environmental quality especially in the developing countries. Whereas the developing countries have manpower they lack in expertise and financial resources to tackle these problems unlike the developed nations that lack manpower resources. By imitating the development pattern of the industrialized nations, the environment of the developing countries is deteriorating at an alarming rate, which in turn threatens the capability of the environment to meet the demands of present and future generations.

There may indeed be instances where the relocation of polluting industries in developing countries may contribute to an increase in their Gross National Product (GNP). As in UN States, there is a trend of relocating industries producing asbestos, mercury, pesticides and other environmentally hazardous substances. For example, installation of asbestos factories in India, Pakistan and Brazil. But before the validity of this possibility, the adverse effects of pollution on the economic sectors and on human productivity must be scrutinized sensibly. Available evidence does show an improvement in environment quality as generating significant benefits without, in most cases, negative effects on the economy. The benefits of environmental policies include reduced mortality, morbidity, improved productivity of labour, technological innovation and increased amenities. Intensification of agriculture and animal husbandry; more efficient food handling, processing and distribution systems; introduction of newer technologies including appropriate application of biotechnology will all have to be exploited to increase food availability to meet the needs of growing population.

9 Approaches and Solutions

As environment is a vital part of development, much organized and systematic efforts should be made towards achieving environmental sustainability. Third World nations must now learn from the past mistakes of the developed countries regarding pollutants and contaminants to save their environment from pollution damages for future generations. While addressing the complex relationships between poverty, population dynamics, natural resource consumption and environmental degradation, the connections between disadvantaged groups, particularly women and indigenous people, and their supporting environment need to be better understood and acted upon.

Some of the pollution control programmes, which should be pursued or implemented on a worldwide basis just as being, carried out in industrialized countries:

(a) Developed countries should influence public opinion and work as catalyst and source (of financial resources and technical assistance), to speed up the transition from desirable principles of sustainable development to workable action.
(b) All out efforts to reduce pollutants/contaminant loads into the environment through improvements in manufacturing techniques that could recycle waste products or destroy high levels of pollutants and contaminants before they can reach the environment.
(c) Prevention of careless dumping of wastes into surface waters and the poorly engineered subsurface burial of wastes.
(d) Better coordination among various professionals involved in pollution control and research. This could be achieved by training therefore improved education of all professionals, planners and managers of pollution problems.
(e) Developed countries should assist the developing ones through expertise and financial resources aid them in planning and management of waste disposal programme.
(f) Developing nations must discard their polluting tendencies in an attempt of industrialization and cooperation in pollution control. Nations should have a

common interest in the stability of the world's environment, whose natural systems are also linked.

(g) Environmental pollution control laws must be effectively enforced by the developing countries.
(h) Public health and education programmes must be highlightedl.
(i) Even though pollution is widespread in developing countries, there is still a paucity of data and poor information exchanges. This should be encouraged between nations, among specialists/professionals/experts, governments and aiding agencies.
(j) Synchronized monitoring and sampling programmes are necessary by zones; nations and regions to check widespread regional pollution.
(k) Sources and types of pollution, modes of occurrence and spread, dynamics of transport and dispersion, pollutants life expectancy and means of waste disposal should be more focused.
(l) Development of effective control technology to reduce environmental pollutants.
(m) The socio-economic costs of environmental damage triggered by a spurt in the industrial growth are estimated to be much higher than the required expenditure of 0.5–1.0% of the GNP for pollution control. Governments have to formulate effective policies for ensuring sustainable industrial development.

Last but not the least, developed nations and the developing ones have one common feature, i.e. they share the same planet, and pollutants have no geophysical boundaries. Therefore, nations should take care and not pollute the airs and waters of other nations or dump upon them substances that they do not tolerate themselves. Today's generation should have a duty of caring for tomorrow's. It is generally noticed that the priority of many developing countries is towards economic growth as compared to basic environmental factors. Therefore, it has been rightly concluded from the Declaration of the United Nation's Conference on the Human Environment, Stockholm (1972) that, "To defend and improve the human environment for present and future generations has become an imperative goal for mankind-a goal to be pursued together and in harmony with, the established and fundamental goals of peace and of world-wide economic and social development". The developing countries have a great responsibility in achieving this.

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Chapter 12
Eco-Friendly Post-Consumer Waste Management Utilizing Vermitechnology

M. Vasanthy, V. Sivasankar, M. Prabhakaran, A. Karthika, D. Tamilselvi, and Kiyoshi Omine

Abstract Solid waste could be defined as the unwanted solid fractions which are generated from domestic and commercial sectors, trade centres, industrial activities, agricultural practices, various institutions and mining activities. Out of the various categories of municipal solid waste, post-consumer waste was of our concern as these wastes are no longer recycled and have the possibility of creating aesthetic pollution in particular. One of the post-consumer wastes is the paper cups which are found in large quantum occupying the MSW. Though there exists many numbers of techniques to manage these wastes, vermitechnology was found to be the simplest, cost-effective methodology for its management. Equal ratio of paper cup waste and cow dung was formed to get decomposed into manure with a C/N ratio<20 within a period of 19 weeks due to the activity of *Eudrilus eugeniae*. The bacterial strains such as *Bacillus anthracis* (KM289159), *Bacillus endophyticus* (KM289167), *Bacillus funiculus* (KM289165), *Virigibacillius chiquenigi* (KM289163), *Bacillus thuringiensis* (KM289164), *Bacillus cereus* (KM289160), *Bacillus toyonensis* (KM289161), *Acinetobacter baumannii* (KM289162) and *Lactobacillus pantheries* (KM289166) were identified and are confirmed by 16srRNA sequencing. The enzymes such as amylase, cellulose and protease were assayed both qualitatively and quantitatively. Further the cellulose degradation was confirmed with the bacterial consortia using high performance liquid

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chromatography(HPLC) analysis. The korearetention (380,620–245,696) and height reduction (6061–3303) confirmed the same. The change in the catalase, glutathione-S-transferase, and glutathione peroxidase and superoxide dismutase was recorded. On comparison, the SOD is found to vary during the paper cup decomposition and thus the parameter acts as a biomarker. During the plastic separation from the paper cup by the earthworm in the 8th week, the morphological and histological changes were also recorded. But it was clear that the earthworms required their lost weight when introduced into fresh waste again. Hence, vermicomposting is one of the eco-friendly methods for the post-consumer waste degradation.

1 Introduction

1.1 Municipal Solid Waste and its Components

Over the past few years, solid waste management has emerged as one of the major concerns in Indian cities. Municipal solid waste (MSW) is defined as the waste materials including of everyday items that are discarded by the public (Maria et al. 2011) from commercial and residential sectors (MoEF 2000). It includes waste products such as packaging, furniture, clothing, bottles, food scraps, newspapers, appliances, paint and batteries, grass clippings, yard trimmings and worn out durable items such as refrigerators and computers. Almost many of the Asian countries are currently concerned about the MSW management due to the unchecked increase in its production rate. The total MSW generated in urban India alone accounts to 68.8 million tons per year (TPY) or 1,88,500 tons per day (TPD) (Ranjith et al. 2012). However, the components of the MSW generated by a particular community vary depending on the people’s socio-economic status, culture, population and their commercial activities. One must now acknowledge that the Central and State Government of India including municipal bodies are taking necessary steps to improve the MSW management in India. The Indian government has formulated MSW management and handling rules (2000) and has been revised in 2016, for managing the total solid waste generation.

1.2 Composition of Urban Municipal Solid Waste in India

Of the total MSW generated in urban areas of India, 51% is reported to be organic in nature, 17.5% recyclables and about 31% is reported to be inert wastes (Esakku et al. 2007). As per Garg and Prasad (2003) and CPCB (2000), the composition of MSW is reported to consist of 3–6% of paper(3–6%) . Of the various types of wastes, paper waste is one of the major component and the household activities generate about 2.6 million tons of recyclable waste per annum. However, the paper waste normally includes newspapers, magazines, books, etc. (MoEF 2011).

1.3 Post-Consumer Wastes

Post-consumer wastes are defined as a type of waste that could not be involved in the production of any other product (http://en.wikipedia.org/wiki/Post-consumer waste). Such waste includes packaging material, recycled paper, paper cup, etc. The common and highly generated post-consumer wastes like disposable paper cup wastes are widely seen in canteens, restaurants, festival spots, bus and railway stations, etc. Hence this type of waste needs much more steps for disposal in developing countries like India.

1.4 Disposable Paper Cup Wastes

Early in the 20th century, people had the habit of sharing glasses at public places such as trains, festival spots, schools and railway stations. This practice led to hygiene-related issues, hence the use of disposable paper cups began. In 1909, Lackawanna, the first railway company began to use disposable paper cups. Normally the paper cups are coated with a plastic resin called polyethylene terephthalate (PET) so as to keep the beverages warm and to prevent the liquid from leaking. PET is a very lightweight material, strong, impact resistant and naturally colourless with a high transparency which acts as a moisture barrier (http://www.rsc.org/Education/Teachers/Resources/Inspirational/resources/6.2.2.pdf). According to the Survey of railway waste management (2003), 14,73,500 paper cups waste was generated by each Express train (Indian environment portal). A survey reported that per day consumption of paper cups in India is about 1.0 crore (Think beyond World Sept 4th, 2013) and about 14 billion paper cups are thrown away every year. Of the generated waste, only 1.3% of disposable cups are reported to be reusable (http://www.sustainablelafayette.org).

1.5 Environmental Impacts of Paper Cups:

The manufacturing of 16 billion paper coffee cups needs about four billion gallons of water, 6.5 million trees and 4884 billion BTUs of energy and results in 253 million pounds of waste (Rob 2007). A significant amount of toxins is released during the manufacturing of PET. The composition of paper cups coated with the polyethylene terephthalate makes them complicated to recycle (http://earth911.com/recycling/paper-cups/). Further, there is no advanced technology to separate the plastic from the paper cup. To recycle 60,000 paper cups, it is estimated that 12 trees, 26,000 L of water and 1750 L of oil are required and the process is reported to create 250 kg of air pollutants and is estimated to consume 4100 kW/h. of energy (Times of India, Bangalore 2008). They are classified as regular waste and are burnt or discarded in the landfill. Also the decomposition process in the landfill releases methane, a greenhouse gas which is estimated to possess 23 times the heat-trapping power of carbon dioxide (Kennedy 2012). It is also reported that the degradation of PET is to last for about 150 years *(*Bijayani et al. 2013).

2 Municipal Solid Waste Including Post-Consumer Waste Management

The methods used for the treatment of MSW includes landfilling, open dumping, waste to energy (incineration, gasification and RDF plants) and composting (aerobic composting and vermicomposting; anaerobic—biomethanation). The details of all these processes are as follows.

2.1 Landfilling

Approximately more than 90% of MSW are disposed onto the land in an unhygienic nature (Mor et al. 2006). Though the sanitary land filling includes the recommended method for the MSW disposal it is not followed in many number of cities (Kansal 2002). Hence the MSW is disposed of in an unhygienic manner in the outskirts of the city limits irrespective of the types of PCW. When we consider glass, aluminium and certain other metals there exists an extensive recovery and recycling system and are in operation today (Northwood and Oakley-Hill 1999). But for paper cups the management practice does not exist and hence they found their way to the landfills or they are left in the open dumps.

2.2 Open Dumping

Improper or illegal disposal of waste is termed as open dumping. It results in aesthetic pollution and also poses danger to the environment and public health. Such places become the breeding areas of mosquitoes, attract animals, harbour disease, create unpleasant odours and pollute the soil and water. The open dumping of such paper cups is one of the widely practiced techniques in developing countries including India.

2.3 Thermal Treatment Technique

The destruction of MSW using heat energy is called thermal treatment. Such treatment includes the following processes.

2.3.1 Incineration

The controlled and complete combustion of solid wastes at about 980 °C–2000 °C is defined as incineration. It facilitates the energy recovery, destruction of toxic wastes and reduces the volume of the combustible solid wastes by about 80–90% (Jha et al. 2003).

2.3.2 Gasification Technology

Gasification is defined as the incineration of solid waste under oxygen-deficient condition to produce fuel gas. This technology is used for MSW management following the pre-treatment of the wastes. The advantage of this process is that about 25% of fuel gas produced may be recycled back to the system (Ahsan 1999).

2.3.3 Refuse-Derived Fuel (RDF) Plant

RDF Plants produce the fuel pellets from MSW. In India, such plants are situated in Hyderabad, Gujarat and Vijayawada. Such pellets could be utilized for generating power and hence they could be used along with the conventional fuel like coal.

2.4 Anaerobic Digestion

Anaerobic digestion of the MSW, i.e. biomethanation, facilitates its energy recovery through the generation of biogas. About 55–60% methane, the chief constituent of biogas makes the product suitable to be used as a fuel for power generation. It is further estimated that by controlled anaerobic digestion 1 tonne of MSW produces 2–4 times methane in 3 weeks when compared to the landfill which takes about 6-7 years. (Ahsan 1999; Khan 1994). Normally 150 t/day of MSW is estimated to produce 14,000m^3 of biogas which generates about 1.2 megawatt (MW) of power. Several schemes of biomethanation of MSW, vegetable market and yard wastes are currently in practice (Ambulkar and Shekdar 2004; Chakrabarty et al. 1995).

2.5 Aerobic Composting

Aerobic composting refers to the bacterial conversion of organic waste in the presence of air under controlled conditions. The final product is called compost (humus). Following the composting process, the waste volume is reported to get reduced by 50–85%. Government of India (GOI) focused on MSWM by promoting composting of urban MSW. In 1960s, the Ministry of Food and Agriculture encouraged the local bodies by providing soft loans for setting up such MSW composting plants. Large-scale composting plants with capacities of 150–300 tons/day were set during 1975–1980 under the central scheme of MSW disposal. Currently, about 9% of MSW are treated by composting (Gupta et al. 2007; Srivastava et al. 2005).

3 Vermicomposting

Paper cup utilization is increasing day by day all over the world. The disposal of this waste is one of the issues that need much attention. Effective disposal of paper cup waste is very much important to maintain healthy environment. To solve this problem, we have chosen vermicomposting, an eco-friendly, cheap and rapid technique to manage the paper cup wastes. Vermicomposting is an appropriate technique for the safe, hygienic and cost-effective disposal of organic wastes (Hand et al. 1988). Earthworms decompose organic waste leading to the production of manure with high nutrient content. Vermicomposting of organic wastes results in the stabilization of organic matter (Tomati et al. 1995). The resultant vermicompost is an eco-friendly organic fertilizer. It is reported to contain nitrogen, potassium, phosphorus, organic carbon, vitamins, enzymes and antibiotics which help to improve the quality and yield of the plant (Thiruneelakandan and Subbulakshmi 2014).

However, the stabilization of organic wastes during vermicomposting happens through the joint action of earthworm and microbes. Initially primary decomposition occurs through microbial action by decomposing biodegradable organic matter through extracellular enzyme activity. Then the partially decomposing matter consumed by the earthworm is further decomposed with the help of microbes in the gut of earthworm resulting in vermicast. The obtained product is reported to act as a good fertilizer in agricultural fields (Ghosh 2004; Jha et al. 2003; Sannigrahi and Chakrabortty 2002).

Compared to other waste disposal treatment, vermicomposting is considered to be the better option of post-consumer waste management (Karthika 2015). It is one of the biological processes which do not result in soil or ground water pollution. The resultant earthworm cast termed vermicompost is found to be rich in microbial population and plant growth regulators. Vermicompost is believed to have high content of enzymes and plant growth hormones, which stimulates the plant growth.

3.1 Types of Vermicomposting

There are two types, namely non-continuous and continuous. A simple bin, bed and pit system is an example of non-continuous vermicomposting where the worms will not be able to move to another layer (Trasar-Cepeda et al. 2012). Windrow method is an example of continuous method in which the worms can migrate vertically and horizontally to the new food sources.

3.2 Species Advocated for Vermicomposting

Earthworms are invertebrates belonging to the phylum *Annelida* and class *Oligochaeta.* Charles Darwin described earthworms as great benefactors of soil and agriculture. Earthworms aerate the soil and supply organic matter and maintain soil moisture by their burrowing behaviour. Earthworms reduce plant pathogens and are believed to release enzymes and hormones in their excreta. These earthworms are primarily employed for vermicomposting of different types of wastes (Abbasi and Ramasamy 2001; Bhawalkar and Bhawalkar 1993).

The species like *Eudrilus eugeniae, Perionyx excavatus* and *Lampito mauritii* were reported to be best suited for the conversion of paper waste into organic manure (Gajalakshim et al. 2001). These species have high frequency of reproduction and faster rate of growth. They are the effective utilizers of humus, manure and other forms of organic carbon and thereby convert them into vermicast. As these species do not burrow deep into the soil, they are best suited for vermireactors. For these reasons, *E. eugeniae, P. excavatus* and *L. mauritii* have been extensively used for vermicomposting throughout the world (Gajalakshim et al. 2001).

3.3 Scientific Classification

Kingdom: Animalia; Phylum: Annelida; Class: Clitellata; Subclass: Oligochaeta; Order: Haplotaxida; Family: Eudrillidae; Genus: *Eudrilus;* Species: *eugeniae.*

Eudrilus eugeniae originated from West Africa and are popularly referred to as "African night crawler" (Graff 1981) (Fig. 12.1). They are widely distributed in warmer parts of the world. It is an epigeic earthworm which lives on the surface of moist soil with high organic matter content. The worm is reddish brown in colour with its dorsal surface to be convex. The ventral side is flattened with the clitellum paler than the rest of the body. The length of the adult worm is about 25–30 cm,

Fig. 12.1 *Eudrilus eugeniae*

5–7 mm in diameter with about 250–300 segments and with a weight of 5600 mg (Viljoen and Reinecke 1992). They are of greater economic importance in the vermicomposting field for converting a wide variety of organic wastes into value-added product.

3.4 Significance of Vermicompost

Vermicompost has high level of bioavailable nutrients for plants (Arancon et al. 2004). It contains high level of beneficial soil microorganisms thus promoting plant growth (Parmanik et al. 2007). It biochemically promotes the root growth of plants (Atiyeh et al. 2000). Vermicompost is reported to be free of pathogens, toxic chemicals and found to protect the plant against various pests and diseases (Nair et al. 2007). It also induces biological resistance in plants (Suhane 2007). Vermicomposting could help to reduce wastes headed to landfill significantly and is an amazing source of nutrients for large- and small-scale farming applications.

3.5 Preparation of Experimental Media

Three 5-litre vermireactors (diameter 40 cm, depth 9 cm) were filled with the feed mixture as mentioned as follows and were subjected to vermicomposting process. Type A (1:1ratio) contains equal quantities (1000 g) of shredded paper cup wastes and cow dung slurry. Type B (1:1/2ratio) and Type C (1/2:1ratio) of wastes were mixed manually to facilitate pre-decomposition for up to 20 days. On the 21st day, about 20 earthworms with an average weight of 0.15–0.2 g each were introduced into each of the vermireactor. About 60–80% moisture content was maintained throughout the study period by sprinkling adequate quantity of water. The experiments were performed under room temperature in triplicate. Based on the results, the composition of type A was found to be the optimum. And hence the equal proportion of wastes was subjected to further analysis. The decomposition of the waste materials using Eudrilus eugeniae was better, by showing significant reduction in C:N ratio while compared with that by Eisenia fetida.(Karthika et al. 2015)

3.6 Enzyme Activity

Extracellular enzymes target the macromolecules such as carbohydrates (cellulases), lignin (oxidases), organic phosphates (phosphatases), amino sugar polymers (chitinases) and proteins (proteases) (Allison et al. 2007). The enzymes break down complex compounds into simple compounds that are transported into the cells to support the heterotrophic metabolism. Laverack (1963) observed that there are commonly six types of digestive enzymes in the worm gut, namely protease, lipase, amylase, lichenase, cellulase and chitinase. Cellulase enzyme is reported to depolymerize cellulose into fermentable sugars. This enzyme is reported to be synthesized by fungi and bacteria to degrade cellulose. Cellulosic enzyme system include enzymes, namely endo-β-glucanase, exo-β-glucanase and β-glucosidase. During the vermicomposting process, the materials passing through the worm's gut undergoes physico-chemical and biochemical changes. Higher activities of cellulase, amylase, invertase, protease, peroxidase, urease, phosphatase and dehydrogenase are reported by Edwards and Bohlen (1996) and Sharpey and Syers (Sharpey and Syers 1976) has reported that *Bacillus* sp. produces amylase, protease and cellulose enzyme.

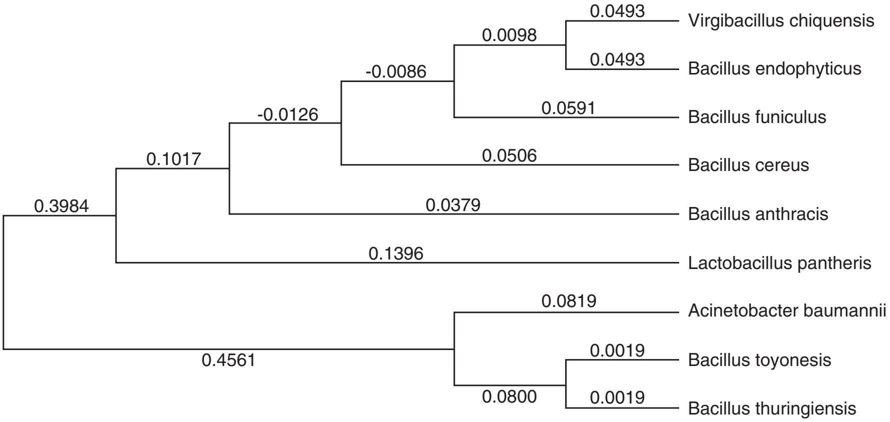
Some microbes have also been found to produce cell-bound enzymes and multiprotein complex expressing celluloses and hemicelluloses called cellulosomes (Miranda et al. 2011). Cellulase production between microbes was reported to be very difficult as the bacteria are reported to produce multiple types of cellulases including endoglucanase, exoglucanase and β-glucosidase which are found to exist as free extracellular enzymes as well as an enzyme complex or cellulosomes which are expressed on the cell membrane.

Shankar et al. (2011) stated the cellulolytic activity of the microbial strains isolated from the midgut of the earthworm *Eudrilus eugeniae* and revealed that this activity might be responsible for the breakdown of cellulose. So both the digestive enzymes and the intestinal microflora of the earthworm play an important role in the digestion of organic matter. Lakshmi Praba et al. (Lakshmi et al. 2007) investigated the level of various enzymes such as amylase, cellulase, xylanase, cellobiase, endonuclease, acid phosphatase, alkaline phosphatase and nitrate reductase to show the activity is higher during the decomposition period.

The microorganisms capable of degrading cellulose, hemicelluloses and lignin are studied by Virendra and Tarun (1981). The authors state that cellobiohydrolase, endo-glucanase and β-glucosidase are involved in the degradation of crystalline cellulose into glucose. Yung et al. (2009) reported that the activity of microbial cellulolytic enzymes, namely cellulose and xylanase is extracellular and the production was dependent on the substrates (xylan, rice husk and rice straw) provided for the growth of the earthworm. The nucleotide sequence of the cell gene of *Bacillus* sp. was overexpressed in *E. coli* and in the product. Sang et al. (1995) proved that the single polypeptide cellulose had both endo- and exoglucanase activities and each activities exists in separate site of the organism.

4 Microbial Diversity and their Significance

Microorganisms are the main agents of biochemical decomposition occurring during vermicomposting process. Totally nine morphologically different colonies were screened from vermicompost samples prepared with type C waste. The 16S rRNA gene from bacterial colonies were amplified and the amplified PCR products were



The evolutionary history was inferred using the Neighbour-Joining method.

Fig. 12.2 Phylogenetic analysis of the isolated bacterial strains

sequenced and compared with 16S rRNA gene database in the Gene bank using BLAST analysis in order to identify the microbes. Nine different species of *Bacillus* were identified from the vermicompost of paper cup waste and the phylogenetic tree is shown in Fig. 12.2.

The microbial flora such as bacteria, fungi and actinomycetes are eventually involved in the conversion of organic waste into humus (Zeng et al. 2001). Earthworm acts as a facilitator of vermicomposting system. Its activity along with the activity of the gut microflora stimulates the decomposition of organic matter. Earthworm could increase the surface area available for the microbes to grow. The microbial population in the vermicompost is reported to be influenced by the appropriate conditions like oxygen, nutrient availability and temperature. Changes in these conditions lead to the reduction in the microbial population which would affect the degradation of the waste (Dominquez et al. 2010). During the final stages of vermicomposting process, a reduction in the microbial population is noticed which may be due to the depletion of nutrient content. Gomez et al. (Gomez-Brandon et al. 2011) reported the reduction in the microbial community at the end of vermicomposting process and substantiated that there may be a relationship between the earthworm gut process and microbial population. However, the proper mechanism is unclear which needs further investigation. Also Ingrid et al. (2014) reported that the vermicomposting and succession of microbial communities are related to the duration of composting process. Even the post-consumer material is decomposed by the earthworm and their gut-associated microbes. The degradation was enhanced only after the addition of the microbial inoculum and that the major role of degradation was played by the microorganisms present in the vermicompost (Karthika et al. 2014a).

During vermicomposting process, detoxification of fats, organic acids and polyphenol is reported to occur and results in odourless product called humus (Echeverria et al. 2011). Federici et al. *(*2011) state that this can be achieved by biotransformation activity of microbes which leads to the rapid succession of special bacterial population during the process. Kumar and Singh (2001) state that the nitrogen-fixing bacteria and phosphate-solubilizing bacteria will increase the percentage of nitrogen and phosphorus during the degradation of organic wastes.

4.1 Effect of Earthworms on the Activity of Microbial Community

The first stage of decomposition during vermicomposting is dominated by the microbes whereas the activities of the earthworm are expected to affect the bacterial growth by reducing the quantity of resource available for microbial communities and consequently the bacterial growth rates. This means that the microbial communities utilize the available energy source efficiently in the presence of earthworms. As a consequence, the system functions much better and increases the rate of decomposition as well as the mineralization (Schönholzer et al. 1999). Brown (1995) states the decomposition of decaying substrate by earthworm decreases the availability of nutrients for the microorganisms, thereby reducing the microbial numbers in the casts and posing alteration in the pattern of microbial population. Hence a thorough investigation regarding the microbial population during vermicomposting of paper cup waste is found to be essential.

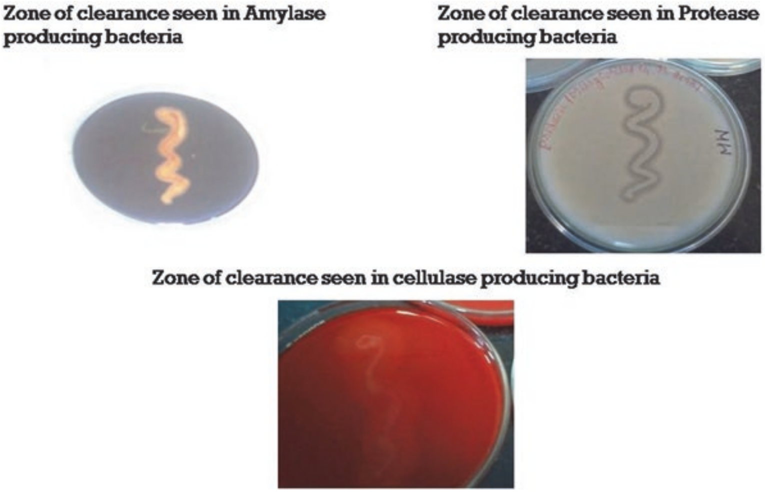
The Total Heterotrophic Bacterial (THB) population of the vermicompost samples was quantified by serially diluting 1 g of freshly weighed sample. Approximately 100 μL of each dilution up to 10^{-7}dilution was spread on a nutrient plate (Yasir et al. 2009). During the initial stage of vermicomposting, there was an elevated level of bacterial count which got declined during the final stage. The vermicompost with relatively high content of humic-like substances, activated microorganisms and enzymes, could greatly enhance the fertility of the soil (Madhuri et al. 2014).

4.2 Qualitative Assay

The bacterial colonies isolated from the vermicompost were subjected for qualitative assay for the production of enzymes. Starch agar medium was employed for amylase production, skim milk agar for protease, LB tributyrene for lipase and Czapek mineral salt agar for cellulose production. The plates were observed for the zone of clearance called halos. The halo diameter was measured for the comparison of the enzyme production among the isolates and is represented in Table 12.1 and Fig. 12.3.

Table 12.1 Qualitative enzyme assay of the isolated bacterial cultures for amylase, protease, lipase and cellulose activity

Enzyme	*Bacillus thuringiensis*	*B. anthrucis*	*B. endophyticus*	*B. funicatus*	*B. cerus*	*B. toyonensis*	*Acinetobacter baumannii*	*Lactobacillus pantheris*	*Virigibacillius chiquenigi*
Amylase	Positive (10 mm)	Positive (8 mm)	Negative	Negative	Positive (8 mm)	Negative	Positive (10 mm)	Negative	Negative
Cellulase	Positive (7 mm)	Positive (8 mm)	Positive (6 mm)	Positive (8 mm)	Positive (5 mm)	Positive (10 mm)	Positive (13 mm)	Positive (7 mm)	Negative
Lipase	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Protease	Positive (5 mm)	Positive (7 mm)	Positive (5 mm)	Positive (8 mm)	Positive (7 mm)	Positive (3 mm)	Positive (4 mm)	Positive (6 mm)	Negative



Zone of clearance seen in Amylase producing bacteria

Zone of clearance seen in Protease producing bacteria

Zone of clearance seen in cellulase producing bacteria

Fig. 12.3 Enzyme activity of the bacterial colonies isolated from the vermicompost

The isolated bacterial strains, namely *Bacillus anthracis* (KM289159), *Bacillus endophyticus* (KM289167), Bacillus funiculus (KM289165), *Virigibacillius chiquenigi* (KM289163), *Bacillus thuringiensis* (KM289164), *Bacillus cereus* (KM289160), *Bacillus toyonensis* (KM289161), *Acinetobacter baumannii* (KM289162) and *Lactobacillus pantheries* (KM289166) were analysed for their enzyme production potential.

4.3 *Quantitative Assay*

The isolates that displayed amylase, protease and cellulase activity in the respective plates were further subjected for quantification of respective enzymes assay by using different substrates. The isolated strains *Bacillus thuringiensis (KM289164)* produced 204 mg of glucose/ml using starch as a substrate thereby thus indicating its potential to produce the amylase enzyme more efficiently compared to other isolates. The *Bacillus cereus (KM289160)* and *Bacillus toyonensis (KM289161)* produced 0.01 mg of tyrosine/ml (Table 12.2) using casein as a substrate thereby indicating its potential to produce the protease enzyme more efficiently compared to other isolates. Evaluating cellulase production between the isolates is found to be a challenge as the bacteria produce multiple type of cellulase, such as endoglucanase, exoglucanase and β-glucosidase which exists as free extracellular enzyme as well as enzyme complexes or cellulosomes expressed on the cell membrane (Maki et al. 2009). From the results *Acinetobacter baumannii (KM289162)* produced 1.98 mg of glucose/ml (Table 12.2) using CMC as a substrate more efficiently compared to other isolates.

Table 12.2 Quantitative enzyme assay of the isolated bacterial cultures for amylase, protease and cellulose activity

Enzymes	Protease units/mL	Amylase units/mL	Cellulase units/mL
Bacillus thuringiensis	0.06 ± 0.002	204 ± 0.002	1.31 ± 0.002
Bacillus anthracis	0.08 ± 0.002	135 ± 0.002	1.93 ± 0.002
Bacillus endophyticus	0.08 ± 0.002	–	0.45 ± 0.002
Bacillus funiculus	0.08 ± 0.002	–	0.82 ± 0.002
Bacillus cereus	0.10 ± 0.002	145 ± 0.002	1.08 ± 0.002
Bacillus toyonensis	0.10 ± 0.002	–	3.49 ± 0.002
Acinetobacter baumannii	0.08 ± 0.002	145 ± 0.002	1.98 ± 0.002
Lactobacillus pantheries	0.08 ± 0.002	–	0.36 ± 0.002
Virigibacillius chiquenigi	–	120 ± 0.002	1.75 ± 0.002

Miranda et al. (2011) have stated that *Bacillus* species have been well characterized for the cellulase production. Pason et al. (2010) have mentioned the presence of various modular enzymes in *Paeni Bacillus*. Further *P. vulgaris, C. freundii, S. liquefaciens* and *Klebsiella* sp., were found to degrade cellulose (Alwin et al. 2004). Based on our results all the nine isolates were reported to be suitable for degrading the paper cup waste materials because of their high potential to produce cellulase enzyme. The authors stated the possible reason for the production of amylase enzyme would be intracellular or induced only by its substrate. At the same time the enzymatic analysis confirms that all isolated organism are capable of producing cellulase enzyme. The result also confirms that these isolates had facilitated organic matter decomposition. It may be because earthworm could digest the organic matter only with the enzymes such as amylase, protease and cellulase produced by the gut microflora (Aira et al. 2005). Similarly, Lattaud et al. (1997) reported that the amylase activity was higher during the degradation of soil organic matter by an endogeic geophagous earthworm from tropical areas, namely *Polypheretima elongata.*

4.4 Role of Microbes in PCW Degradation

Microorganisms can degrade substrates by producing certain extracellular enzymes during vermicomposting process. Enzymes are the main agents for the degradation of different types of wastes (Tiquia 2001). The enzyme activity could apparently give interesting information on the rate of decomposition of organic matter and therefore the product stability. Ben-David et al. (2011); Portillo et al. (2011) reported that the hydrolytic enzymes such as cellulase, hemicellulases, proteases, lipases, phosphates and aryl sulphatase are the major enzymes produced by the dominant microorganisms present during the process of composting. These enzymes help the microbes to depolymerize various organic waste constituents (Marx et al. 2001). Urbasek and Pizl (1991) reported that earthworm itself can produce numerous enzymes to digest soil organic matter. Recently, researchers put more effort to detect the functional role of hydrolytic enzymes involved in biogeochemical cycle like β-glycosidase, cellulase, ureases, proteases, phosphomonoesterase, and phosphodiesterase and aryl sulphatase. The monitoring of enzyme activities throughout the composting process is found to be useful to understand about the dynamics of carbon and nitrogen and to understand the transformation that occurs during composting (Vargas-Garcia et al. 2010). The earthworms derive their nourishment from microorganisms that grow upon these materials and at the same time they promote further microbial activity since the faecal material or 'casts' they produce is more fragmental and with higher microbial activity (Karthika et al. 2014b).

4.4.1 Amylase

Amylase is an extracellular enzyme which hydrolyses starch into glucose and maltose or specific malto-oligosaccharides or mixed malto-oligosaccharidase (Hashim et al. 2005). Though amylase can be derived from different sources, amylase derived from microbes in particular is highly preferred in industrial sector for commercial purposes. Amylase enzyme has been purified and characterized for the biotechnological and industrial application. Microorganisms like fungi and bacteria are reported to be suitable candidates for starch hydrolysis (Sahnouna et al. 2012). Hence the microbes present in the earthworm gut may help to degrade the waste during the vermicomposting process.

4.4.2 Protease

Paul and Clark (1996) interpreted that the protease activity is linked to the nitrogen cycle as the enzyme catalyses the hydrolysis of protein. Apart from the microorganism, earthworm itself has been reported to secrete the protease enzyme such as earthworm fibrinolytic enzyme, tissue plasmogen activators, etc. in their alimentary tract. Among microorganisms *Bacillus thuringiensis, Bacillus cereus* and *Bacillus endophyticus* were reported to produce protease which has facilitated the degradation dung the vermicomposting process (Karthika et al. 2014a, b, c).

4.4.3 Lipase

Lipases are the enzyme used to convert lipid into fatty acid. The presence of lipase in the organic waste is low so their role is limited in the carbon cycle while compared with other kind of waste which shows high fat content (Gea et al. 2007)

4.4.4 Cellulase

Cellulase is the one of most abundant biopolymer which constitutes large pool of carbon source for the microorganisms which perform the decomposition of organic matter. The bacterial community present in the gut of earthworm is reported to be responsible for the breakdown of cellulose into reducing sugars with the help of cellulase enzyme. Bacterial species like *Pseudomonas, Bacillus, Micrococcus* and fungal genera like *Trichoderma and Aspergillus* were reported to have cellulolytic activity (Immanuel et al. 2006).

The vermicompost with relatively high content of humic-like substances, activated microorganisms and enzymes could greatly enhance the fertility of the soil (Madhuri et al. 2014). In several strains the amylase activity was higher compare to protease and cellulase. This may be due to the higher availability of carbonate source. Similarly, Lattaud et al. (1997) reported that the amylase activity was higher during the degradation of soil organic matter by an endogeic geophagous earthworms from tropical areas, namely *Polypheretima elongata.* The authors stated the possible reason for the production of amylase enzyme would be intracellular or induced only by its substrate. At the same time the enzymatic analysis confirms that all isolated organisms are capable of producing cellulase enzyme. The result also confirms that these isolates had facilitated organic matter decomposition. It may be because earthworm could digest the organic matter only with the enzymes such as amylase, protease and cellulase produced by the gut microflora (Aira et al. 2005).

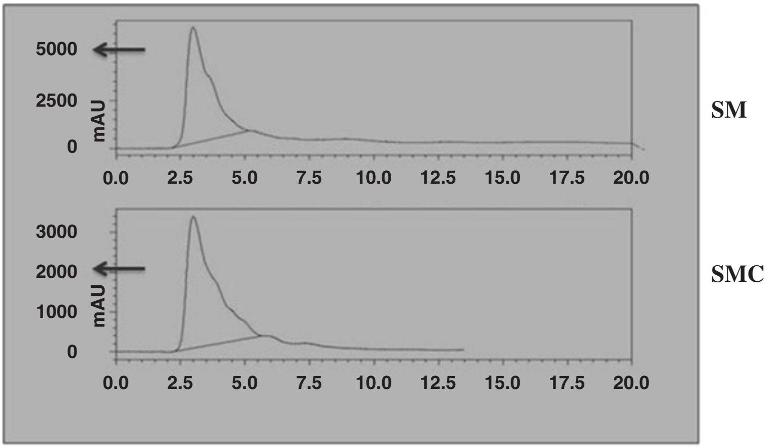
4.5 Degradation of Cellulose:

Consortia of nine isolates were inoculated into the supplemented medium (SM) containing paper cup as substrate. The composition of the supplemented medium is given as follows.

L.Glutamicacid—0.03 g, Ammonium nitrate—0.14 g, Potassium dihydrogenphosphate—0.2 g, Calcium chloride—0.03 g, Magnesium sulphate—0.03 g, Protease peptone—0.75 g, Ferrous sulphate—0.5 g, Manganese sulphate—0.16 g, Zinc sulphate—0.14 g, Tween 80—2%, Paper cup—3 g.

Accurately 100 ml of consortia of bacteria were prepared and inoculated into the supplemented medium. The medium was maintained at three different pH (4, 7 and 9) and the temperature was also stabilized at 4 °C, 35 °C and 60 °C. The optimum pH and temperature for cellulase production are considered as the most important factors. Generally enzymes need optimum pH and temperature at which their activity is either higher or lower (Lehninger et al. 1993). The maximum production of cellulose was obtained at pH 7 and minimum production was reported at pH 4 and pH 9. Similar reports were reported for cellulose production by *Aspergillus niger* (Akiba et al. 1995 and Bansal et al. 2012).

The cellulase enzyme production was compared between the supplemented medium having consortia, namely SMC and the other one which is not having consortia, namely SM. Mixed bacterial culture resulted in a substantial reduction in colour intensity which might be largely attributed to the degradation of cellulose in the paper cup by bacterial cellulase enzymatic action.



Peak	Retention time/min	Area	Height
1	2.987	380620	6061
2	2.988	245696	3303

Fig. 12.4 High performance liquid chromatography (HPLC) of the bacterial consortia

HPLC analysis is used to examine the hydrolysis of cellulose present in paper cup by the consortia of nine isolates. In Fig. 12.4, a single peak was detected in SM at 254 nm with the intensity of peak range at more than 5000 with the retention time of 2.9. But in the case of SMC the intensity of peak decreased to 3000 with the same retention time and in the same wavelength, after period of 20 days. The area of the peak reduced from 380,620 to 245,696. Similarly the height of the peak also reduced from 6061 to 3303. This variation may be attributed to the activity of cellulose-producing bacteria. HPLC analysis indicates that the consortia including all the nine isolates have the ability to degrade the cellulose present in the paper cup.

5 Antioxidant Enzymes

The exposure of animals to chemical toxicities is found to result in disturbed activity along with the marked changes in their enzyme activity too. Therefore, enzyme studies are very important to understand the biochemical indices of normal metabolism. Sabatini et al. (2009) reported about the generation of reactive oxygen species (ROS) in living organism on exposure to various environmental contaminants. The enhanced production of free radicals and oxidative stress can be induced by various factors like radiation, heavy metals and many environmental toxins. In order to prevent the damage caused by the free radicals, tissues develop an efficient antioxidant defence system that includes enzymatic activities like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and the non-enzymatic antioxidant glutathione S-transferase (GST). Superoxide dismutase is found in the cytosol as a zinc/copper-containing enzyme and in mitochondria as a manganese-containing enzyme (Michiels et al. 1994; Simibe et al. 2001). Catalase is found within the peroxisomes and cytosol of the cell and it decomposes hydrogen peroxide to water and oxygen. Glutathione peroxidase is selenium-containing metallo enzyme, partially located within the cellular membrane that can effectively remove hydrogen peroxide by converting reduced glutathione into oxidized glutathione.

The antioxidant enzyme was reported to reduce ROS to stable compounds. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) constitute a mutually strong system to defence against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by reducing the steady-state level of O_2^-. CAT is a hemeprotein, localized in the peroxisomes or the microperoxisomes and catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and OH. sGPX is a seleno enzyme, two-thirds of it is present in the cytosol and one-third in the mitochondria. GPX needs reduced glutathione for its action. Glutathione reductase (GSR) reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH. This GSH is an important cellular antioxidant (Mannervik, 1987). Glutathione peroxidase can also terminate the chain reaction of lipid peroxidation by removing lipid hydroperoxides from the cell. The GPX and GST activities cause a reduction in the GSH level and thus decrease the cellular antioxidant status (Flohe 1982).

5.1 Changes Observed in Earthworm During Vermicomposting

The earthworm *Eudrilus eugeniae* employed to degrade paper cup waste separated the plastic lining present inside the cups and was found around the rim of the tub as a layer. It was curious to note the behaviour of the tiny worm which has rejected the non-biodegradable material.

5.2 CAT

Catalase enzymes eliminate free radicals thus protecting the cell from damage. Decrease in the activity of the enzyme may be due to stress and hence the stress may reduce the defensive effect of antioxidative enzymes. The CAT was normal during the 1st week of the vermicomposting process. Later during the 12th week of the process, there was an increase in the activity of CAT. In the final stage of composting, there was a drastic decrease in the activity of CAT enzyme.

5.3 GST

GST is involved in xenobiotic metabolisms in living organism (CanesiL et al. 2007). GST too is an important detoxification enzyme and is used as a potential biomarker in the earthworms exposed to pollutants. GST activity increased up to the middle of the process and it got decreased at the end of the process. During 1st week of exposure, the activity of the GST is stimulated in earthworm which catalyses the conjugation of glutathione with both endogenous and exogenous substrates.

5.4 GPX

GPX helps in the conversion of lipid hydroperoxides to alcohols and to further reduce free hydrogen peroxide to water. GPX activity gets increased during the middle stage.

5.5 SOD

SOD removes the O_2^- produced during biological oxidation. The decrease in SOD may be due to O_2 converted to OH^- ions ($O_2^-OH^-$) by GSH and GST and the excess O_2^-could stop the activity of SOD. SOD activity is too considered as biomarker for determining the effect of pollutants on the different components of the ecosystems. The SOD activity reported was initially lower and increased during the middle stages and decreased at the end of the process. The antioxidative enzymes in earthworm in the test group were significantly higher than those observed in control group after the 8th week of vermicomposting the paper cups. During the early stages of vermicomposting, earthworm has had the capacity to tolerate the oxidative stress and to further activate the antioxidant enzymes. But during the later stages, the activity of the enzymes decreases with an increase in toxic stress. This demonstrated that the defensive effect of antioxidative enzymes might be lost. The earthworm had started the separation of plastic from the 8th week of the process and continued till the 19th week. During this period, the papers have been completely decomposed.

6 Conversion of Post-Consumer Waste into Manure (Waste to Wealth):

6.1 Characterization of the Resultant Vermicompost

Homogenized samples (free from earthworms, hatchling and cocoons) from feed mixture (Type A, B and C) were drawn after 1, 4, 8, 12, 16 and 19 weeks. pH, EC, total organic carbon (TOC), total organic matter (TOM), C/N ratio, total kjeldahl nitrogen (TKN), total phosphorus (TP), total potassium (TK), Ca, Na, Mg of the samples were analysed (Tandon 2009; Senesi 1989).

6.2 *Macronutrients*

The macronutrients like carbon in the vermicompost would be very low because of loss of CO_2 in the final vermicompost. The total nitrogen content would be high in the vermicompost due to the mineralization of organic matter. According to Senesi (1989), the C/N ratio <20 is reported to indicate the advanced degree of stabilization of organic matter and it reflects the satisfactory degree of maturity of the organic waste subjected for the decomposition. In vermicomposting process, the reduction in carbon and an increase in nitrogen were directly proportional to one another.

6.3 *Toc*

During vermicomposting process, TOC reduction was noticed in the range of 20–45%. This may be attributed to the microbial respiration as CO_2 and also due to the mineralization of organic matter.

6.4 *TKN*

The total nitrogen content of the compost was increased. Vermicomposting results in an increase in the nitrogen content which could be due to the addition of nitrogenous excretory substances and enzymes too (Tripathi and Bhardwaj 2004).

6.5 *C/N Ratio*

The C/N ratio is an important parameter in the vermicompost because the loss of C/N ratio shows the maturity of the compost. The C/N ratio is considered to be an important to assess the manural quality of the vermicompost. The C:N ratio of type A feed mixture decreased from 46.6 ± 0.001to 15.02 ± 0.001 following the19th week of vermicomposting period. Maboeta and Van (2003) have reported an advanced degree of organic matter stabilization when the C/N ratio happens to be less than 20. The increase in the quantity of cow dung is thought to infest higher microorganisms which could reduce the C/N ratio. This could also reduce the earthworm population (Aira et al. 2005) thereby reducing the manural value of the resultant vermicompost. Another post-consumer waste, namely artificial paper banana leaf (APBL) is manufactured in such a way similar to the shape and size to the natural plantain leaf in order to compensate the enormous need for natural banana leaf. This paper leaf is used in large quantities both in private and public sectors such as hotels, marriage functions, etc. After usage this waste is ultimately thrown into the landfill site as it has no recycling value till now. These cellulosic wastes were subjected for vermicomposting utilizing two different genus of earthworm such as *Eisenia fetida* and *Eudrilus eugeniae* (Seetha et al. 2012) and the APBL was found to be efficiently degraded by *Eudrilus eugeniae* resulting in final C/N ratio to be < 20 (Sai 2013). Yet another PCW, namely the cotton waste was subjected for vermicomposting process by employing *Eudrilus eugeniae* which could got converted into organic manure containing enriched nutrient composition (Habibu Nisha 2014).

6.6 Total Phosphorous

The release of phosphorous might be due to the existence of phosphate-solubilizing microorganism in the vermicasts. Lebayon and Binet (2006), Senesi and Brunetti (1996) already reported that the earthworms would have mediated the phosphate enhancement in the worms' gut and might have finally excreted through the cast deposition.

6.7 Magnesium

Vermicompost derived from paper cup waste is found to contain higher magnesium content. The activities of the earthworm may affect the magnesium availability in worm casts. This may be due to the fact that the fresh cast encourages colonization of fungal and micro algal hyphae available which may lead to increase in the magnesium content of the final vermicompost (Suthar 2010.)

6.8 Total Potassium

The increase in the potassium content is facilitated by the activity of acid-producing microorganism present in the earthworm gut which involves solublization of potassium present in the vermicompost. At the same time Kaushik and Garg (2004) reported that potassium level is high in vermicompost because of the excess water drained from the feed mixture contain potassium leachate.

6.9 Sodium

Sodium can stimulate plant growth and can be used as an alternative in cases where potassium is deficient. The same result was obtained by Ansari and Sukhraj (2010). Sodium contents slightly get reduced after the processing of the waste by the earthworms. A similar reduction has been reported by Kaur et al. (2010).

7 Morphological and Histological Changes of the Earthworm:

7.1 Morphological Studies

Environmental disturbances cause structural and functional impairments in tissue of organisms and its associated metabolism. When exposed to stress, physical and deleterious changes are reported to set in the metabolism of organisms (Vaidya 2014). Attempt was carried out to observe the morphological modification in the earthworm during the degradation of the paper cup waste. During the process of vermicomposting of paper cup waste, there is a pattern in the morphology of earthworm. Both the length and the weight of the earthworm were modified. During the 1st week of the process, equal number of earthworms was introduced. The number of earthworm got increased during the middle stage and at the final stage there was a drastic reduction in the number of the earthworms in the vermin reactors. Table 12.3 presents the variation in the weight and length of the earthworm during the decomposition process.

7.2 Histological Analysis

The histopathological examination indicates that vermicomposting of paper cup may affect the external barrier of the earthworm.

Damages noticed in the outer skin and the intestine reflect the health effects of the earthworm. This is because these type of damages interfere with the functioning of these tissues and may affect the homeostasis of the earthworms (Lapied et al. 2010). Some erosion of epithelium, granular changes in the coelom and fibrotic

Table 12.3 Variation in weight and length of the earthworm

Stages	Weight (g)	Length (cm)
Control	0.72 ± 0.14	8.1 ± 0.24
1st week	0.79 ± 0.24	15.5 ± 0.24
12th week	1.66 ± 0.17	23.2 ± 0.14
19th week	0.34 ± 0.09	5.2 ± 0.07

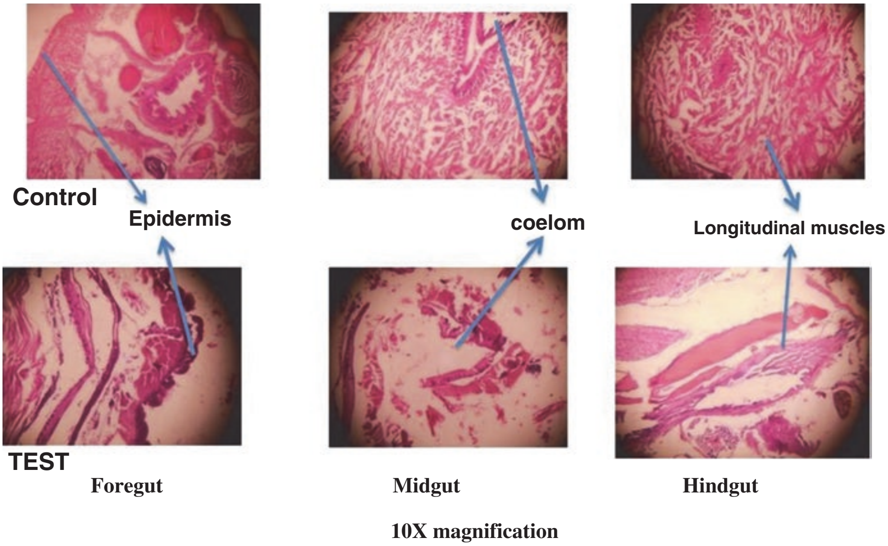


Fig. 12.5 Transverse section of segments from the anterior region of *Eudrilus eugeniae.* (Control and Test)

changes in the circular muscle and longitudinal muscle were noted in the earthworm exposed to the vermicomposting of paper cup waste compared to that of the control and shown in Fig. 12.5. This suggests that some inflammation has occurred. The result indicates that the immune system is trying to respond to the exposure of stress during the separation of plastic from the paper cup. Similarly (Lapied et al. 2010) reported that the external barrier of the earthworm *L. terrestris* was affected by AgNP exposure.

During this process of decomposition of the paper cups, the earthworms engaged in the separation of the plastic lining material could have been exposed to the toxicity of the HDPE lining of the cup. This may be the reason for the loss in the body weight and height of the organism during the middle and final stages, when compared with the initial stages. Fortunately, this earthworm has the ability to regain the metabolic rate when those organisms are inoculated into fresh waste material. Figure 12.6 shows the regaining of the length and weight of the earthworm after the 1-week period of the completion of the process.

8 Future Perspectives

The suitability of the process has been checked and found to be suitable for the post-consumer wastes like dry litter, recycled paper, artificial banana leaf, paper plates, etc.; managing such wastes reduces the possibility of aesthetic pollution too.

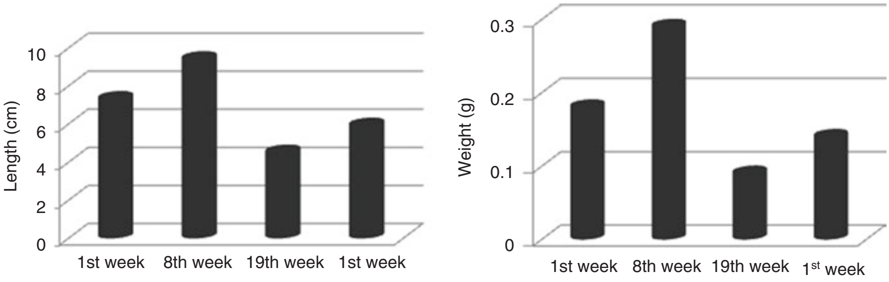


Fig. 12.6 Regaining the length and weight of the earthworm after the vermicomposting of paper cup waste

Earlier people employed microorganisms for the manufacture of bread, cheese, alcohol even though the phenomenon of fermentation was not clear. But at the present scenario, researchers work on living organisms and transfer their genetic material between different organisms, generating transgenic plants/animals. Nowadays, the applications of biotechnology are practiced in many fields of science to produce new and improved foods, produce drugs for many of the dreadful diseases and also for control of pollution. Further work in this direction would guarantee the post-consumer waste management effectively and in eco-friendly manner in a cost-effective manner.

9 Concluding Remarks

Higher degree of organic matter stabilization was achieved when the paper cup wastes were subjected to vermicomposting process in different ratios along with the carbon source, namely the cow dung. Hence, vermicomposting pave the way for managing the paper cup waste, one of the post-consumer wastes produced at higher quantities in developing countries and also convert the waste into value-added product.

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Chapter 13
Recent Advances in Green Sustainable Nanocellulosic Fiber: An Overview

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Abstract The current scenario of economic and social aspects led to the development of smart and new biomaterials. Sustainable bio nano approaches are focusing on environmentally friendly biomaterials from renewable resources. The renewable bio nano materials are often produced directly from natural or recycled products. The natural products are biodegradable and mostly consist of cellulose, chitosan, starch, collagen, soy protein, and casein. The cellulose is a grade one biomaterial with appealing features including biocompatibility and biodegradability. These renewable materials play an important role in reducing global warming by preventing the release of carbon dioxide to the atmosphere. The cellulose microfibrils are made up of a linear chain of nanofibrils of amorphous and crystalline character. Natural cellulose represents the cellulose I type polymorph which is thermodynamically metastable. The isolation of nanocellulose from the cellulose involves several methods. Nanocellulose possesses unique propensities such as high surface area-to-volume ratio, young modulus, high tensile strength, and coefficient of thermal expansion. Nanocellulose is mainly of two types, nanofiber and nanocrystals. Nanocellulose could be altered in long fibers, suspension, and film through various processes and modifications.

The extraction of nanocellulose involves multistage processing with vigorous chemical and mechanical treatment. Chemical methods involve alkali pretreatment combined with acid hydrolysis, ultrasonication, enzymatic hydrolysis, high pressure homogenization, cryocrusing, TEMPO-mediated oxidation, and so on. The choice of selected method strongly influences the aspect ratio, surface features, and mechanical stiffness. Recently most cellulosic material has been involved in fabrication into a biopolymer composite system but cellulose's intrinsic hydrophilic character of the original surface features hampers the interfacial interaction with other hydrophobic polymeric structures. Therefore the modification of the surface

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introduces new functionalities into the cellulosic chain to convert it into active nanocellulose. The surface can be modified via two approaches: (i) physical interaction between cellulose and other macromolecules through adsorption on the surface, and (ii) alteration in the chemical bonding between cellulose and other chemical agents. A high surface area and the presence of an hydroxyl group provide a classic condition for surface mediation. TEMPO-mediated oxidation, amination, silylation, acetylation, oxidation, esterification, surfactant, or polymer grafting are the methods most often applied for surface modification of nanocellulose.

Apart from this current physical treatment, surface fibrillation, electric discharge (corona, cold plasma), irradiation, ultrasonic, electric currents, and the like have been applied to create a modified surface. Thus the use of modified reinforced biopolymer fibers instead of traditional fibers provides several advantages in different sectors including pharmaceuticals, paper, biomedicine, and the development of other novel smart materials. The presence of exceptional mechanical properties, surface groups, and biological properties makes it a suitable material for tissue scaffolds, drug delivery agents, and enzyme and protein immobilizing material. In addition to this, in the development of aerogel, biofoam, nanofiber, and additives for new devices or material, nanocellulose plays an important role. The application of nanocellulose in different sectors needs the proper assessment of biodegradability, toxicological profiling, and biocompatibility. Development of a new research platform for the creation of various supramolecular structures and engineered biobased material by the utilization of nanocellulose is the need of the hour. The economic and scientific points of view suggest that nanocellulose is a promising reinforcing green sustainable biomaterial that might be helpful in creating revolutionary changes in current technology and helping in advancement in various sectors.

1 Introduction

The applications of renewable material have been known since ancient times. However, renewables attract the attention of the scientific community due to the various environmental issues. Renewable materials can be manufactured from natural products or obtained by synthetic processes, and often include recycled products. Cellulose, chitosan, starch, collagen, soy protein, and casein are the most abundant and biodegradable sources used for the preparation of sustainable and renewable materials from natural products. Recycling of plant biomass waste is a promising option for production of renewable material. It also prevents a large amount of carbon dioxide from being released into the atmosphere due to open burning of plant waste (Kim et al. 2015).

Cellulose is one of the most abundant renewable polymer resources of the biosphere. It has an annual production of up to 7.5×10^{10} tons. It is an inexhaustible raw material and has served as an important sustainable material for usage on an industrial scale for millennia. The fascinating property of cellulose allows its application in different forms for a wide range of materials and products such as cellulosic fibers in lumber, textiles, paper, cellophane films, and dietary fibers. In addition to these applications, a more sophisticated usage of cellulose is as reinforcement in plastic-based composites. After mechanical shearing or controlled acid hydrolysis of cellulose fibers, they yield elongated fibrillar or defect-free rod-like crystalline particles with at least one dimension in the nanoscale range. Targeting the nanotechnology and keeping these cellulosic substrates has led to an enormous level of attention being generated because of their exceptional physical and chemical properties (Habibi 2014; Rebouillat and Pla 2013).

Cellulose has a large number of hydroxyl groups in its template that provide an exclusive platform for the modification of surfaces and grafting of myriad functional groups. It leads other materials with its wide range of highly sophisticated applications. Various chemical transformations, modifications, and derivatizations are carried out to improve the performances of nanocellulose-based materials and for obtaining application-specific propensities. Generally, in these modifications the integrity of the original, that is, the morphology of nanocellulose, should be preserved.

The nanoscale polysaccharide nanocrystals serve as promising candidates for the preparation and applications in the field of materials science due to their low weight, stiffness, high strength, and biodegradability. However, the safety issue of these natural nanocrystals is in the spotlight for the evolution of polysaccharide nanocrystal materials. The toxicological profiling of cellulosic nanocrystals is very important due to the inherent toxicity and potential ecotoxicological evaluation of biomedical materials (Lin and Dufresne 2014).

2 Sources and Types of Nanocellulose

Cellulose is a plentiful natural material made from renewable and sustainable resources that is biodegradable and carbon neutral with low environmental, health, and safety risks. Due to these fantastic properties, there is ongoing intensive research and development of nanocellulosic material by applying upcoming nanotechnologies. More recently, the design of new cellulose nanoparticles (CNs) from natural resources for the production of innovative cellulose-based biomaterials and composites has been reported (Eichhorn et al. 2001; Habibi et al. 2010). Cellulose, which is naturally produced in plants, can also be synthesized by algae, tunicates, and bacteria (Klemm et al. 2006; Iwamoto et al. 2007). Agriculture residue and waste by-products can be good options for production of nanocellulose as they are largely low-cost unused sources. The limited association of cellulose with other polysaccharides and lignin turned it into a very complex and heterogeneous structure. Figure 13.1 presents the hierarchy of cellulose in a plant cell wall.

Bacterial celluloses (BC) have better features such as higher purity, degree of polymerization, crystallinity, mechanical stability, and water content as compared to plant cellulose. This could be due to biosynthesis of cellulosic fibers in bacteria

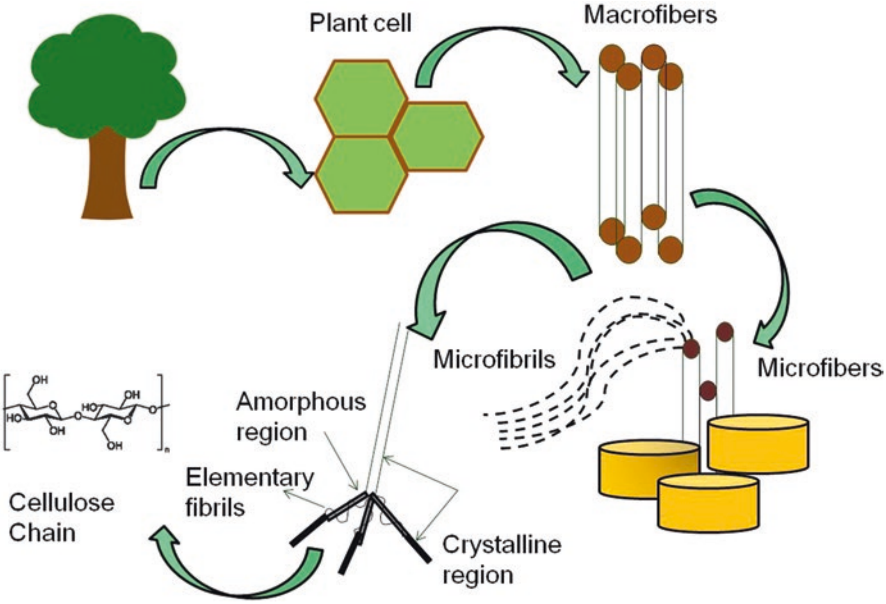

Fig. 13.1 Hierarchical structure of cellulose in a plant cell wall

without the other components (Klemm et al. 2011). Each cellulose consists of repeating units (called cellobiose) due to connections of two anhydroglucose rings, which are joined with a β-1,4 glycosidic linkage, turning to linear homopolymer, and has a stiff rod-like conformation; it has a fascinating hydrophilic nature and functionality (Azizi Samir et al. 2005).

The three-dimensional cellulose crystal structure allows intra- and intermolecular hydrogen bonding only at secondary and primary hydroxyl groups and therefore generation of semi-rigid cellulose polymer occurs that is insoluble in water and other traditional organic solvents, with no melting point. The high tensile strength and rigidity of the cellulosic cell wall is due to holding of elementary microfibrils side by side through multiple hydrogen bonds. Depending on their origin, these microfibrils are generally made up of fibrous structures (Siró and Plackett 2010) and consist of crystalline and noncrystalline domains positioned along their main axis. Cellulose properties mainly incur supramolecular order and specific assembling. Table 13.1 comprises various studies revealing the isolation of nanocellulosic materials from green resources.

3 Types of Nanocellulose

Different types of cellulosic nanomaterials can be obtained by ensuring a clear material purification step followed by an isolation method and characterization process. Different routes for the production of nanocellulosic fibers are given in Fig. 13.2.

Table 13.1 Source and types of nanocellulosic biomaterials

Type	Synonyms	Potential Sources	Method of Extraction	References
Microfibrilated cellulose (MFC)	Cellulose nanofiber (CNF), Microfibrils, nanofibrilated cellulose (NFC)	Date palm, Abaca, Sisal, Hemp, Rice Straw, Bagasse, Cotton, Woody plants	Enzymatic pretreatment, Mechanical disintegration, High pressure homogenization, Chemical oxidation	Hassan et al. (2014), Abidin et al. (2016), Hassan et al. (2015), Adel et al. (2016), Alila et al. (2013)
Cellulose nanocrystals (CNCs)	Nanocrystals (CNC), nanowhiskers, microcrystals	Banana pseudostem, Pineapple peel waste, Oil palm, Tomato peels, Poplar wood	Acid hydrolysis, Ultrasonication	Pereira et al. (2014), Anwar et al. (2016), Shanmugarajah et al. (2015), Jiang and Hsieh (2015), Chen et al. (2013)

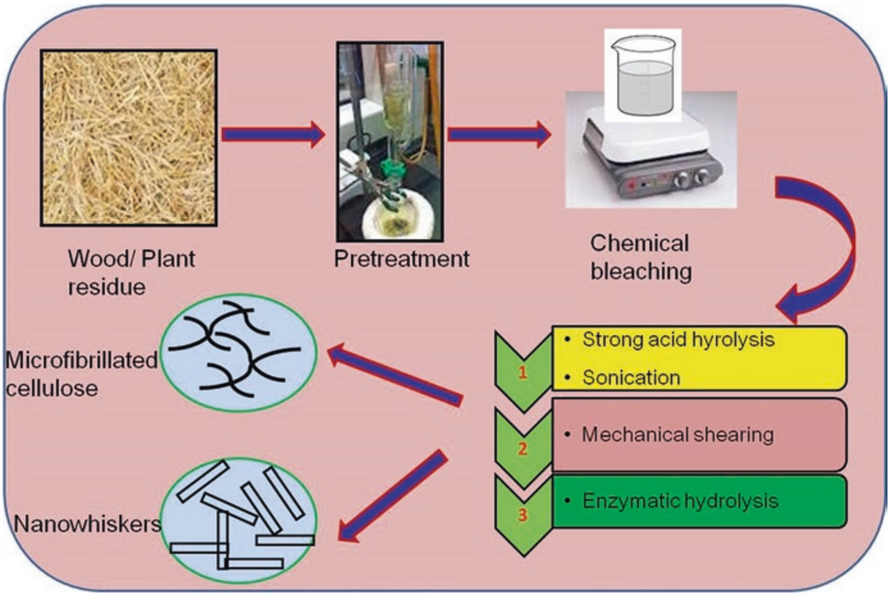

Fig. 13.2 Method used in preparation of nanocellulose from plant biomass

3.1 *Cellulose Nanocrystals*

Cellulose nanocrystals (CNC; nanocrystalline cellulose) can be produced by acid hydrolysis. The nanocrystals originated from wood pulp are shorter and thinner than the microfibrilated cellulose. CNC has been isolated from a wide variety of cellulosic sources, including plants (Klemm et al. 2011), microcrystalline cellulose, animals, bacteria, and algae (Chirayil et al. 2014). Tunicin whiskers have been a favored source because of their high crystallinity and length (Klemm et al. 2011), although their pandemic application may be restricted by the high cost of harvesting and finite availability. Wood, cotton (94% cellulose content), filter paper, and other products were the elected substrate for fundamental research on CNC, because of their large quantity, purity, and ready accessibility in laboratories (Klemm et al. 2011).

The presence of hemicellulose and lignin inside the cellulose backbone is forced for delignification for extraction of CNC. The kraft pulping process is mainly applied for lignin removal, which utilizes a hot solution of sodium hydroxide and sodium sulfide in a digester. Acid hydrolysis can sufficiently break the amorphous cellulose and results in CNC in the suspension (George and Sabapathi 2015).

3.2 *Cellulose Nanofibers (CNF)*

Excessive mechanical forces effectively break interfibrillar hydrogen bonding of cellulose microfibers. Amorphous domains of cellulose or hemicellulose can easily break through a different level of mechanical forces by breaking interfibrillar hydrogen bonding followed by Van der Waals force. The high bonding strength of intramolecular hydrogen bonding of cellulose means it can rarely break. Cellulose nanofibers (CNF) can separate into nanofibers by swelling microcrystalline cellulose through dissolution. Enzymatic pretreatment can also attack the amorphous regions of cellulose fibers, helping in the extraction of nanofibrillated cellulose (Yu et al. 2014).

N,N-Dimethylacetamide (DMAc) with lithium chloride (LiCl) is a well-known solvent for dissolving cellulose (Zhang et al. 2014). 1- Butyl-3-methylimidazolium chloride ionic liquid was checked for dissolving and regenerating cellulose for application in electroactive paper (Isik et al. 2014). An electrospinning process is also used for the generation of fine cellulose fibers. 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation under moderate conditions has been applied for extracting 3–4 nm wide and a few microns long cellulose nanofibrils (Kim et al. 2015). TEMPO-mediated oxidation is a type of regioselective surface modification wherein original morphologies are preserved during oxidation (Isogai et al. 2011).

3.3 Bacterial Nanocellulose (BNC)

Acetobacteraceae are Gram-negative, rod-like, aerobic bacteria of unusual acid tolerance (growing well below pH 5.0), active motility, and universal and also with the ability to produce high-yielding natural polymer cellulose. They are found wherever a low pH (below 5) is found such as a fermentation place, that is, on damaged fruits, on flowers, and in unpasteurized or unsterilized juice, beer, and wine.

Bacterial nanocellulose (BNC) has the same molecular formula as plant cellulose but is fundamentally different because of its nanofiber building blocks (Klemm et al. 2011). BNC has no impurities and no functional groups yet the nanoscale features are quite different from plant cellulose (Lee et al. 2014). Because of the appealing features of BNC, the biofabrication of cellulose materials should be investigated in the future. For commercial applications, cost-efficient processes for the mass production of BNC are required. The static and agitated cultivation of BNC generates new concepts in research in this field, thereby developing cost-efficient processes for mass production.

4 Production Cost

The preparation of CNC from biomass generally occurs in two primary stages. The first stage is a purification of the biomass to remove most of the noncellulose components present in the biomass such as lignin, hemicelluloses, extractives, and inorganic contaminants. The first stage of purification of the biomass is to remove most of the noncellulose components by conventional pulping and bleaching. The second stage uses an acid hydrolysis process to dissect the "purified" cellulose material into its crystalline parts by removing the amorphous regions of the cellulose microfibrils. In this process the cellulose yield might reach up to 50%. The Technical Association of the Pulp and Paper Industry (TAPPI) Nanocellulose Division estimates the commercial sales price of CNC produced from this method to be $10–50/lb. The commercial sales price of CNC produced by the TEMPO method to said be $ 4/lb. The USDA's Forest Products Laboratory nanocellulose pilot plant also produces TEMPO-pretreated cellulose nanofibrils on the 2–3.5 kg scale. The principal disadvantage of the process is the high cost of TEMPO, which is not recycled.

5 Unique Properties of Nanocellulose

As a natural nanoscaled material, nanocellulose possesses unique characteristics such as special morphological and geometrical dimensions, high specific surface area, crystallinity, rheological properties, barrier property biocompatibility, biodegradability, and so on. On the basis of these extraordinary properties, both

Table 13.2 Comparison in the characteristics of nanocellulosic materials

Source Material	Method Applied	Diameter (nm)	Crystalinity (%)	Degradation Temperature (°C)	Reference
Sugarcane bagasse	High pressure and Homogenization	10–20	36	238	Li et al. (2012)
Soy hulls	Acid hydrolysis	4.43 ± 1.2	73	294	Neto et al. (2013)
Commercial MCC	Ultrasonic-assisted hydrolysis	10–20	73	250	Tang et al. (2014)
Citrus waste	Enzymatic Hydrolysis	10	63	NA	Mariño et al. (2015)
Poplar wood powder	Ultrasonic-assisted hydrolysis	4–14	76.5	NA	Chen et al. (2013)
Palm tree	TEMPO-mediated homogenization	40	62–72	NA	Benhamou et al. (2014)
Banana peel	Chemical treatment and ultrasonication	20–35	30–63.4	260–295	Khawas and Deka (2016)
Mango seeds	Chemical treatment and Acid hydrolysis	4.6–2.2	74.3	271	Henrique et al. (2013)
Agave	Acid hydrolysis	8–15	82.4	361	Rosli et al. (2013)
Oat hulls	Acid hydrolysis and Ultrasonication	70–100	49–86	357	Paschoal et al. (2015)
Lotus leaf stalk	Ultrasonication	25	70	217	Chen et al. (2014)
Commercial MCC	Enzymatic hydrolysis and Ultrasonication	5–10	70–82	250	Cui et al. (2016)
Ushar seed fibers	Acid hydrolysis TEMPO Oxidation	10–20 14–20	70 59	275–350	Oun and Rhim (2016)

“nano-enhanced” and completely smart “nano-enabled” products have been predicted. The potential and successful usage of cellulosic nanomaterials (CNs) in different applications needs an in-depth understanding of their structure and properties. A brief summary of the characteristics of nanocellulose obtained from different resources is presented in Table 13.2.

5.1 Structural and Morphological Properties

The most important structural and molecular properties of CNs, that is, geometrical dimensions (average length and width), crystallinity, and average degree of polymerization vary with the source of the cellulosic material and the conditions applied for production.

Filtration and centrifugation steps help in the reduction of heterogeneity arising within particle sizes. When functional groups are added to the CNs' surface, such as charged entities, hydrophobic groups, and the like, the obtained degree of modification as well as the presence of impurities and other polymers is to be checked.

5.2 Mechanical Properties

Properties such as geometrical dimensions, crystal structure, anisotropy, and defects are the governing factors for the mechanical properties of nanocellulosic material. Theoretical evaluations mainly involve the role of cellulose crystalline features, the critical arrangement of intra- and intermolecular hydrogen bonding, and different theoretical approaches to designing the elastic properties. The elastic properties of the crystalline part of cellulose have been evaluated by experimentation using a tensile test along with the XRD tests. The elastic modulus of individual CNC, t-CNC, and BC particles have been determined, either in the axial direction, E_A, and/or in the transverse direction, E_T. Tunicate CNCs have been used as classical particles because of their longer length, crystalline properties, larger cross-section, higher volume fraction of $I_{\beta,}$ and uniform particle morphology. The mechanical properties of several cellulose particle types are summarized in Table 13.3.

5.3 Thermal Properties

The differences among hemicellulose, cellulose, and lignin structure can be seen in their thermal degradation. The degradation is divided into three steps derived from a thermogravimetric analysis (TGA) curve. Most of the nanocellulose first stage occurs due to decomposition of hemicelluloses at around 225–300°C. The second stage of cellulose decomposition lies between 310 and 385°C. At the final stage

Table 13.3 Mechanical properties of cellulosic fibers

Material	E_A (GPa)	E_T (GPa)	Technique	Reference
CNC	57, 105	—	Raman	Rusli and Eichhorn (2008)
Acid treated CNC	151 ± 29	—	AFM-3 pt. bend	Iwamoto et al. (2009)
TEMPO treated CNC	145 ± 31	—	AFM-3 pt. bend	Iwamoto et al. (2009)
BNC	78 ± 17	—	AFM-3 pt. bend	Guhados et al. (2005)
Cellulose Iβ	120–138		XRD	Diddens et al. (2008)
Cellulose Iα	128–155	5–8	Modeling based	Eichhorn and Davies (2006)
Cellulose II	9–90	—	Raman	Eichhorn et al. (2005)

E_A = elastic modulus in axial direction, E_T = elastic modulus in transverse direction

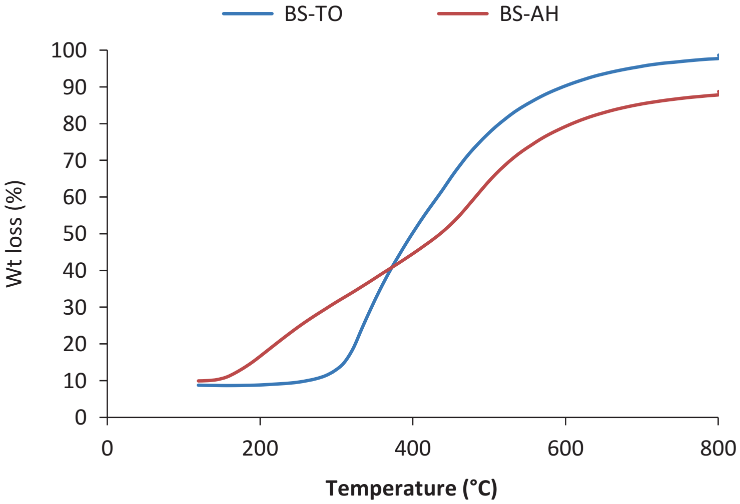

Fig. 13.3 TGA curve of nanocellulose: AH, TO

above 385°C mostly lignin degrades. Actually, hemicellulose, lignin, and other noncellulosic substances start their decompositions at low temperature. The reduction of these substances causes the decomposition to occur at higher temperature. Otherwise, the removal of these noncellulosic substances through the treatments would make the structure of cellulose more dense and compact. These factors might be the reasons cellulose fibers have better thermal stability. The thermal degradation temperature (T_d) of CNC lies between 200 and 300°C. The thermal characteristics of CNC depend on the source, preparation method, and nature of surface modification, which further results in a significant reduction in degradation temperature. A comparison was made between the TGA curves of nanocellulose isolated from banana stem (BS). Thermal degradation behavior was checked for acid hydrolyzed (AH) and TEMPO oxidized (TO) nanocellulose (Fig. 13.3) and it was concluded that thermal decomposition of BS-AH was shifted to higher temperature due to a more crystalline nature whereas introduction of a carboxylic group in BS-TO samples resulted in their becoming thermally unstable.

6 Methods for Surface Transformation of CNs

Various surface modification of CNs can be performed by adsorption of various chemical extremities on the surface through chemical modification, processing during extraction, or by covalent bonding of molecules on the surface. Generally, these modifications are application specific.

6.1 Transformation During CNs Extraction

Various methods used for the CN extractions may produce nanocellulose with additional functional groups. The presence of functional groups on the CN surface turns it into a stabilized form. Also cellulose with different functional groups has different types of applications. Acid extraction (HCl and H_2SO_4) and TEMPO-mediated oxidation are the most common methods used for the purpose. Sulfuric acid hydrolysis promotes the formation of sulfate esters there with high surface acidity with stabilized nanocrystal dispersion, whereas hydrochloric acid hydrolysis results in hydroxylated surfaces (Jonoobi et al. 2015). TEMPO-mediated oxidation previously described in Section 13.3, is also used during CN extraction. This is helpful in promoting a carboxylic acid-rich surface.

6.2 Transformation by Adsorption

Noncovalent surface modifications of CNs are accomplished through adsorption of surfactants such as stearic acid (Spoljaric et al. 2009), xyloglucan block copolymers, cetyltetramethylammonium bromide (CTAB) (Padalkar et al. 2010), and mono- and diesters of phosphoric acid with alkylphenol tails (Heux et al. 2000). Most commonly, layer by layer deposition is used (Cranston and Gray 2006), thus the obtained surfactant-coated CNs disperse very well in nonpolar solvents. The main objective behind this is to improve their dispersibility/compatibility within nonpolar solvents by reducing surface energy.

6.3 Chemical Transformations of CNC

The presence of hydroxyl groups on the surface of nanocellulose extends the possibility of surface modification. The modification aims towards (i) improvement in aggregation behavior via reducing hydrogen bonding for better dispersibility, and (ii) the compatibility inside different solvent systems used during production. Various examples of chemical modifications of CNC are carboxylation by TEMPO-mediated oxidation, cationization, fluorescent labeling, esterification, silylation, and polymer grafting (Missoum et al. 2013). Apart from improving surface energy, adhesion behavior, and polarity, several pernicious outcomes are reported, concerning the mechanical performance of nanocomposites.

6.4 Polymer Grafting

Two methods for polymer grafting are applied; the first is “grafting-onto” and the second one is “grafting-from” (Dufresne 2013). In grafting-onto a coupling agent is used that helps in the grafting of polymer on the CNC surface. The main advantage of this technique is that the properties of the resulting material can be controlled. The main problem arises due to steric hindrance and high viscosity of the medium. The grafting-from involves mixing cellulose with monomer and initiator, followed by growth of the polymer taking place. The reaction is fast and easy due to the lack of steric hindrance and the low viscosity of the medium. The nanocellulose does not dry in this process until the exchange of water to the organic solvent takes place. Both modifications exhibit a large effect on the individualization of the nanocrystals due to reduction in the hydrogen bonding and polar nature of the particles.

7 Applications of Cellulosic Nanomaterial

Different functional nanocellulosic materials with special applications can be produced through various processes. These smart materials can be used directly or are helpful in the development of other unique materials. Figure 13.4 shows various sectors for nanocellulose applications.

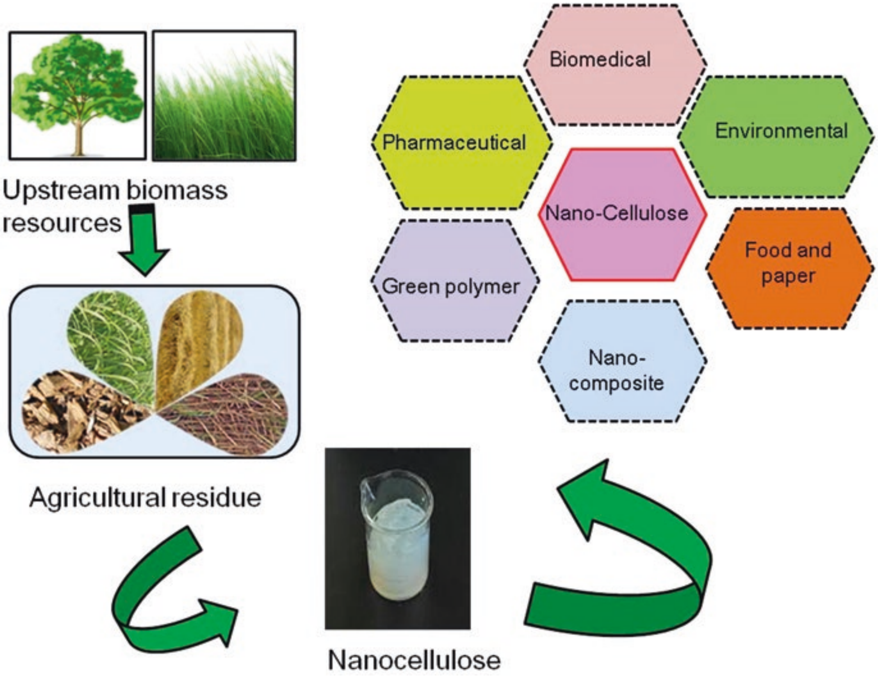

Fig. 13.4 Applications of nanocellulose

7.1 Biomedical Applications

7.2 Drug Carriers

Nanocellulose-based materials including microspheres, hydrogels, and membranes are largely used as drug carriers. They are able to trap the drug inside the matrices and control their regulation at the effective site. A pH-sensitive CNC/sodium alginate microsphere-based controlled release system was developed for drug delivery. Due to the alginate, more swelling, higher encapsulation efficiency, and improved sustained-release profiles were achieved (Lin and Dufresne 2014). Regarding the application of nanocellulose in the fabrication of hydrogels, cyclodextrin-grafted nanocellulose was used as a fabrication of hydrogel obtained via in situ inclusion. The in vitro release of doxorubicin was determined by using hydrogels as the carrier and it was found that the drug exhibited a sustained-release system with special kinetics whereas the obstruction and locking effects resulted in good dispersion of the nanoparticles (Lin and Dufresne 2013). CNC also showed better and controlled release of camptothecin in a physiologically relevant solution (Mishra et al. 2016). The presence of CNC (only 0.1 wt%), used as a costabilizer, positively affected the decrease of bead size and affected narrower size distribution of beads. At the same time, beads containing CNC had good flow properties, which were stable during the compression process. The usage of cellulose as a costabilizer offered narrower distribution of beads with improved flow properties. In another work, Jackson et al. concluded the controlled release phenomenon of CNC with the surface modification using cationic surfactant cetyl trimethyl ammonium bromide (CTAB) on the drug molecules. CTAB-modified CNC was used as a carrier for anticancer drug molecules, which exhibited prolonged release over a two-day period. (Jackson et al. 2011).

Huang et al. reported the extended release duration of berberine hydrochloride and berberine sulfate by using BC membranes in comparison to commercial tablets (Huang et al. 2013). The polyelectrolyte macroion complex was introduced by modifying CNC through the addition of chitosan matrix for application in drug delivery (Wang et al. 2011). Recently direct attachment of the drug molecule on the surface via covalent bonding has been in progress. Apart from application of nanocellulose as a drug carrier several points should be kept in mind, including the interaction mechanism of the drug with nanocellulose, drug activity, and regulation in release of the drug from the matrix. Table 13.4 shows the potential uses of nanocellulose as a drug delivery vehicle.

7.3 Cellular Bioimaging

Fluorescent dye grafted nanocellulose proved an effective agent for cellular bioimaging. Rhodamine B isothiocyanate (CN–RBITC) and fluorescein isothiocyanate (CN–FITC) grafted nanocellulose introduced active amino groups on the surface (Mahmoud et al. 2010). Due to the presence of a cationic amino group of dyes, the

Table 13.4 Application of cellulosic material in drug delivery

Carrier Form	Matrix	Drug Used	Release Time	Model Applied	Reference
Microsphere	CNC + EA; MMA; BMA	Propranolol hydrochloride	12 h in pH 6.8 PBS	—	Villanova et al. (2011)
Microsphere	CNF	Indomethacin; nadolol; atenol	10–14 days in pH 7.4 PBS	Baker-Lonsdale	Kolakovic et al. (2012a)
Suspension	CNC+ CTAB	Paclitaxel; Docetaxel; DOX; TET;	1–4 days in PBS	—	Jackson et al. (2011)
Hydrogel or gel	CNC+ Cyclodextrin/ Pluron	DOX	Cyclodextrin/ Pluron	Ritger-Peppas equation	Lin and Dufresne (2013)
Hydrogel or gel	CNC + Regenerated cellulose	BSA	48 h in simulated body fluid	Fickian diffusion law	Wang and Chen (2011)
Hydrogel or gel	BC	BSA	48 h in pH 7.4 PBS	Ritger-Peppas equation	Müller et al. (2013)
Membrane or coating for tablet	CNF	Lysozyme	10 h in pure water or water/ ethanol solution	Fick's second law	Cozzolino et al. (2013)
Membrane or coating for tablet	BC	Berberine hydrochloride,	24 h in pH 2.1 HCl or H_2SO_4	Ritger-Peppas	Huang et al. (2013)
Nanofiber	CNC+ Poly(lactic acid)	Columbia blue	48 h in water	Higuchi equation	Xiang et al. (2013)
Film	CNF	Itraconazole	Last up to three months	Zero order kinetics	Kolakovic et al. (2012b)
Suspension	CNC	Camptothecin	Last up to 12 h	Ritger- Peppes	Mishra et al. (2016)
Sponge	Nanocellulose + calcium phosphate	Bone morphogenetic proteins	Last up to 30 days	—	Sukul et al. (2015)
Cryogel	Microcrystalline Cellulose + Epichlorohydrin	Procaine hydrochloride	Last up to 3 h in water at pH 6.5	—	Ciolacu et al. (2016)

DOX= doxorubicin hydrochloride, TET= tetracycline hydrochloride, PB= phosphate-buffered solution, BMA = butylmetacrylate, EA= ethyl acrylate; MMA = methylmethacrylate, CTAB = cetyl trimethyl ammonium bromide, BSA= Bovine serum albumin electrostatic interaction was favored between nanocrystals and cell membranes and they were easily taken up by the HEK 293 cell line and Sf9 insect cell line without any membrane damage and cytotoxocity.

7.4 Permselective Nanostructured Membranes

Nanocellulose may be used for the permselective nanostructured membranes that could be used for electrodes. Thielemans et al. utilized a suspension obtained from cotton CNC (1.5 wt%) to design the membranes at glassy carbon electrodes with simple drop-coating procedures (Thielemans et al. 2009). The suspension of nanocrystals was also used as a membrane in a glassy carbon electrode. The porous membrane was formed due to the interparticle hydrogen bonding at the electrode surface. Due to the negatively charged surface, this nanostructured membrane performed the permselective role for substances with different charge properties. The negative charge present on the surface of nanocellulose favors the permselective membrane function, which inhibits the diffusion of the negative charge particle, and the positive charge particle moves easily.

7.5 Emulsion Nanostabilizer

The application of emulsion is a regular practice in different fields including food, cosmetics, and pharmaceuticals. The ability of BC as a stabilizing agent was checked by Kalashnikova et al. (2013) in a hexadecane water interface, which was able to disperse for a period of up to several months. CNC were dispersed in water with the required concentration, and hexadecane was added afterward. Emulsions are of great practical interest because of their extensive occurrence in the pharmaceutical and biomedical industries, food, and cosmetics. Research efforts on the development of sustainable, biodegradable, and nontoxic particles, emulsions, and stabilizers have not stopped since they began several decades ago.

8 Smart Nanomaterials

Naturally isolated nanocellulosic fibers offer a new platform for sustainable production of a wide range of advanced nanomaterials. These biobased materials are envisaged to be used for applications with great economic value in various sectors. These smart materials constructed by using nanocellulose can be applied in the food and pharmaceutical industries, electronics, composite materials, rheology modifiers, and engineered structural materials, among others. Figure 13.5 represents various sectors for the potential utilization of cellulosic biomaterials.

Fig. 13.5 Strategies for construction of smart nanomaterials

9 Future Prospective

Rapid advancement in nanocellulose applications motivated the current research for isolation and modification of nanocellulose from environment-friendly resources. A wide range of cellulosic products such as nanofibrillated cellulose, CNC, and bacterial cellulose are current research topics in the materials science community. The abundance of biomaterials allows the utilization of nanocellulosic material in diverse fields due to their various chemical functionalities and physical attributes. Smart materials and structures are fabricated by nanocellulose which can adjust its shape, morphologies, and material properties so as to acclimatize its environmental change. These materials based on nanocellulose exhibit intelligent behavior in response to electrical input, light, temperature, pH, and magnetic force to develop sensory-actuation systems. Simple chemical alteration of nanocellulose can permit inorganic hybrid materials in order to extend the functionality of cellulose. Nanocellulose production technology is still facing problems such as reducing the quantity of harsh acids used, the aggregation problem, and reduction in energy input during nanocrystal production.

The large-scale production process of nanocellulose includes the development of new research and development groups, pilot plant establishment, and augmentation of new smart engineered nanoproducts. The progressive building up of modern transformational routes is essential for evaluating different sectors of nanocellulose production so that accrued benefits will be extremely high. There are many scientific and technological challenges that need to be overcome for scaling up this ultramodern technology for simplicity and reduced cost. Therefore from both scientific and economic viewpoints, nanocellulose is a nature-gifted resource recently on the verge of development through phenomenal discoveries in various sectors.

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Chapter 14

Metal NPs (Au, Ag, and Cu): Synthesis, Stabilization, and Their Role in Green Chemistry and Drug Delivery

Prashant Singh, Kamlesh Kumari, Vijay K. Vishvakrma, Gopal K. Mehrotra, Ramesh Chandra, Durgesh Kumar, Rajan Patel, and Vaishali V. Shahare

Abstract Nature is very powerful and strategic and has tailored various materials with sizes in nanometers. This phenomenon is observed with the passage of time. In the current picture, the most important thing is to control the changes in biological processes at the nanoscale. Due to this behavior of nature, scientists and academicians have been inspired and encouraged towards nanoscience and nanotechnology and reproducing the manufacture and controlled synthesis of nanomaterials in bulk. Nanoscience is the interdisciplinary science where basics and the advancement of the discipline of the fundamental principles of atoms and molecules have been discussed regarding their structures in various dimensions where the particle size is less than 100 nm. Nanoparticles are popular due to their high specific surface area and good dispersion in various solvents. Therefore, metal nanoparticles (NPs) have been applied in different areas of science including medicine, electronics, electrical, and catalysis, among others. The synthesis of metal NPs in bulk is a challenge to researchers due to their aggregation behavior. Thus, the stabilization of metal

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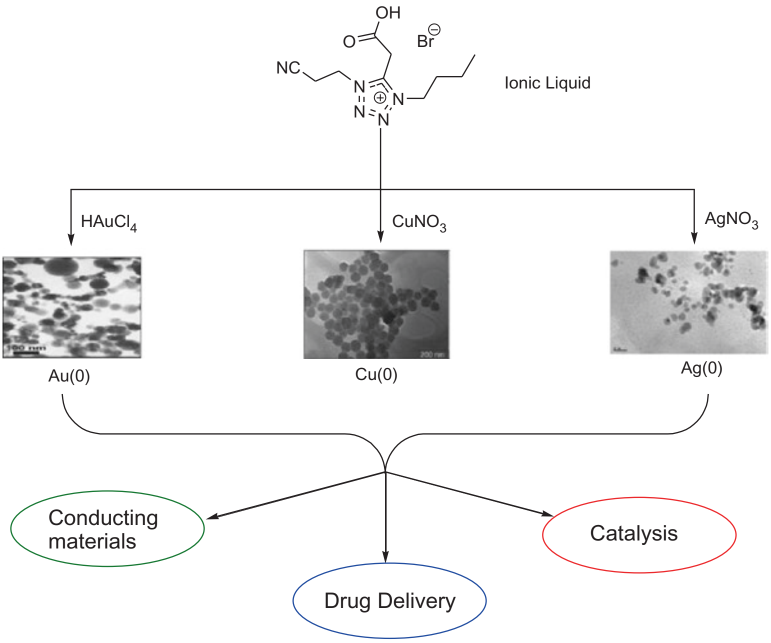
nanoparticles becomes a challenging job. But in the last decade ionic liquids (ILs) were found to be potent alternatives for the stabilization of metal nanoparticles.

As per the literature, ionic liquids are organic salts. They are based on the ammonium or phosphonium cation and different anions. Ionic liquids melt at low temperature, that is, 100°C. Their hydrophobicity or polarity can be tuned easily via changing the alkyl part of the ammonium or phosphonium cation as well as the anion together or independently. ILs have low vapor pressure and are preferred over conventional organic solvents. Gold, silver, and copper NPs can be synthesized in ionic liquid (FIL) as a solvent and stabilizer. Synthesized metal NPs can be used in the synthesis of composite materials of PANI, gelatin, and triblock copolymers to explore their conducting and biomedical applications. Synthesized metal NPs were used to catalyze various organic reactions including the Knoevenagel reaction, oxidation of thiols, and acetylation of alcohol and amines. The products obtained are in high yields and of short duration using metal NPs in ionic liquids as catalytic media. This methodology is much cheaper than the others and also does not require high temperature. Hence, ILs serve in a green and efficient method. Further catalytic applications of metal nanoparticles for various reactions are currently under investigation.



In the present era, the curiosity to learn or understand the science of the very small size of nanoparticles and their application calls for correlating the various disciplines of science. Actually, the preparation and isolation of metal nanoparticles is a tedious process, mainly when the interest is in particle size, shape, and composition. Varying the composition of dispersion of metal nanoparticles in a solvent alters their applications in the areas of physics, biology, and the like (Ibanez and

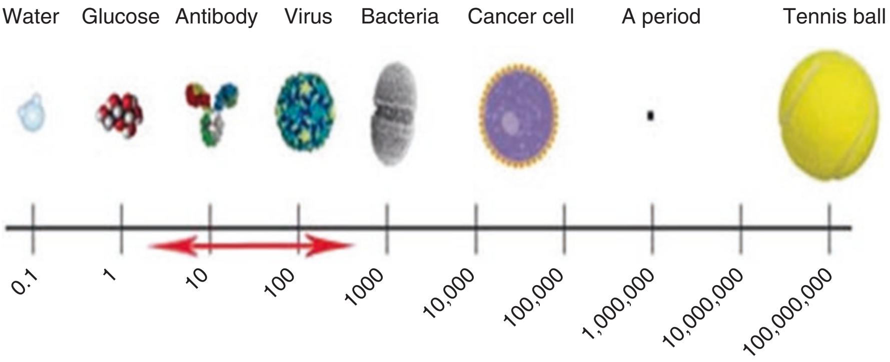


Fig. 14.1 Comparison of various objects with the nanoparticle

Zamborini 2012; Usman et al. 2012; Sun et al. 2008; Conde et al. 2012; Du et al. 2013; Yu et al. 2012). Researchers have developed various methods for the synthesis of size- and shape-controlled metal NPs with different morphology (Misawa and Takahashi 2011; Tsubaki 2013). However, obtaining monodispersive metal NPs in bulk is a challenge, as monodispersive metal NPs exhibit unique applications, better than polydispersive metal NPs. In view of these, researchers try to conceptualize the requirement of metal NPs by developing new methodologies to synthesize metal NPs in bulk (Offenhäusser and Rinaldi 2009; Kumar 2009).

Matter can be placed in several categories according to size. Tailoring of the material at infinitesimally very small size is usually governed by Nature with due passage of hive whatever it present today. The field of nanoscience and nanotechnology was born of inspiration from Nature to mimic the selective change in the nanomaterial of a particular size (Laurentius et al. 2011; Zheng et al. 2012; Ungureanu et al. 2010; Cho et al. 2011).

Term nanometer can be explained in various ways like it is a billionth part of a meter (Fig. 14.1) or the size of six carbon atoms in a row. The word nano" has been taken from the Greek word nanos and it means "dwarf". A famous Scientist gave a clue towards the possible existence of the field of nanoscience and nanotechnology in a speech entitled, "There's Plenty of Room at the Bottom." From that time, many significant contributions have been made towards tailoring of materials at small lengths of scale. But still there is huge complexity levels in the biological world and are still to achieve (Brancolini et al. 2012; Zakhidov et al. 2012).

1 History of Metal Nanoparticles (NPs)

The science of metal colloids (in contrast to alchemical and aesthetic research) can be supposed to have begun on gold sols in the middle of the nineteenth century. Ruby color solutions of colloidal gold NPs (0) were synthesized by reducing tetra chloroaurate $[AuCl_4]^-$ on exposure with phosphorus which behaves as a reducing agent (Leu et al. 2011; Brust and Gordillo 2012; Campbell 2013; Feng et al. 2011; Banerjee et al. 2011; Etame et al. 2012; Sun et al. 2013).

The world of nanotechnology is not new to mankind. The history of noble metal colloids can be traced back to ancient times. Cosmetic use of lead-based chemistry was initiated in ancient Egypt about 4000 years ago. Historical proofs suggest that gold NPs were also recommended and used as medicines by the Chinese in 2500 BC. Colloidal gold was used as Swarna Bhasma and Makaradhwaja in the Indian traditional medicinal system and known as Ayurveda. Colloidal gold NPs were probably the first spiritual health recipe. In the sixteenth century AD, researchers claimed to have created a potion called *aurum potabile* (Latin: potable gold), also called the elixir of life. The German-Swiss physician Paracelsus also developed formulations from metallic minerals, including gold for health rejuvenation. The great Cyrus, King of Persia, used to have water for his soldiers carried in silver containers that kept water fresh and fit for consumption for several months. The use of silver plates to improve wound healing was used by the Macedonians and may have been the first attempt to avoid surgical infections. In the treatment of ulcers, a silver recipe was used by Hippocrates. Medical uses of silver nitrate can also be found (Offenhäusser and Rinaldi 2009; Kumar 2009).

The first clear record of silver nitrate being present in medicinal use was formed by Gabor in 702–705 AD. Silver filings were used as a blood purifier around 980 AD. Furthermore, silver was used to avoid heart palpitations and also to treat violent breath in 1520 AD. In 1614, silver nitrate was frequently used as a purgative and for the treatment of brain infections. There are number of reports where an epileptic stopped having seizures after swallowing a large coin of silver that was used to prevent him biting his tongue during seizures (Offenhäusser and Rinaldi 2009; Kumar 2009; Heilmann 2003).

Since the era of advances in civilization gold–silver alloys have been used to decorate many historical buildings including temples, monasteries, and other places worldwide. The optical properties of colloidal gold and silver nanoparticles have especially drawn the attention of researchers and academicians due to their absorption maxima in the visible region of the electromagnetic spectrum, which leads to colored colloidal suspensions. It would not be out of place to mention the different colors seen in drinkable gold. The pink or purple color of the solution is due to the very small uniform size of gold NPs, and the yellow color is due to the aggregation of particles. In earlier days, colloidal gold and silver were also used as colorants. The colorant in glasses, "Purple of Cassius," is a colloid with heterocoagulation of gold nanoparticles and tin oxide. Similarly, the Lycurgus cup dating to fourth century AD, which looks green in reflected and red in transmitted light, has been reported to contain colloidal gold as well as silver and the "Damascus Sword" contains nanoscale carbon particles (Dirote 2007; Feldheim and Foss 2002) (Fig. 14.2).

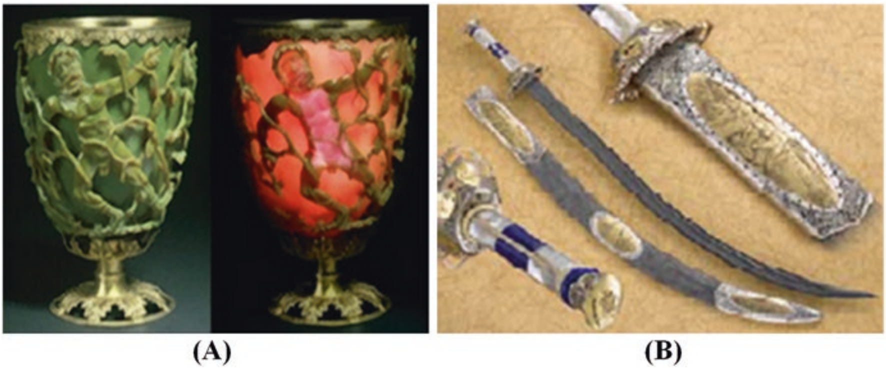


Fig. 14.2 Examples of use of nanomaterials in ancient times: (**a**) the Lycurgus Cup, (**b**) the Damascus Sword

2 Generation of Metal Nanoparticles

The key to the successful development of this relatively new area of inorganic materials chemistry has been the control of nanoparticle size, morphology, and composition during the synthesis and methodology by which metal NPs can be produced chemically in significant quantities. Researchers faced the problem of isolation of metal nanoparticles with good yields as well as uniform size and it was key to synthetic organometallic cluster chemistry (Polte et al. 2012). Any process or reaction that causes the formation of metal NPs is a potential source. Such methodology may generally lead to the preparation of a metallic precipitate. Although they remain dispersed in the solution only in the presence of an appropriate stabilizing agent, the aggregation of metal NPs can be stopped at an early point, and the metal NPs can be well kept in suspension form.

Metal nanoparticles have gained significant research interest in recent years due to their unique electrical, optical, and mechanical properties. They are suitable for various applications in different areas of science including optics, catalysis, sensor design, drug delivery, and antimicrobial coatings, among others (Larguinho and Baptista 2011; Herrero et al. 2010; Yao et al. 2012; Zhang et al. 2012a, b; Niikura et al. 2013). Impressive advances have occurred in the synthesis of isolated nanostructures, which have opened the possibility for finding a new class of advanced materials with the required properties, not only by varying the amount of chemicals, but also by monitoring the size and shape of the chemicals. Metal NPs have been explored for a number of advanced applications. Metal NPs show optical absorption that correlates to electronic vibration patterns on the metal NP surface, known as plasmons. However, the morphology of metal NPs affects the optical properties significantly (Zamiri et al. 2011; Reil et al. 2008; Kai et al. 2013; Smith et al. 2013; Neudeck et al. 2011; Neudeck et al. 2011).

A great deal of research has been done on the size, shape, surface chemistry, and optical properties of metal NPs. Gold and silver NPs can be observed in the visible range and they are chemically noble and environmentally friendly. Gold NPs have an interesting model system for exploring the world of colloids. Gold NPs have a wide range of applications, ranging from charge storage systems to sensing of organic and biomolecules, and to photonic device fabrications. The ability to synthesize monodisperse metal NPs with predetermined size and shape of a specific composition is still limited and is an ongoing challenge for the scientific community. Even more limited is knowledge about NP formation mechanisms. A reason for this is the difficulty regarding real-time observation of the formation of NPs. Nucleation and growth processes are often very fast, making direct observations difficult. Furthermore, the melting point of metal nanoparticles depends upon the size. The smaller the size of the nanoparticles, the more the ratio of surface atoms will be. As the size of nanoparticles decreases, it was found that the number of surface atoms may be equal to or more than the number of inner-core atoms (Ma et al. 2011; Redel et al. 2011).

3 Distinctive Properties of Metal Nanoparticles (Lattice Constant, Melting Point, Chemical, Catalytic, Mechanical, Optical, and Biological Properties)

Nanoparticles derived from various metals exhibit unique physical and chemical properties that have been explored for innumerable applications. These exotic properties of metal NPs are significantly different from their bulk counterparts. It has been reported in the literature that with change in the size and shape of nanoparticles, the properties change (Juve et al. 2013; Von White et al. 2012).

The *lattice parameter* of metal NPs is inversely proportional to the NP size. A decrease in particle size can be easily explained by the theoretical model, which explains the reduction of the lattice constant in terms of the surface stress caused by the decrease in particle size. As the size of the nanoparticles decreases, the surface energy of the nanoparticles increases simultaneously due to the high percentage of atoms exposed to the surface. The stress induced by the increase in surface energy causes the elastic contraction of the lattice parameter (Von White et al. 2012; Yu et al. 2013; Zhao et al. 2012).

The *melting point* of metal NPs is directly proportional to their size, therefore, small-size nanoparticles may melt at a low temperature significantly below the bulk melting point (Zhao et al. 2012). It is known that a high quantity of surface energy is related to surface atoms in solid phase as these surface atoms are coordinatively unsaturated. The process of melting begins from the surface and the nanoparticle arrangement is highly stable in the liquid phase due to the decrease in surface energy. The melting point of gold metal is 1064°C, for gold NPs having the size of 11–12 nm, a melting point of 1000°C, and furthermore, for Au NPs with nanoparticles the size of 5–76 nm the melting point is up to 900°C and then it decreases more rapidly to 700°C for 2–3 nm nanoparticles (Fig. 14.3).

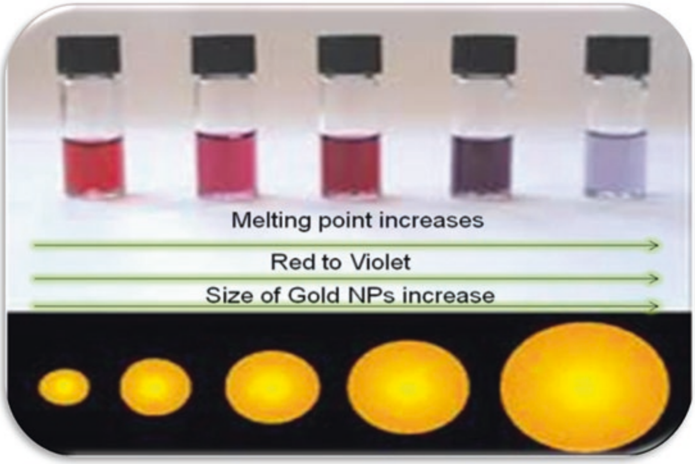


Fig. 14.3 Effect of size on the melting point of Au NPs

The *chemical properties of metal NPs* show the variation in structure that is a function of NP size and is inherently related to the changes in electronic properties of metal NPs. In general the ionization potential for small atomic clusters is more than for the corresponding material in bulk. It may exhibit obvious fluctuations and is considered a parameter of cluster size (Hao et al. 2013).

Catalysis is a well-known surface phenomenon and the catalytic efficiency of a given material is directly governed by the available surface area to carry out various organic reactions (Ai and Jiang 2012). As the particle size for a given volume of material decreases, a greater number of atoms form on the surface resulting in the increase in surface area. This increase in surface area results in the enhanced catalytic activity of metal NPs. The distinctive metal surface composition and structure, electronic pattern, and surface area render metal NPs suitable candidates for homogeneous and heterogeneous catalysts in solution and solid support, respectively. Various crystallographic facets have been established on NPs with different shapes, size, reactivity, and the like (Alonso et al. 2011; Alonso et al. 2013).

Mechanical properties or toughness are largely dependent on the simplicity of formation or the occurrence of imperfections within a material. On decreasing the size of metal NPs, the capability to sustain such defects increases and consequently the mechanical properties will be transformed significantly. Nanostructures are fairly different from bulk structures in terms of atomic structural arrangement and show different mechanical properties. Metal NPs are highly ductile, but have been found to be not elastically hard. Therefore, in consideration of the mechanical point of view, nanotechnology gives methods for improving the properties of metals (Amin et al. 2013; Boisselier et al. 2010; Chandra et al. 2013; Hao et al. 2013; Thomas et al. 2013; Sato et al. 2013; Lopez-Abarrategui et al. 2013; Ludwig et al. 2013; Shi et al. 2013).

Optical properties include silver and gold which are colored when present in nano size. All finely divided silvers are yellowish brown to black due to high maximum absorption of light by the large surface. Distinctive colors of gold and silver NPs occur because of the surface plasmon absorbance. In this phenomenon, incident light makes oscillations in the conducting electrons present on the surface of the metal NPs and thereby electromagnetic radiation gets absorbed (Juve et al. 2013; Kai et al. 2013; Kohno and Ohno 2012; Krpetic et al. 2011). Metal NPs are treated, have free electrons, and are known as plasma. Quantized plasma oscillations or collective oscillations of the free electrons are known as plasmons. On imposing an electromagnetic wave on the surface of metal NPs with a depth of less than 50 nm for Ag/Au, the electrons present on the surface play an important role in oscillation, a phenomenon known as surface plasmons (SPs). These plasmons generated in metal NPs interact with visible light, which is called surface plasmon resonance (SPR). In this phenomenon, the size of the metal NPs must be much smaller compared to the wavelength of incident lightfalls (Zhang et al. 2009; Bahshi et al. 2009; Grabtchak et al. 2013; Hartland et al. 2013; Fent et al. 2009; Goel et al. 2009; Kumar et al. 2012a, b; Springer and Homola 2012; Sunkar and Nachiyar 2013; Wang et al. 2013a, b).

4 Synthesis and Stabilization of Metal Nanoparticles

Various routes are reported in the literature for the preparation of colloidal noble metal NPs including physical, chemical, and biological routes as illustrated in Fig. 14.4. A few of the popular and significant physical methods for the preparation of metal NPs are physical vapor deposition (PVD), chemical vapor deposition (CVD), thermal decomposition, spray pyrolysis, solvated metal atom dispersion,

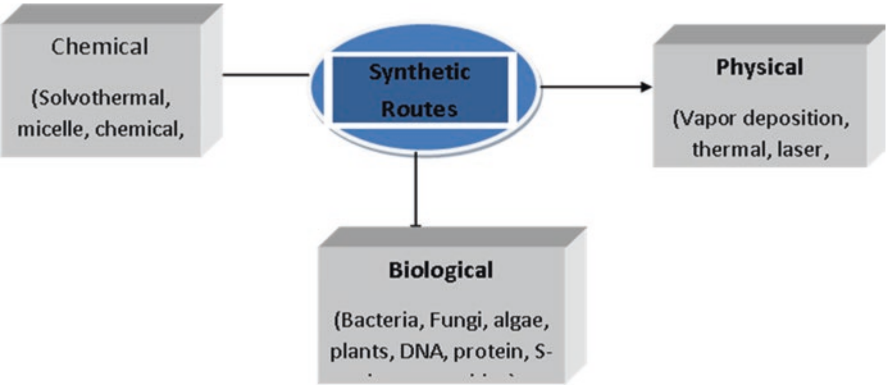


Fig. 14.4 Schematic diagram of the various well-established approaches for the fabrication of metal NPs

photo irradiation, ultrasonication, radiolysis, and laser ablation. However, chemical methods are preferred over other methods for the preparation of metal NPs because the methodology is simple in implementation. Some of the chemical methods are solvothermal synthesis, micelle-based synthesis, sol–gel method, electrochemical, galvanic replacement, and so on. Even chemical methods such as reduction methods are used for metal NPs and provide a high yield in a very short time (Sanyal and Jagirdar 2012; Ye et al. 2013; Ando et al. 2013; Li and Xia 2009).

A common aspect of metal colloid chemistry may be the methodology for the stabilization of metal NPs in a dispersing medium inasmuch as metal NPs are highly unstable due to the agglomeration of metal NPs to the bulk. In the case of a short distance between NPs, where two NPs are attracted towards each other by Van der Waals forces (and without repulsive forces to oppose these forces), the unprotected sol may be aggregated. This opposition can be done by one of the two most popular methods, electrostatic or steric stabilization. In conventional gold sols, sodium citrate has been used in the reduction of aqueous [$AuCl_4$]. The synthesized gold NPs are surrounded by an electrical double layer created due to citrate and chloride ions present in the solution. It results in Coulombic repulsive forces among the metal NPs.

The minimum potential energy at a moderate distance between metal NPs describes a stable planning of colloidal NPs and can be easily disturbed by medium effects such as heat. Therefore, if the electric potential on the double layer is more than the electrostatic repulsion, it can avoid the accumulation of NPs. It is electrically stabilized, has high ionic strength, and it may be coagulated. On the decreasing surface charge on metal NPs due to mobility of anions, the colloidal NPs collide and aggregation occurs. Hence, for metal NPs in liquid suspension (nonpolar solvents), the probability cannot be ignored because the electrostatic stabilization reinforces the stability of the sol (Hermes et al. 2012; Baek et al. 2013) (Fig. 14.5a). In another approach, metal NPs can be kept from agglomerating by the adsorption of molecules such as polymers, as well as surfactants on the surface of the metal NPs via creating a protective layer. Regarding surfactants, polymers are widely used as stabilizers in order to be effective, but must also be sufficiently solvated by the

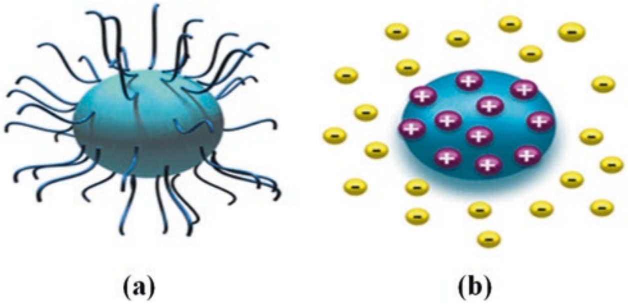


Fig. 14.5 Stabilization of metal NPs: (**a**) steric hindrance, (**b**) electrostatic charge

Table 14.1 List of various precursors, reducing agents, and stabilizers for the preparation of metal NPs

Precursors	
Metal anode	Pd, Ni, Co
Palladium chloride	$PdCl_2$
Hydrogen hexachloroplatinate (IV)	H_2PtCl_6
Potassium tetrachloroplatinate (II)	K_2PtCl_4
Silver nitrate	$AgNO_3$
Silver tetraoxylchlorate	$AgClO_4$
Chloroauric acid	$HAuCl_4$
Rhodium chloride	$RhCl_3$
Reducing Reagents	
Hydrogen	H_2
Sodium citrate	$Na_3C_6H_5O_7$
Hydroxylamine hydrochloride	$NH_4OH + HCl$
Citric acid	$C_6H_8O_7$
Carbon monoxide	CO
Phosphorus in ether	P
Methanol	CH_3OH
Hydrogen peroxide	H_2O_2
Sodium Carbonate	Na_2CO_3
Formaldehyde	HCHO
Sodium tetrahydroborate	$NaBH_4$
Stabilizers	
Poly(vinylpyrrolidone)	PVP
Polyvinyl alcohol	PVA
Polyethyleneimine	PE
Sodium polyphosphate	Na-PP
Sodium polyacrylate	Na-PA
Tetraalkylammonium halogenides	TAA-X

solvents and the polymers. They are known as amphiphilic (Fig. 14.5b). The selection of the polymer is important and done by consideration of the solubility of the precursor of metal NPs, the selection of solvent, and the skill of the polymer to stabilize the metal NPs in the colloidal state (Table 14.1). Natural polymers such as gelatin, agar, cellulose acetate, cellulose nitrate, and cyclodextrins have been used in the last decade (Susumu et al. 2011; Li et al. 2011; Shen et al. 2013; Chen et al. 2012; Krpetic et al. 2011; Cui et al. 2012; Wu et al. 2013; Conde et al. 2011). Thiele proposed a theory to determine the protective value that is a measure of the capability of a polymer to stabilize metal NPs. Various researchers reported the potential of polymers to behave as steric stabilizers. It has been observed that vinyl polymers having polar sidegroups such as poly(vinylpyrrolidone) and poly(vinyl alcohol) are very useful in this regard (Table 14.1) (Chen et al. 2012; Krpetic et al. 2011; Cui et al. 2012; Wu et al. 2013; Conde et al. 2011).

The use of copolymers provides another approach of variability to colloidal NP stabilization on varying the ratio of comonomer. For example, the use of vinylpyrrolidone– vinyl alcohol copolymers was reported for the synthesis of gold and silver NPs. Silver sols were stable only in the presence of copolymer and the size of the Ag NPs decreases with an increase in vinylpyrrolidone content in the copolymer (Hartland et al. 2013; Harne et al. 2012; Greshnykh et al. 2009; Yoon et al. 2013). It was reported in the literature that a facile method for the preparation of (co)polymer-stabilized transition metal colloids was accomplished with the "grafting-to" approach. When the reduction is performed, with $NaBH_4$ in aqueous media in the presence of an appropriate transition metal species, (co)polymer-stabilized metal nanoparticles are formed in which the size and size distribution are dependent upon the individual transition metals (Lowe et al. 2002).

Ionic liquids (ILs) are interesting solvents with a wide range of applications due to various possible cation–anion combinations, side chains, and functional groups (Hozumi et al. 2010; Kohno and Ohno 2012; Matsuyama et al. 2011; Zhao et al. 2011). There has been enormous interest by both academia and industry. Ionic liquids are explored in a broad range of fields and used in a multitude of applications, such as catalysis, electrochemistry, and nanotechnology to name a few. Because of their unusual complexity, ILs challenge concepts of common solvents for various purposes that are widely accepted. Fundamental understanding of the liquids will render new insights and will help to advance and embrace the huge range of possibilities of ILs (Wu et al. 2011; Zhang et al. 2012a, b). Metal NPs can be formed in ILs by chemical reduction of metal ions or metal complexes. The negligible vapor pressure of ILs enables them to be used in vacuum conditions and gives rise to a whole new research domain. As a result, new unexplored methods for metal nanoparticle synthesis can be developed. The advantage of the method for the synthesis of metal NPs is the cleanliness, whereby no impurities are introduced into the NPs/IL system. This is interesting for both fundamental research and for possible applications. This chapter aims to contribute to the understanding of the synthesis of metal nanoparticles via ionic liquids (Cho and Park 2011; Liu et al. 2013; Gyton et al. 2011; Li et al. 2013).

5 Synthesis of Metal Nanoparticles

5.1 Synthesis of Au NPs

Synthesis of gold nanoparticles with the help of reducing agents including sodium borohydride, ascorbic acid in the presence of cetyl-trimethyl-ammonium bromide (CTAB), and sugar (glucose, fructose, and sucrose) has been reported by researchers. One common method to synthesize gold nanoparticles is the reduction of hydrochloroauric acid $HAuCl_4$ with sodium citrate $C_6H_5O_7Na_3$. Spherical gold NPs are surrounded by a layer of citrate ions providing a negative charge to the NPs preventing aggregation by electrostatic repulsion (Ma et al. 2011; Gajan et al. 2009; Yi et al. 2010; Chen et al. 2010; Tsukamoto et al. 2012; Buonerba et al. 2011; Manju and Sreenivasan 2011; Moriggi et al. 2009; Brown et al. 2010; Sumbayev et al. 2012).

5.2 Synthesis of Ag NPs

Many chemical reduction methodologies have been commonly used to prepare silver NPs from silver nitrate or other precursors of silver. Chemical reduction methodologies that have been used to prepare silver NPs from silver nitrate have the variation selection of reducing agent, the relative amount and concentrations of reagents, temperature, mixing rate, and duration of reaction, and so on. These parameters affect the size and shape of metal nanoparticles. Greenish-yellow (λ_{max} 420 nm) colloidal silver with particle sizes from 40 to 60 nm has been reported from the reduction of silver nitrate with sodium citrate. Silver colloids have been described as brownish or yellow-green, with absorption maxima at 400 nm and particle size of about 10 nm. A method reported that the use of both sodium citrate and sodium borohydride under boiling conditions gives a greenish colloid absorbing at 438 nm with particle size 60–80 nm. Clear yellow or greenish-yellow of colloidal silver can be obtained depending upon the duration of the reaction with ice-cold sodium borohydride (Jacob et al. 2011; Ponarulselvam et al. 2013; Zhang et al. 2008).

5.3 Synthesis of Cu NPs

In contrast to noble metals (silver and gold), synthesis of Cu NPs is more challenging because Cu NPs are highly unstable in aqueous solution as they become oxidized in the presence of air. However, Cu NPs cost significantly much less than silver and gold NPs. Therefore, Cu NPs are economically attractive for academicians and researchers. When Cu NPs are exposed to air, oxidation on the surface of metal NPs occurs frequently and ultimately aggregation occurs in a very short time. To avoid oxidation of metal NPs, the reduction procedures are usually carried out under an inert atmosphere of argon or nitrogen, in organic solvents, or via microemulsion systems, and in the presence of protective polymers, surfactants, molecules, and the like. In the chemical reduction method, copper salt is reduced using a strong as well as weak reducing agent, for example, sodium borohydride, hydrazine (N_2H_4), ascorbate, polyol, isopropyl alcohol with cetyltrimethylammonium bromide (CTAB), or glucose. Most commonly used methods are coprecipitation, thermal decomposition, hydrothermal synthesis, microemulsion, and sonochemical, and other synthetic routes can also be used for the synthesis of Cu NPs (Yu et al. 2013; Kwon et al. 2011; You et al. 2012).

5.4 Role of Ionic Liquid in the Synthesis of MNPs

The huge interest in ionic liquids only took off in the last two decades. The first synthesized room-temperature ionic liquid was ethylammonium nitrate, $[EtNH_3][NO_3]$, with a melting temperature of 12°C. The literature revealed that the arbitrary border between low melting organic salts and high melting inorganic salts was 100°C. At that time, not much interest was shown in these curious liquids. Since then, a broad range of stable ILs has been developed for various applications. This exponential development gave ionic liquids a large boost and made them popular in industry and academia. New ILs having different cations such as imidazolium, pyridinium, pyrrolidinium, piperidinium, phosphonium, and more hydrophobic anions including bis(trifluoromethylsulfonyl)imide and trifluoromethane-sulfonate were developed. Currently, more than one million different anion–cation combinations are possible (Gyton et al. 2011; Li et al. 2013; Du et al. 2011; Mandal and Nandi 2013; Kumar et al. 2012a, b; Lin et al. 2013; Tietze et al. 2012; Zang et al. 2010; Venkata et al. 2012).

A typical IL has a bulky, asymmetrical organic cation and an organic or inorganic anion, such as methanesulfonate, nitrate, or triflate, among others, and their strong hydrogen bonds increase the melting point. The imidazolium cation carries acidic hydrogen on the C_2 position, which forms strong hydrogen bonds with halide anions (Kumar et al. 2012a, b; Lin et al. 2013; Tietze et al. 2012; Zang et al. 2010; Venkata et al. 2012). For this reason, ILs having halide as an anion have higher melting points than $[BF4]^-$. Furthermore, the conformational freedom in the ions has an influence. Anion Tf_2N has *trans*- and *cis*-conformers. The *trans*-conformer seems to predominate whereas in crystal structures the *cis*-conformer predominates. This may lower their viscosities and melting points. The size of the chain length plays a very important role. The chain lengths need to be long enough (4–6 carbon atoms) to undo the symmetry from the core, that is, cation. But if the chains become too long, dispersive interactions take place between the alkyl chains that will increase the melting point. Because of the many possible cation–anion combinations, the variations in side chains and the possibility of incorporating functional groups in the ions, make them popular as "designer solvents" (Kohno and Ohno 2012; Matsuyama et al. 2011; Cho et al. 2011; Shim et al. 2011). Ionic liquids have been explored in many applications including solvents in chemical synthesis, catalysis, electrochemistry, and nanotechnology. Because of their unusual complexity, ILs challenge concepts that are widely accepted in common solvents. Fundamental understanding of these ILs will render new insights and will help to advance and embrace a huge range of applications (Gyton et al. 2011; Li et al. 2013; Zhang et al. 2011a, b, c; Allen et al. 2013).

The uncommon behavior of their intermolecular interactions advances the molecular-based conclusion and also creates many controversies and speculations. Comparison of ionic liquids with common solvents is not as easy as it might seem. After different combinations, some will be hydrophobic and others hydrophilic with relatively low or high viscosity. It was claimed that ILs are nonflammable, nonvolatile, intrinsically “green,” and thermally and electrochemically stable. IL challenges are widely accepted in aqueous and organic solvents, for example, electric permittivity. Most of the ILs have a wide liquidus range that can be as broad as a few hundred Kelvin. The liquidus range of $[C_4C_1Im][Tf_2N]$ is 184–723 K. This is large compared to common organic solvents such as hexane. The vapor pressure of ILs is very low and cannot be detected at room temperature (10^{-15} bar at 298 K for $[C_4C_1Im][PF_6]$ compared with 3×10^{-2} bar at 298 K for H_2O). This behavior of ILs makes them useful as greener alternatives for common volatile organic solvents and even enables their use in vacuum systems. The vapor pressure of IL can be detected at high temperature. At 500 K, the vapor pressure is on the order of magnitude of 10^{-8} bar.

As mentioned above, both ionic liquids and NPs are currently hot research topics as noted by the last decade’s output regarding them. Needless to say, the combination of these two topics is also currently an active field of research. There have been a total of more than 100,000 publications on this subject (Greshnykh et al. 2009; Lee et al. 2010; Chakraborti and Roy 2009).

Researchers emphasized the stabilization mechanism of the metal nanoparticles that until recently had not been clear. It was stated in the literature that metal NPs prepared in ILs were stable and did not require any stabilizing agent. The interactions in simple salts between the net charges of the ions are controlled by long-range Coulombic forces. In ILs, the bulky asymmetric molecular ions and their delocalized charge distribution diminish the Coulombic forces. Researchers claimed that metal NP suspensions in ILs are stable without any additional stabilizers. Metal NPs can be formed in ILs by chemical reduction of metal ions or metal complexes. In these methodologies, by-products and impurities can enter the IL during synthesis which will have an influence on the stability and possible applications, that is, catalysis. (Zhao and Bond 2009; Ji et al. 2013)

5.5 *Characterization of Metal Nanoparticles*

Metal NPs are characterized by most commonly used techniques including transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), X-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), infrared spectroscopy (IR), UV-visible spectroscopy, and nuclear magnetic resonance (NMR). SEM provides high magnification images for detailed study of surface structures. TEM is valuable for studying thin sections of samples to examine internal structures. It also provides useful data on the size and shape distribution of the nanoparticles. Both techniques use an electron beam for imaging. EDX and XPS are used to study the elemental analysis of the particles. Powder X-ray diffraction is an important technique providing patterns for crystalline and amorphous solids that can be used for identification. IR and NMR spectra can provide evidence for functionalization of metal NPs (Table 14.2, Usman et al. 2012 Heuer-Jungemann et al. 2013, Piret et al. 2012, Pham et al. 2012, Wang et al. 2013a,b).

Table 14.2 List of techniques used to characterize metal NPs

Method	Name	Primary Beam	Detected Signal	Energy Range	Uses
Reflection spectrometry		V/UV photon	V/UV photon		Film thickness *t*, refractive index *n*
Ellipsometry		Vis photon	Vis photon		Film thickness, index
Low-energy e^- diffraction	LEED	e^-	e^-	20–200 eV	Surface structure
Scanning electron microscopy	SEM	e^-	e^-	~0.3–50 keV	Surface morphology
Electron microprobe	EDX	e^-	X-ray	1–30 keV	Surface composition
Transmission electron microscopy	TEM	e^-	e^-	50–400 keV	High-resolution atomic structure
Secondary ion mass spectroscopy	SIMS	ion	Ion	1–15 keV	Composition versus depth
X-ray diffraction	XRD	X-ray	X-ray	>1 keV	Crystal structure, composition
X-ray fluorescence	XRF	X-ray	X-ray	>1 keV	Composition near surface
XPS	XPS	X-ray	e^-	>1 keV	Surface composition
Atomic force microscopy	AFM	solid probe tip	probe height		Surface topography
Vibrating sample magnetometer	VSM	RF signal	RF response		Magnetization, coercivity, hysteresis

5.6 Shape of Particles

Metal NPs of various shapes and sizes have different plasmon properties. For example, the absorption spectrum of spherical copper NPs, 10 nm in size, produced via treating copper dodecyl sulfate ($Cu(DS)_2$) with sodium borohydride as the reducing agent, has a plasmon peak at 558 nm. The maximum obtained in the absorption spectrum for Cu NPs is 564 nm (Santiago-Rodriguez et al. 2013; Habibi et al. 2011; Sankar et al. 2012).

6 Applications of Metal NPs in Drug Delivery, Electrochemistry, and Catalysis

There are several applications of metal NPs as mentioned in Fig. 14.6 but in this section, only a few of them are explained.

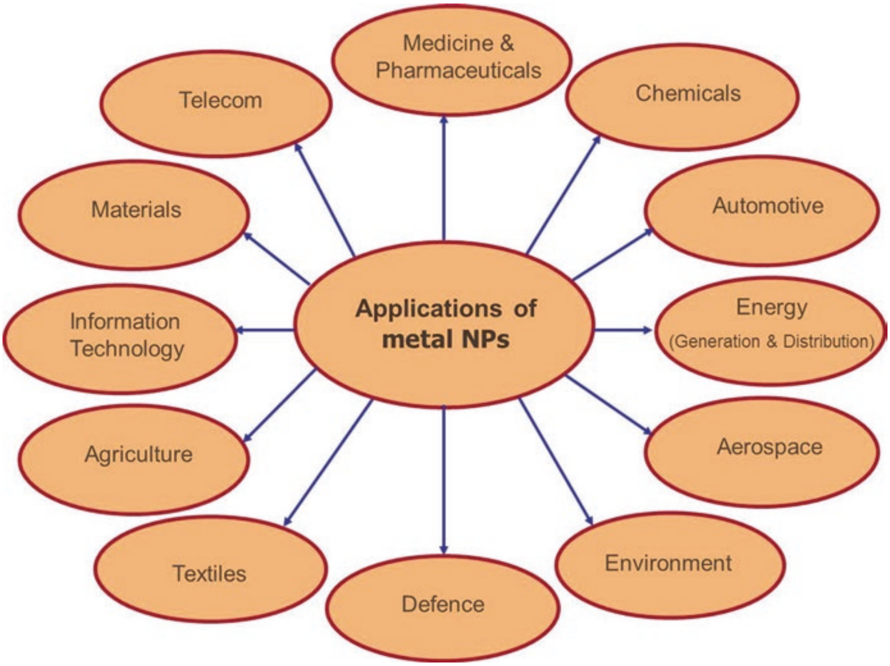


Fig. 14.6 Applications of metal NPs in various disciplines

6.1 Role of Metal NPs in Composite Materials

A common approach for the preparation of metal nanoparticle–polymer composites involves mechanical dispersion of premade nanoparticles by reduction of metal salts. Another technique involves polymerizing the matrix around a metal nanocore by using chemically compatible polymers. The photogeneration of silver nanoparticles in PVA matrix with the silver ions reduced using the polymeric benzophenone ketyl radicals was reported in the literature (Sakai et al. 2013; Sundaravadivelan et al. 2012; Samundeeswari et al. 2012; Ahmad et al. 2011). Metal NPs are prone to aggregation because of their high surface free energy and oxidation by air or moisture. This factor can change the properties of the composite materials where the NPs are embedded dramatically. Therefore, it is important to explore new methods for preparation of stabilized metal nanoparticle–polymeric composites (Chen and Liu 2011; Liu et al. 2011; Ojea-Jimenez et al. 2012; Pineider et al. 2013; Lopez et al. 2013).

6.2 Biomedical Applications of Metal NPs

From their first use of metal NPs in ancient cultures in different applications, gold NPs have continued to be used in various modern medical treatments. The claims for the medical welfare of gold NPs date back several centuries. Metal NPs are related to the excellent biocompatibility of gold as a material (Sakai et al. 2013; Sundaravadivelan et al. 2012; Samundeeswari et al. 2012; Ahmad et al. 2011). Recently, interesting developments have been observed in the use of gold NPs for the delivery of various vaccines and pharmaceuticals into the human body. Gold NPs have the compulsory adsorptive features and biocompatibility for such uses. Au NPs are used in the finding of deadly poisons, in the progress of gold-coated lasers to aid in skin rejuvenation, and in the testing of vaccines. Au NPs are also used in biomedical analyses. Gold NPs are a cheap, not reusable, membrane-based technique that gives visual proof of the availability of an analyte in a liquid sample. Au NPs have superiority due to their stability, sensitivity, and reproducibility. Gold NPs have the ability to be layered with particular organic ligands, DNA, which makes it feasible to engineer nanostructures and to modify properties for different applications. The ability to control the size of metal NPs and ligands enables fine-tuning for selective sensing applications. Pregnancy testing kits based on gold NPs are already on the market. Au NPs have been used to locate tumors and, when exposed to X-rays, kill the tumor. It appears to be a capable technique for cancer treatment, and the use of Au NPs on silica nanoshells altered specifically for use in blood diagnostics and even the use of gold NPs to destroy unpleasant odors in socks are examples of possible medical applications (Huang et al. 2011; Wang et al. 2012; Stratakis and Garcia 2012; Alani et al. 2012; Sriram et al. 2012; Venkatpurwar and Pokharkar 2011; Zhang et al. 2011a, b, c).

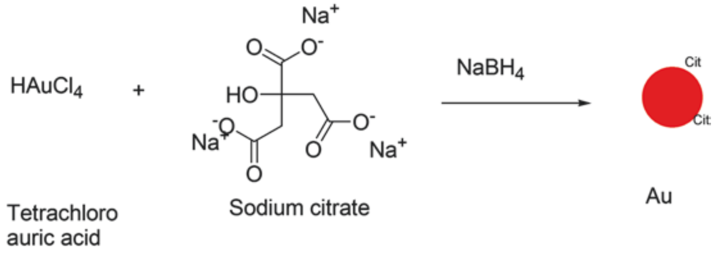
Considering its extensive past, it is a little astonishing that the chemistry of Au NPs is not well established compared to other valuable metals and, therefore, its applications have been held up. However, its chemistry is now being well researched and interesting behaviors are developing such as luminescence and antitumor activity (Fent et al. 2009; Goel et al. 2009; Kumar et al. 2012a, b; Springer and Homola 2012; Sunkar and Nachiyar 2013). Au NPs are also objects of curiosity in optoelectronics, nonlinear optical materials, sensors, and devices. Gold compounds have been used for the treatment of arthritis for a long time. Recent research has shown that some compounds display antitumor activity, making them a potentially proper candidate for the treatment of cancer. Au NPs are biocompatible and have antimicrobial properties, increasing awareness of the use of Au NPs in medical and related fields.

6.3 Electrochemical Applications

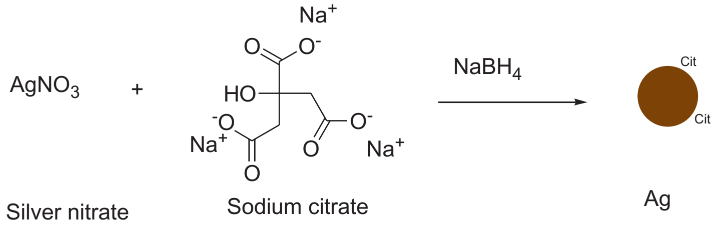
Exhaustive research has been carried out on the preparation of "synthetic metals" due to their excellent properties such as electronic and optical among others. Electrical conductivity is attained in the conjugated polymers via delocalization of the π-electrons that permit the charge mobility along the back of the polymer chain. Synthesis of conducting polymers has been achieved by oxidizing or reducing the process either through chemical doping or electrochemical doping. Polyaniline (PANI) has considerable interest due to its electrical and optical properties that can be varied by oxidation and protonation of the amino group. Polyaniline has garnered plenty of attention, because of both necessary interest and potential applications in the electrochromic devices, energy storage and conversion systems, sensor anticorrosive coatings, and electrocatalysts. PANI showed a broad range of electronic structure change and depends on the doping.

6.4 Metal NPs as Potent Catalysts in Organic Synthesis

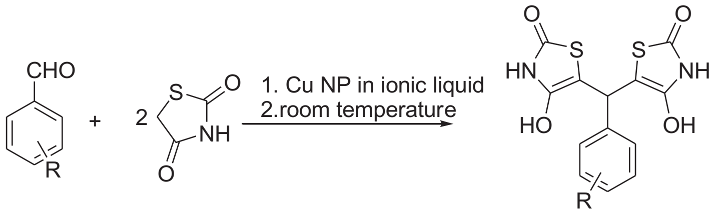
The small size of metal NPs has attracted the attention of researchers for their potential in catalysis. They have high surface tension and it makes their surface atoms very active. The field of nanocatalysis has been very active in both heterogeneous catalysis (NPs are supported on solid surfaces) and homogeneous catalysis (colloidal NPs). They give a high yield and decrease the reaction duration, due to the good dispersion and high surface area of metal NPs. Metal NPs can be recycled and this leads to reduction in the cost of the catalyst. Gold NPs were used in various applications and many applications have yet to be reviewed. Catalysts drive many reactions, as they have the ability to lower the activation energy of the reaction and therefore an increase in the reaction rate, and also give the desired products in high yields. The use of metal nanoparticles as catalysts has increased exponentially in the last two decades due to their potential catalytic behavior. The possibility of using small-range metal nanoparticles having various properties for different shapes makes them very attractive. Catalysts are important to the chemical industry, with over 90% of all the chemicals manufactured (Ai and Jiang 2012; Huang et al. 2011; Wang et al. 2012; Stratakis and Garcia 2012). Among metals, gold, silver, and copper have traditionally been viewed as exceptions in comparison with other precious metals. In the twenty-first century, researchers reported that gold nanoparticles were very active catalysts for carrying out various reactions. The key to the preparation of gold nanoparticles of around 5 nm in diameter is using oxide-supported material. A major attribute is that gold NPs are catalytically active at low temperatures. It has been reported in the literature that the oxidation of carbon monoxide is important in automotive pollution control and this reaction was catalyzed by gold nanoparticles at temperatures of −77°C. Gold nanoparticles can also catalyze a range of reactions, including oxidation, hydrogenation, coupling, condensation, and so on. Their properties allow them to be utilized in new applications that are not possible with other catalytic systems, making the commercial future of gold NPs very promising. The bulk amount of these NPs is concerned with chemical processing, but pollution can be controlled where the catalyst is manufactured, and their application as fuel cells is also significant (Ai and Jiang 2012). The small size of metal NPs increases their surface area and therefore they can also be recycled. For condensation reactions, Schemes 14.3–14.10 describe the reaction between thiazolidine-2,4-dione and aryl aldehydes to prepare biologically potent compounds using copper nanoparticles as catalysts dispersed in ionic liquid.



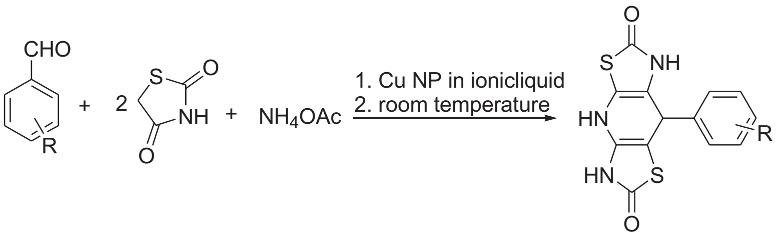
Scheme 14.1 Synthesis of Au NPs using citric acid



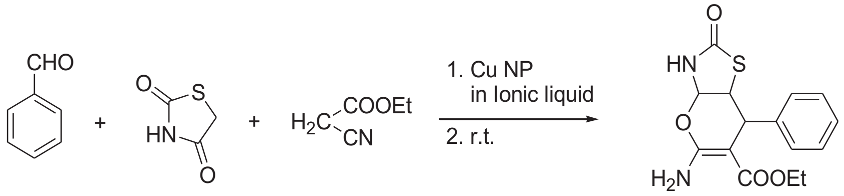
Scheme 14.2 Synthesis of Ag NPs using citric acid



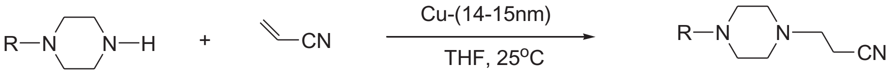
Scheme 14.3 Condensation reaction type 1



Scheme 14.4 Condensation reaction in one pot three-component synthesis

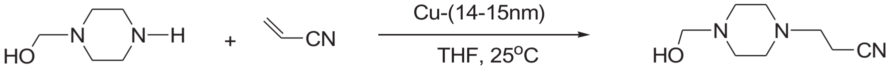


Scheme 14.5 Condensation reaction in one pot three-component synthesis

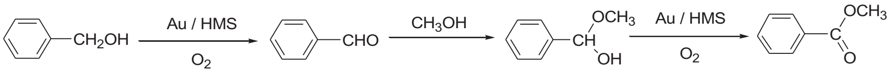


R=H, Ph, $PhCH_2$, 2-$MeOC_6H_4$, 2-MeC_6H_4, 4-MeC_6H_4

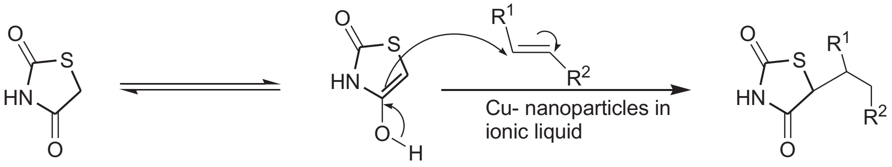
Scheme 14.6 Addition reaction between secondary amine and acrylonitrile



Scheme 14.7 Addition reaction between substituted piperizine with acrylonitrile



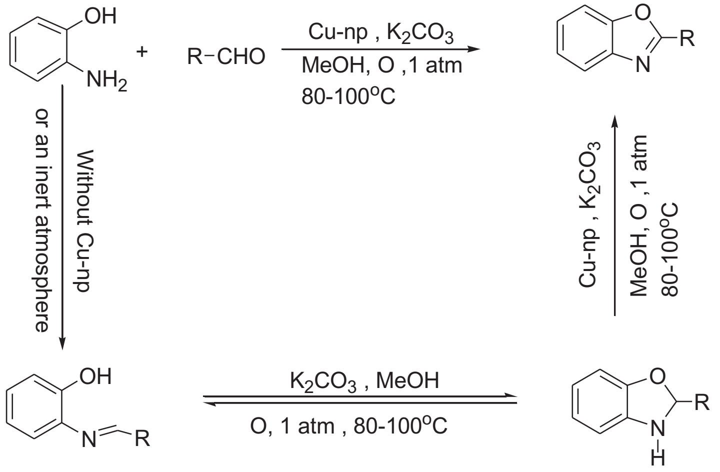
Scheme 14.8 Au NP-catalyzed oxidation reaction



Scheme 14.9 Cu NP-catalyzed carba-michael addition reaction

6.5 Biocompatibility

One of the important aspects in the study of nanoscience and nanotechnology is to assess the cytotoxicity levels of metal NPs in living systems. Some attempts have been made towards this aim and studies have been undertaken to address these issues with metal nanoparticles. Gold nanoparticles have especially been extensively studied for their cytotoxicity effects. Au NPs have been exploited for various



Scheme 14.10 Cu NPs catalyzed synthesis of benzooxazole

applications in the field of biomedical science (Polleux et al. 2011; La Torre et al. 2012; Aili et al. 2008). Researchers have studied the uptake of gold nanoparticles by mammalian cells by pinocytosis and their compartmentalization in lysosomal bodies. They have shown that chemically synthesized gold nanoparticles do not show any visible cytotoxicity to human cell lines until a concentration of 100 μM. They reported up to 85% of cell viability even after 72 h of exposure of human cells to the gold nanoparticles. These reports clearly suggest that the gold nanoparticles are biocompatible and thus are useful candidates for various applications in nanomedicine (Zhang et al. 2013; Lamsal et al. 2012).

6.6 *Other Applications of Silver, Gold, and Copper Nanoparticles*

On comparison with organic dyes, the light absorption by Au and Ag NPs is about 5–6 orders of magnitude higher. It implies that a metal NP is over 10^6-fold more and changes it into thermal energy. This results in these metal NPs being used in thermal therapy and optical imaging of tumors (Eck et al. 2010). Silver and gold nanostructures were thoroughly investigated as important colorimetric sensors because of their large extinction coefficient. The sensitivity of optical sensors is mainly dependent on the size and shape of metal NPs, and the surrounding medium. Furthermore, an increase in the refractive index frequently causes the surface plasmon peak to move towards the longer wavelength (Wang et al. 2013a, b; Meli and Green 2008).

Silver has gained more consideration due to the high bacterial resistance to antibiotics caused by overuse of this pharmaceutical. Silver NPs can be used in medicine to decrease infections in burn treatments, to avoid bacterial colonization on dental and stainless steel materials, to eliminate microorganisms on textile fabrics, and they can also be used for water treatment (Jacob et al. 2011; Ponarulselvam et al. 2013).

7 Conclusion

In the process of the synthesis and stabilization of metal NPs, ionic liquids play a very crucial role. Metal NPs can be formed in ILs by chemical reduction of metal ions or metal complexes. The advantage of the method for the synthesis of metal nanoparticles is the cleanliness, whereby no impurities are introduced in the NPs/IL system. High yields are obtained using metal NPs in ionic liquid as catalytic media for a short duration. This methodology is much cheaper than the others and also does not require high temperature. Hence, ILs serve as a green and efficient method. The negligible vapor pressure of ILs enables them to be used in vacuum conditions and gives rise to a whole new research domain.

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Chapter 15
Green Synthesis of Nanoparticles: An Emerging Phytotechnology

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Abstract The metallic particles whose size ranges between 1 and 100 nm in any one of the dimensions are termed as nanoparticles (NPs). Nanoparticles pose a great interest to chemists, physicists, biologists, and engineers for the development of new generation nanodevices, electronics, catalysis, chemistry, energy, and medicine. "Green synthesis" refers to the use of green material (i.e., plants) for the synthesis of any material. Nanoparticles can be synthesized by various ways, viz., laser ablation, gamma irradiation, electron irradiation, chemical reduction, photochemical methods, and microwave processing, which however produce hazardous chemicals as by-product. Green synthesis of NPs is presently becoming popular due to its eco-friendly and cost-effective approach with no use of any toxic chemicals. At the same time, synthesis of NPs through biological methods is not as easy and lots of open challenges are there. Green synthesis of NPs involves the use of water in closed reactors, which is a nontoxic solvent, and applies green techniques like ultrasound and microwave. The reagents used for the synthesis of NPs also include natural compounds such as sugars, vitamins, biodegradable compounds, and microbes. Among these reagents, plant-based materials are the most suitable

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candidates for large-scale synthesis of NPs. Several plants and their compounds are being used for green synthesis of NPs. This chapter focuses on the range of plants being used for various NP biosynthesis and also gives a view of various applications of NPs in diverse fields.

1 Introduction

Nanotechnology is encompassing all spheres of human life day by day. Nanoparticles (NPs) are gaining importance for their applications in daily life such as medicine, food and water quality, defense, security, electronics, energy, and information storage (Prasad 2004). Hence, there is an increasing commercial demand for NPs for applications in areas such as electronics, catalysis, chemistry, energy, and medicine.

The size of nanoparticles ranges between 1 and 100 nm and this brings change not only in their physical properties (e.g., size, distribution, and morphology) but also in chemical properties (e.g., higher specific surface areas, higher mechanical strengths, lower melting points, optical and magnetic properties) than their corresponding bulk metals. The optical property of NPs has been an area of prime interest for scientists. Since ancient times, NPs were used for the glass painting in church for producing different colors. Gold NPs have a characteristic wine red color, silver NPs are yellowish gray, and platinum and palladium NPs are black.

Several techniques have been successfully applied to produce NPs. However, these techniques are expensive, viz., laser ablation, ultrasonic fields, or use of toxic chemicals during the process, e.g., aerosol-based methods and photochemical reduction. A critical development needed in the field of nanotechnology is the standardization of a process for synthesis of NPs that uses eco-friendly processes. Over the traditional methods of NP synthesis mentioned earlier, nowadays, the reagents, enzymes, or compounds of biological origin such as vitamins, sugars, and microbes are getting popular (Kharissova et al. 2013). Green synthesis of NPs through biological methods has demonstrated certain benefits like slower kinetics of the reaction that allows the use to effectively control the growth and stabilization of NP crystal. These advantages have motivated an increase in research on analyzing various synthesis routes to effectively control shape and size of NPs (Singh et al. 2011). Nonetheless, these syntheses too have certain limitations and have led successfully to the fabrication of limited number of inorganic NPs (mainly metal NPs).

Plant-based materials appear to be the best choice as they offer opportunities for large-scale ‘biosynthesis’ of NPs (Iravani 2011). Plant parts ranging from root to leaf, and stem to seed have been used for the purpose and the list of plants and their extracts used in the purpose is quite large. Plant extracts and plant-based compounds are appropriate when the need to synthesize NPs for medical applications must be free of any toxic contaminants. The plant extract-based synthesis can also provide NPs of desired size and morphology. The field of green synthesis of NPs is certainly a burgeoning one. An approximate estimate of number of papers in the area (based on Pubmed) suggests increase from 60 in 2006 to nearly 2638 in 2016. The present review provides a brief overview of process of NPs by biological methods and a brief description of application of NPs.

2 Biosynthetic Process of Nanoparticles

Nanoparticle biosynthesis is an open challenge for biologists. Though there are several physical and chemical methods for NPs synthesis, biological methods have been the focus of attention. The advantage of biological methods over others is its nonhazardous and environment friendly nature. Plant-based biosynthesis is an easily operable process that can be utilized even for the large-scale production of NPs.

There are two basic approaches for NPs synthesis: “top down” and “bottom up.” In “top-down” approach, NPs are prepared by decreasing the size of large pieces of a material until only a NP remains through mechanical and chemical processes, viz., lithographic techniques, etching, grinding. In bottom-up approach, biological synthesis of NPs is prominent, where NP is “grown” from simple molecule reaction precursors. Size and shape of NPs can be controlled through variation in precursor’s concentrations, reaction conditions (temperature, pH, etc.), functionalizing the NP surface, using templates, etc. Plants have the ability to hyperaccumulate and biologically reduce metallic ions (Kulkarni and Muddapur 2014), which makes them suitable for environment friendly green synthesis of nanoparticles. Green synthesis of NPs is a cost-effective process and is thus a preferable process to adopt when large-scale synthesis of NPs is required. Innumerable types of NPs having different shape and size were synthesized with various parts of plants in diverse physical conditions. Table 15.1 presents list of various work done on biological synthesis of NPs. Fig. 15.1 represents the in vitro and in vivo approaches used in biosynthesis of nanoparticles

2.1 In Vitro (Lab) *Biosynthesis of Nanoparticles*

Plants take up metal ions from their surroundings through transporters. Inside the plants, these metal ions may be transformed into as smaller nanosize structures (Singh et al. 2011). Thus, plants are the biological factories that cannot only recover metals, viz., from industrial wastes but also capable of preventing and cleaning environmental pollution.

Different types and parts of plants having different biochemical processing capabilities synthesize a particular type of NP due to their specific enzyme activities and intrinsic metabolic processes. Hence, investigations are made to develop a NP with specific properties, well-defined size and morphology with a specific plant or other biological source.

Table 15.1 Different nanoparticles biosynthesis using various in vitro plants extract

S. No.	Plant name	Plants	Extract part	Metal name	Physical condition	Time of synthesis	Size of particle	Reference
1	*Abutilon indicum*		Leaves	Ag	–	15 min	7–17 nm	Kumar et al. (2015)
2	*Acacia*		Gum	Silver	120 °C And pressure of 15 Psi	2 min	–	Venkatesham et al. (2012)
3	*Acalypha indica*		leaf extract	Ag	–	30 min	20–30 nm	Krishnaraj et al. (2010)
4	*Acalypha indica*		Leaves	Ag	37 °C under static condition	Development of yellowish brown color	0.5 nm	Kumarasamyraja and Jeganathan (2013)
5	*Acorus calamus*		Aqueous rhizome extract	Ag	room temperature	24 h	31.83 nm	Nakkala et al. (2014)

6	*Albizia lebbeck*		Leaf	Cu	Dark at 37 °C	color changes from pale yellow to brown	<100 nm	Jayakumarai et al. (2015)
7	*Allium sativum*		Bulb	Silver	Under bright sunlight	15 min	7.3 ± 4.4 nm	Rastogi and Arunachalam (2011)
8	*Allium sativum (garlic clove)*		Clove extract	Ag	–	30 min	4–22 nm; average diameter of 12 nm	Ahamed et al. (2011)
9	*Aloe vera*		Leaf	Cu	100–120 °C	10 min	20–30 nm	Vijay Kumar et al. (2015)
10	*Alternanthera dentate*		Leaves	Ag	–	–	31.83 nm	Nakkala et al. (2014)
11	*Anacardium occidentale*		Aqueous extract and dried powder of leaf	Au/Ag	Room temp and boiling	–	~6 nm and 17 nm	Sheny et al. (2011)

(continued)

Table 15.1 (continued)

S. No.	Plant name	Plants	Extract part	Metal name	Physical condition	Time of synthesis	Size of particle	Reference
12	*Anacardium occidentale*		Essential oils	Gold	Room temperature	–	36 nm	Sheny et al. (2012)
13	*Argemone mexicana*		Leaf extract	Ag	Boiling	–	30 nm	Singh et al. (2011)
14	*Boerhaavia diffusa*		Aqueous extract of *B. diffusa* powder	Ag	100 °C with continuous stirring	24 h	25 nm	Kumar et al. (2014)
15	*Boswellia ovalifoliolata*		Dried stem bark	Ag	500–950 °C	Until change in the color	30–40 nm	Ankanna et al. (2010)
16	*Boswellia serrata*		Gum	Ag	–	–	7.5 ± 3.8 nm.	Kora et al. (2012)

17	*Brassica rapa*		Leaves	Ag	–	–	16.4 nm diameter of ca. 39.5 nm	Narayanan and Park (2014)
18	*Breyniar-hamnoides*		Stem extract	Au, Ag	Au under dark conditions	~7 min for AuNPs 2 h for AgNPs	~25 nm for AuNPs 64 nm for AgNPs	Gangula et al. (2011)
19	*Calotropis gigantea*		leaf extract	Zinc nitrate	Boiled	Until solution reduced to a deep yellow colored paste.	30–35 nm	Vidya et al. (2013)
20	*Calotropis gigantea*		Leaf	Cu	Boiled	60 min	20 nm	Sharma et al. (2015)
21	*Calotropis procera*		Whole Plant	Ag	Room temp	24 h	19–45 nm	Gondwal and Pant (2013)

(continued)

Table 15.1 (continued)

S. No.	Plant name	Plants	Extract part	Metal name	Physical condition	Time of synthesis	Size of particle	Reference
22	*Caulerpa peltata, Valencia,* and *Sargassum myriocystum*	  	Whole plant	Zinc nitrate	Heating (80 °C) and stirring	5–10 min and 1 h	300 nm and 600 nm.	Nagarajan and Kuppusamy (2013)
23	*Centella asiatica*		Leaves	Ag	Room temperature	–	30–50 nm	Rout et al. (2013)
24	*Cinnamomum camphora*		Leaf broth	Pd	–	–	3.2–6.0 nm	Yang et al. (2010)

25	*Citrus medica Linn. (Idilimbu)*		Fruit juice	Cu	Boiling	15–20 min	10–60 nm	Shende et al. (2015)
26	*Citrus sinensis*		Peel extract	Ag	Room temperature (25 °C) and 60 °C	–	35 and 10 nm	Kaviya et al. (2011)
27	*Cochlospermum gossypium*		Gum	Ag, Au, Platinum	Ag: 45 °C Au: 75 °C at Pt: autoclaved at 15 psi	Ag, Au:250 rpm for 1 h Pt: 15 min	Ag, Au: 5.5 ± 2.5 nm and 7.8 ± 2.3 nm Pt: 2.4 ± 0.7 nm	Vinod et al. (2011)
28	*Cocos nucifera*		Inflorescence	Ag	–	–	22 nm	Mariselvam et al. (2014)
29	*Eucalyptus hybrid*		Leaf	Ag	Room temp. (dark)	–	50–150 nm	Dubey et al. (2009)
30	*Euphorbia milii*		Latex	Silver	Xenon lamp followed by ultrashort laser pulses	–	10–50 nm	de Matos et al. (2011)

(continued)

Table 15.1 (continued)

S. No.	Plant name	Plants	Extract part	Metal name	Physical condition	Time of synthesis	Size of particle	Reference
31	*Ficus carica*		Leaves	Ag	–	–	13 nm	Ulug et al. (2015)
32	*Gloriosa superba L.*		Plant extract	Cu	Constant stirring	10 min	5–10 nm	Naika et al. (2015)
33	*Jatropha curcas*		Latex	Silver	Heated at 85 °C with constant stirring for	4 h	Radius 10–20 nm	Bar et al. (2009)
34	*Malva sylvestris*		Leaf	Cu	Room temperature, followed by 80 °C heat for 2 minutes	until color change	5–30 nm	Awwad et al. (2015)
35	*Melia dubia*		Leaves	Ag	–	–	35 nm	Kathiravan et al. (2014)
36	*Moringa oleifera*		Aqueous leaves extract	Ag	60–80 °C	20 min	57 nm	Prasad and Elumalai (2011)

37	*Nelumbo nucifera (lotus)*		Aqueous leaf extract	Ag	–	24 h	25–80 nm	Kumar et al. (2011)
38	*Nyctanthes arbortristis*		Leaf	Titanium	Stirring	4 h	100–150 nm	Sundrarajan and Gowri (2011)
39	*Ocimum sanctum*		Leaf	Ag	Boiling	5 min	3–20 nm	Mallikarjuna et al. (2011)
40	*Phyllanthus amarus*		Secondary metabolites	Ag	–	–	10–20 nm	Ahamed et al. (2011)
41	*Pistacia atlantica*		seed aqueous extract	Ag	–	35 min	27 nm	Sadeghi et al. (2015)
42	*Pogostemon benghalensis*		Leaves	Ag	Shaking and boiling	Yellowish black color appearance	>80 nm	Gogoi (2013)
43	*Rhododedendron dauricam*			Ag	–	–	25–40 nm	Mittal et al. (2012)

(continued)

Table 15.1 (continued)

S. No.	Plant name	Plants	Extract part	Metal name	Physical condition	Time of synthesis	Size of particle	Reference
44	*Rubus glaucus*		Leaf and fruit	Cu	Stirred at 75–80 °C	6 h	43.3 nm	Kumar et al. (2015)
45	*S. vulgare*		–	Ag	Stirring at room temperature	3 h	5–15 nm	Govindaraju et al. (2015)
46	*Seaweed (Sargassum muticum)*		Whole plant	Iron oxide	Stirring at room temperature	60 min	18 ± 4 nm	Mahdavi et al. (2013)
47	*Sesuvium portulacastrum*		Tissue culture derived callus and leaf	Silver	–	–	5–20 nm	Nabikhan et al. (2010)
48	*Swietenia mahogani (mahogany)*		Aqueous mahogany leaf extract	Ag/Au bimetallic	Continuously stirred at ~40 °C	30 min	50 nm Ag at pH 7; 20 nm Ag and 100 nm Au at pH 12.5; 50 nm Au/Ag bimetallic at pH 12.5	Mondal et al. (2011)

49	*Tabernaemontana divaricata*		Leaf	Cu	–	–	48 nm	Sivaraj et al. (2014)
50	*Tea extract*		Dried green tea leaves	Ag	Vigorously stirring 25 °C	120 min	20–90 nm	Sun et al. (2014)
51	*Terminalia arjuna*		Leaf	Se	Shaking	72 h	10–80 nm	Prasad and Selvaraj (2014)
52	*Thevetia peruviana*		Plant extracts	Ag	–	–	10–30 nm	Rupiasih et al. (2015)
53	*Tinospora cordifolia*		Water extract	Cu	Solution combustion method	–	6–8 nm	Nethravathi et al. (2015)
54	*Trachyspermum copticum*		Extract	Ag	Constant mixing in a rotary shaker at 120 rpm at 28 °C	35 min	6–50 nm	Vijayaraghavan et al. (2012)

(continued)

Table 15.1 (continued)

S. No.	Plant name	Plants	Extract part	Metal name	Physical condition	Time of synthesis	Size of particle	Reference
55	*Tribulus terrestris*		Dried fruit	Ag	Dark room	Until color changes	16–28 nm	Gopinath et al. (2012)
56	*Vitex negundo*			Ag	–	–	10–30 nm	Shabanzadeh et al. (2015)
57	*Vitex negundo L.*		Leaves extracted with methanol	Ag	25 °C	48 h	10–30 nm	Zargar et al. (2011)
58	*Ziziphora tenuior*		Leaves	Ag	Room temperature	Deep red	8–40 nm	Sadeghi and Gholamhoseinpoor (2015)

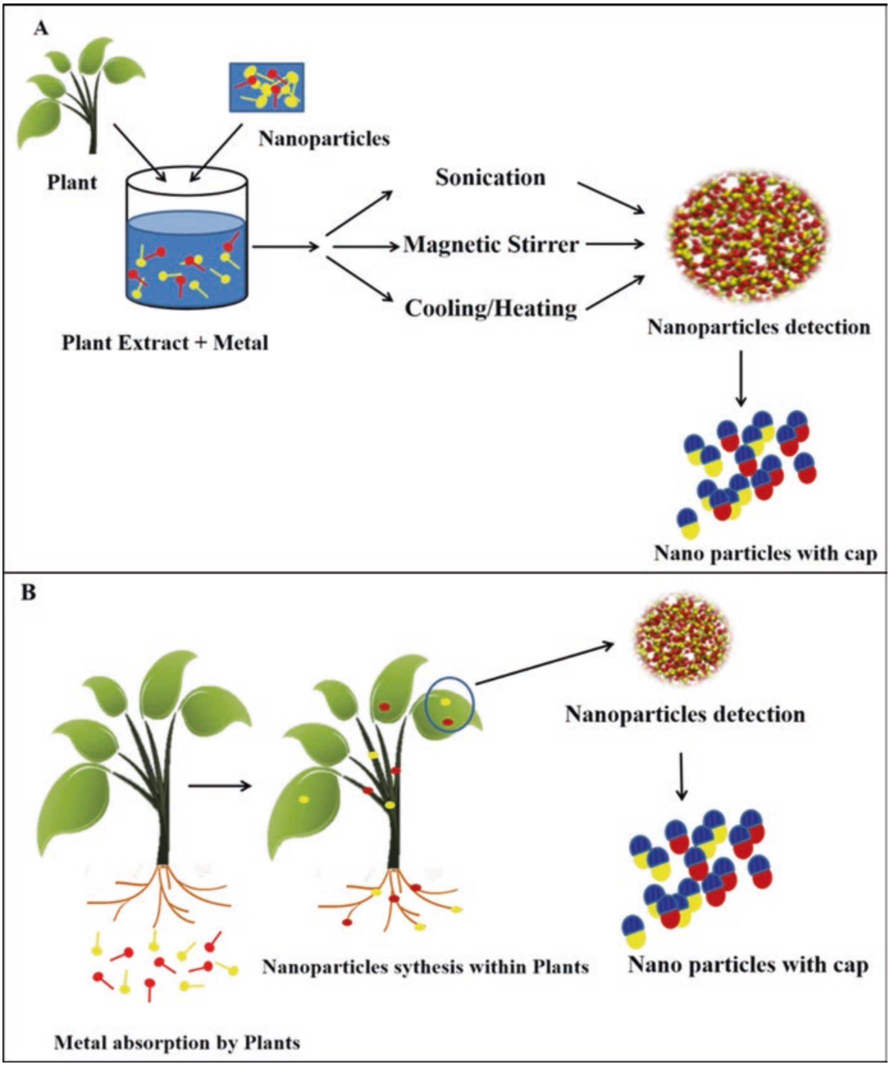

Fig. 15.1 (**a** and **b**) Biosynthesis of nanoparticles by different approaches (**a**) in vitro (**b**) in vivo

2.1.1 Nanoparticles from Different Plant Parts

Nanoparticles of various size and shape are being synthesized by using different parts of plants such as root, stem, leaf, floral parts, seed, callus, peel, and fruits, which do possess variable metabolic activities and biochemical profile. Biosynthesis reactions may also be altered by amount of plant extract in the reaction medium to transform the shapes and size of the NPs (Dubey et al. 2010).

Suman et al. (2014) have reported synthesis of gold NPs using root of *Morinda citrifolia.* Stem is also used to make an extract for production of gold and silver NPs (Rodriguez et al. 2007; Tahir et al. 2015). Several reports have already been published on green synthesis of metal oxide nanoparticles using leaf extracts (Ref. from Table 15.1). Reproductive parts of plants are also reported to synthesize variety of NPs. Floral extract of *Lantana camera* is used to synthesize the silver NPs (Kumar et al. 2016) while fruit extract of *Prunus serotina* and *Vaccinium floribundum* have the capability to synthesize the iron NPs (Murgueitio et al. 2016).

Plant extracts comprise of bioactive phenolic acids, proteins, sugars, polyphenols, alkaloids, and terpenoids having a significant role as reducing agents along with capability of a stabilizer in the process of NPs biosynthesis (Castro et al. 2011). The composition of active biomolecules and their concentrations from different parts of plants affects the interaction with metal ions. These variable interactions are the major factor contributing to the variety of NPs produced with different sizes and shapes (see Table 15.1).

2.1.2 Nanoparticles from Different Physical Conditions

Type of NPs depends upon the biological entities used for the synthesis while shape and size of NPs depends upon the physical conditions such as temperature, pH, and time duration for the synthesis procedure. The ability of plant extracts to stabilize the NP also determines its morphology after synthesis is complete (Mittal et al. 2013). After the synthesis, NPs aggregate to form several kinds of morphologies such as triangles, cubes, rods, hexagons, spheres, and pentagons (Akhtar et al. 2013). Smaller neighboring particles amalgamate to form larger NPs immediately after a period of growth and these are thermodynamically more stable. Temperature has a significant role in controlling the aspects such as ratio, amount, and adequate regulation of the shape and size of NPs. Other than temperature, the pH of the medium influences the size of NPs at great concern. The reaction mechanism for the formation of magnetite NPs has been found to be controlled by pH when coprecipitation method was followed. Nanoparticle size was found to be pH dependent; larger nanoparticles (25–85 nm) in small quantities were formed at higher acidic pH, while smaller nanoparticles (5–20 nm) in large quantities were formed at medium acidic pH.

2.2 *In Vivo (Plants) Biosynthesis of Nanoparticles*

The plants as a whole can also be used for the synthesis of various kinds of NPs. However, the dynamics of NPs formation in living plants is yet an emerging area to be understood. Plants store bioavailable elemental form of metal in their tissues and are able to produce NPs in themselves. In this scenario, phytoremediation has been considered useful to be applied in nanotechnology. The elemental form of metal, absorbed by root is transported across the root membrane via transporters and then translocated via symplastic and/or apoplastic movement from cell to cell throughout the plants (Haverkamp and Marshall 2009). Subsequently, transported metal gets reduced and deposited in the form of NPs of different shapes and sizes.

The root system of intact 16 plant species of 11 different families was used to synthesize the gold NPs of the size 5–100 nm in the purple colloidal salt solutions turning to pale yellow (Pardha-Saradhi et al. 2014). The authors were the first to demonstrate the bulk synthesis of gold NPs in an aqueous medium by using the roots of intact plants exogenously. *Brassica juncea* and *Medicago sativa* are the hyperaccumulators of silver. Harris and Bali (2008) demonstrated that these species accumulate silver in the form of nanoparticles with a mean size of ~50 nm.

Another study reveals the formation of silver nanoparticles in *Brassica juncea*, *Festuca rubra* and *Medicago sativa* plants grown in Hoagland's solution. Plant fractions showed the in vivo formation of in the roots, stems, and leaves of the plants (Marchiol et al. 2014). Alfalfa plants were grown in an AuCl4-rich environment and live plants were used for the fabrication of gold nanoparticles in crystalline state inside the plant. X-ray EDS studies corroborated that the nanoparticles are pure gold (Gardea-Torresdey et al. 2002).

3 Applications of Nanoparticles

Nanoparticles play a critical role in various biomedical applications. Nanoparticles have an array of applications and this array is continuous with growing research and changing requirements. The important applications of NPs are described as follows.

3.1 Detoxification by Nanoparticles

Nanoparticles of three main classes such as micellar nanocarriers, liposomes, and ligand-based NPs have been investigated as nanodetoxifiers. Micellar nanocarriers are mainly composed of an amphiphilic shell and hydrophobic core used for detoxification. These NPs can be circulated in a hydrophilic environment due to their specific characteristics. While doing so, these NPs may contain encapsulated hydrophobic compounds.

Liposomes are nontoxic, degradable, and nonimmunogenic NPs composed of phospholipid bilayers. These liposomes self-assemble into spherical shape with a hydrophilic core. Liposome carriers can encapsulate a wider range of toxins as compared to micellar ones. Various ionizable drugs may be extracted with the use of liposomes. To cite an example, when liposomes are prepared with anionic phospholipids, they may capture cationic drugs such as amitriptyline, bupivacaine, and imipramine. Ligand-based NPs are fabricated with several moieties for specific purposes, such as to scavenge specific toxins. These moieties can be proteins, chelators, and antibodies (Graham et al. 2011). Detoxification can be made by reduction, precipitation, biosorption, biomineralization, and bioaccumulation.

3.2 Antimicrobial Properties

In the pathogenic organisms, NPs have the ability to disrupt the polymer subunits of cell membrane and disturb the protein synthesis mechanism in the bacterial system. Menthol extracted from leaves of *Cassia fistula* acts as a very good bioreductant for the synthesis of silver and gold NPs. The synthesized NPs have been found to be effective against *Staphylococcus aureus* and *Escherichia coli,* which are the human pathogens (Sondi and Salopek-Sondi 2004). Similarly silver nanoparticles synthesized from *Citrus sinensis* peel extract effectively disrupted *Escherichia coli*, *Pseudomonas aeruginosa* (gram-negative) and *Staphylococcus aureus* (gram-positive) (Kaviya et al. 2011). In another report, *Acalypha indica* plant leaf-synthesized silver nanoparticles effectively controlled water-borne pathogenic bacteria with lower concentrations of 10 µg/ml (Krishnaraj et al. 2010). Silver nanoparticles have different bactericidal properties when they interact with *E. coli* in a concentration-dependent manner (Kant Awasthi et al. 2013).

3.3 Biological Sensing and Drug Delivery

A sensor consists of two components: a recognition element and a transduction element. The recognition element specifically binds to a target and this signal is then transferred through the transduction element. Biosensor is defined by its biological receptor unit with unique specificities toward corresponding biomolecule like DNAs of bacteria or viruses, or antibodies versus antigens. The unique physicochemical properties of nanoparticles coupled with biomolecule make these systems promising candidates for sensing applications. Several approaches have been employed in sensors for a variety of nanoparticles functionalized with different biosensing molecules (e.g., DNA, antibodies, proteins, or enzymes) in order to increase specificity of the methods. Biosensors of NPs have been used for a variety of purposes like detection of very low concentrations of analytes, detection of pathogens and cells, and their further separation and capture. The cost-effective sensing of biological agents and diseases would be an important achievement in biomedical diagnosis that will be helpful in timely monitoring of the ailments and its further treatment. Further, NP-based biosensors can find application I analysis of forensic samples and for an array of environmental samples (De et al. 2008).

Nanotechnology has the potential to lead to development of a library of advanced systems for drug delivery integrating the elements of recognition and diagnostics. Nanoparticle-based drug/biomolecule delivery utilizes nanometric carriers comprising of particles (< 1000 nm) of various morphologies, viz., spheres, capsules, micelles, liposomes, etc. (Reis et al. 2006). A number of nanocarriers for delivery of peptides have also been designed and include liposomes, polymeric nanoparticles, and solid lipid nanoparticles. Among these several designs of nanocarriers, polymeric-based NPs have gathered wider attraction as they can both effectively and safely deliver the proteins to desired target cell. Nanoparticle drug delivery system research is mainly based on several attributes which comprises the selection and combination of carrier materials, the surface modification of nanoparticles, the optimization of synthesis procedure of nanoparticles, and the in vivo interaction process of nanoparticles with tissues. Nanoparticles have advantage over other systems due to their physical stability and the opportunity of changing the formulating materials so as to have controlled release characteristics (Emeje et al. 2012).

3.4 Probing of Proteins and DNA Structure

Proteins are the important part of the cell's language, structure, and machinery. Nanoparticle–protein complex formation is a multifactorial process that depends on the characteristics of the nanoparticles as well as on the interacting proteins and the medium. Adsorption of proteins at the nanobio interface is regulated by various bonds, e.g., hydrogen bonds, solvation forces, van der Waals interactions, etc. The other regulatory factors include the affinity of the protein toward the nanoparticle surface and its potential to cover the surface. The organization manner of proteins onto surface of NPs may in turn affect the biological activity of proteins at cellular level (Ge et al. 2011). Metal nanoparticles are widely used in immunohistochemistry to identify protein–protein interaction. The specific association and dissociation rates differ from protein to protein and this decides the duration of their interaction with the NP surface.

Magnetic nanoparticles were used to detect the albumin, apolipoprotein A-1, complement factors, vitronectin, and hemoglobin proteins from bovine serum (Mu et al. 2009). Metal nanoparticles such as gold were used to detect certain proteins such as albumin, fibrinogen chains, transport proteins, coagulation factors, and tissue development proteins from human plasma and bovine serum (Maiorano et al. 2010; Deng et al. 2012). The detection of DNA sequences is a crucial event not only in clinical diagnosis and gene therapy but also in biomedical studies. A sandwich-type DNA biosensor was fabricated by Chen et al. (2011) where an amino-functionalized capture DNA was immobilized on the magnetic bead. This capture DNA hybridizes with the target DNA at one end while at the other end, it binds to a signal DNA probe labeled on the surface of gold nanoparticle (Chen et al. 2011).

3.5 Tissue Engineering

Nanotechnology can be used to create nanofibers, nanopatterns, and controlled release nanoparticles with applications in tissue engineering, for mimicking native tissues such as extracellular fluids, bone marrow, cardiac tissues, etc. Nanofabrication techniques, materials science, surface, micro and nanopatterning in tissue engineering help in providing best microenvironment where cells have to grow. Micro and nanofabrication techniques offer several advantages for tissue engineering. For tissue engineering, the major criterion is to target specific tissues in a particular organ and hence, the size and morphology of nanoparticles become important parameters. To develop protein-based nanoparticles with appropriate features of size and morphology, several methods are available. These include crosslinking, emulsion formation, coacervation, and precipitation. Proteins, due to their precise molecular sizes and amino acid sequences, self-assemble into specific structures like α-helix and β-sheet or other random structures. This helps in the designing of specific nanostructures such as nanoparticles, nanofibers, and nanosheets (Nitta and Numata 2013). Polysaccharides are other biomolecules of choice due to features like biodegradability and biocompatibility. They can also be easily modified at molecular level both covalently and ionically. Some bone-morphogenic proteins have also been delivered with the use of NPs in the field of osteo-engineering (Nguyen et al. 2012).

3.6 Sorting of Biological Molecules and Cells

Various nanoparticles due to their optical and magnetic properties have been explored as sensors for detection and sorting of biological molecules and cells. One of the most common methods used for the detection of biological cells involves direct immunological reactions using magnetic nanoparticles coated with antibodies against surface antigens. Yoon et al. (2006) investigated the possibility of applying an external magnetic field for specific targeting and cell sorting of NPs. For the purpose, NPs were made up of biocompatible magnetic silica-coated core–shell nanomaterials.

Other than size, nanoparticles also have to possess a hydrophobic layer for high stability, hydrophilic layer for biocompatibility, and outermost functional layer for recognition for sorting of cells (Cao and Brinker 2008). Zahavy et al. (2012) applied nano-magnetic particles for immunomagnetic labeling of bacteria and separated them from high and low concentration of bacteria [down to 10(5) cfu/ml).

4 Conclusions

In conclusion, this chapter deals with the green way of synthesizing NPs. Large-scale NPs synthesis could be best drawn by the plant-based materials. We have emphasized the use of different plant parts along with the variable physical condition in this chapter. Among all plant parts and green synthesis methods, leaf extract is the most used strategy to get NPs. Most of the research is also focused on silver NPs; other metal NPs should also get the attention of researcher in future. Furthermore, application of NPs synthesized by green technology must be of wide range for future prospective.

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Chapter 16
Green Analytical Techniques: Novel and Aboriginal Perspectives on Sustainable Development

Elijah M.M. Wanda, Edgar C. Mapunda, Deogratius T. Maiga, V. Sivasankar, Bhekie B. Mamba, Titus A.M. Msagati, and Kiyoshi Omine

Abstract Green analytical techniques refer to approaches that decrease or completely remove preservatives, reagents, solvents, and other substances that are dangerous to man and the environment or and that also have the capacity to enhance speed and produce energy-efficient chemical analyses without affecting the quality and the required level of performance of products. This chapter discusses basic principles of green environmental techniques which aim at reducing the impact of chemical activities on man and the environment. These basic principles include energy and water usage reduction, reagent and solvent usage reduction, minimal production of gaseous, liquid and solid, substances during analytical processes, instantaneous analysis for prevention of pollution and intrinsically safer chemistry for prevention of accidents, synthesis of less harmful chemicals, atom economy, prevention, catalysis, design of benign chemicals, use of solvents and auxiliaries that are safer, designing processes that are energy efficient, usage of renewable resources, derivative reduction, and planning for degradation. Emphasis on green separation techniques, green spectrophotometric techniques, basics of green analytical techniques, the problems associated with the formulation of ideologies of green analytical chemistry to existing analytical laboratories, as well as the evaluation of the impact on man and the environment have also been discussed in this chapter.

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1 Introduction to Principles of Green Analytical Techniques

Chemistry is indisputably a very vital discipline for the sustenance of human's daily lives (Turner 2013). However, chemical processes bring with them new human and environmental health problems as well as harmful unforeseen peripheral effects, which necessitate the formulation of products that are 'greener.' Green analytical techniques refer to inventive designs and processes that minimize or remove the application and/or fabrication of unsafe substances in the scheme, production and use of chemical products (Anastas 1999; Gałuszka et al. 2012; Lawrence 2013; Turner 2013). Green analytical techniques are also known as (Wardencki et al. 2005):

- Sustainable chemical techniques
- Environmentally benign chemistry techniques
- Harmless-by-design analytical techniques
- Techniques that prevent pollution at basic structural level
- Clean chemical techniques
- Atomic economical techniques
- All of the above

Conventionally, people of the Aboriginal origin demonstrated capabilities of virtuous neighborliness in their interaction with their surroundings. The aboriginal perspectives are crucial in greening analytical techniques. The application of analytical chemistry techniques and methodologies has the capacity to minimize or remove preservatives, reagents, solvents, as well as other hazardous chemicals in addition to being faster and efficient in terms of energy consumption during chemical reactions, synthesis and processes without affecting the quality and the required level of performance of products (Wardencki et al. 2005; Tobiszewski et al. 2010; Gałuszka et al. 2012). Basically, green analytical techniques seek to prevent pollution at the scale of molecules through minimization of on-site waste generation, application of catalytic agents instead of reagents, usage of reagents that are not toxic, employment of renewable materials instead of nonrenewable ones, improvement of atom proficiency, and utilization of solvent restricted or solvent systems that are environmentally safe and can be recycled (Wardencki et al. 2005; Tobiszewski et al. 2010; Gałuszka et al. 2012; Turner 2013).

The major underlying principles of green analytical techniques are thus to reduce waste, materials, risks, energy, and costs of chemical designs and processes. Green analytical techniques are generally based on 12 principles postulated by Anastas and Warner (1998). The principles consist of guidelines required for analytical chemists in implementation of new materials, syntheses, and scientific processes. The following subsections describe the 12 principles of green analytical techniques (Wardencki et al. 2005; Tobiszewski et al. 2010; Gałuszka et al. 2012):

1.1 Prevention

This principle of green analytical techniques seeks to prevent waste from being generated other than handling or dealing with waste after it is formed. For example, prevention would involve the design, production, procurement, or consumption of materials to minimize their amount or toxic effects prior to discharge into the waste stream. Another example could be sample preparation techniques using less solvents. There are three major basic ways to achieve prevention in green analytical techniques:

- Reduction of the amount of material used to manufacture a product without compromising the quality utility of the product.
- Increasing the lifetime of a chemical product and designing chemical products that last longer so as to reuse products and materials.
- Elimination of the need for a given product by substitution.
- Increasing the lifetime of a chemical product and designing chemical products that last longer so as to reuse products and materials.
- Elimination of the need for a given product by substitution (Fig. 16.1).

A good example of how pollution can be prevented is through the use of CO_2 as follows:

(a) Carbon dioxide substitution

Carbon dioxide (CO_2) substitution exemplifies a very important opportunity to replace current plausible substance for a wide variety of more toxic substances. The recyclability and reuse of CO_2 in many processes can make closed-loop systems possible.

(b) Solvent substitution

Carbon dioxide acts as a solvent that could be utilized for gelatinous coating thinning up to a desirable application level when it is heated to 33.11 °C and compressed to 1100 psi (Ohio Environmental Protection Agency 2010). Owing to its solvent-like properties, CO_2 has the virtue of replacing hydrocarbon solvents which are hazardous to man and the environment. In addition, the amount of solvent used could be remarkably reduced by 50.0–85.0%. Furthermore, utilization of harmful air contaminants, e.g., toluene and xylene, could be entirely removed in some instances.

1.2 Atom Economy

This principle of green analytical techniques recognizes that synthetic methods ought to be formulated in such a way that will maximize the conversion of all starting materials (i.e., the reactants) used in chemical reactions and process into the

Fig. 16.1 Essentials of Green analytical techniques

resulting products based on the formula weight (FW) of atoms utilized and formula weight of reactants expressed as a percentage as follows:

$$\%\text{Atomic economy} = \frac{\text{FW of atoms utilised to produce the final product}}{\text{FW of all reactants}} \times 100$$

Let us consider the following reaction Eq. 16.1:

$$C_4H_9OH + NaBr + H_2SO_4 \rightarrow C_4H_9Br + NaHSO_4 + H_2O \qquad (16.1)$$

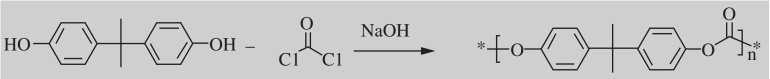
From the equation, the FW of atoms used to formulate products is 137 and that of the reactants is 275. We now compute the % atomic economy as follows:

$$\%\text{Atomic economy} = \frac{137}{275} \times 100$$

$$= 50\%$$

1.3 Less Hazardous Chemical Synthesis

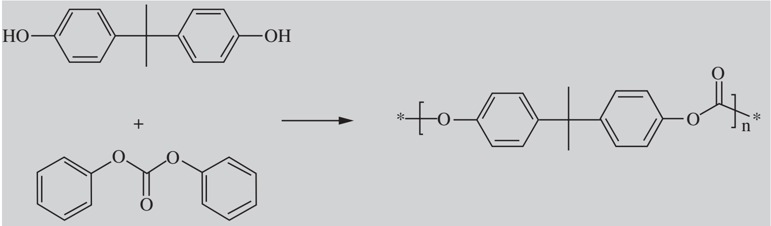
This principle advocates that wherever probable, man-made approaches should be schemed to utilize and create materials that have minimal or no detrimental effects on the environment and the health of humans. A good example is the synthesis of polycarbonates such as the diphenyl polycarbonate using phosgene ($COCl_2$) as shown in Equations (16.2 and 16.3).



(16.2)

Phosgene is known to be an insidious poison, with its odor detection threshold being 0.4 ppm. In addition, phosgene is corrosive and highly toxic. The toxicity of phosgene emanates from its action on proteins in the site of gas exchange, i.e., the pulmonary alveoli, where it can cause suffocation due to the disruption of the blood–air barrier. Phosgene also causes urea-like cross-linkages by reacting with the amino-proteins. In addition, this reaction requires huge amounts of the reactant, dichloromethane (CH_2Cl_2) in addition to the polycarbonate being at risk of contamination with chloro- impurities. Nevertheless, the polycarbonate can be produced by using the solid-state process, which is a less hazardous process.

In the solid-state process, the diphenyl polycarbonate is produced without the toxic phosgene and also the employment of CH_2Cl_2. In addition, the solid-state process produces polycarbonates of better quality (Eq. 16.2).



(16.3)

1.4 Designing Safer Chemicals

This principle advocates that products of chemical reactants, processes, and syntheses ought to be premeditated so as to preserve the products' function efficacy while minimizing toxicity. The majority of commercial chemical substances are associated with diverse hazardous effects to man and the environment. This has led to the formulation of several local and international policies, conventions, and agreements aimed at identifying and controlling the said harmful chemical substances that are widely used. For example, chlorine is one of the commonly used disinfection products in water treatment processes. However, chlorine reactants with natural organic matter (NOM) during the disinfection step during the water treatment process are able to form disinfection by-products (DBPs). The resulting disinfection by-products have been known to be associated with a number of mutagenic and carcinogenic effects in addition to attacking the skin, eyes, respiratory system, and mucous membrane systems. Another example is the use of tributyltin oxide, an organotin compound that has traditionally been used as an antifoulant. However, tributyltin oxide is chronically toxic to aquatic life, persists in the environment because it has a long half-life (i.e., > 6 months in seawater). In addition, tributyltin oxide bioaccumulates and bioconcentrates in marine organisms at concentrations >104 times that in immediate water environment. Organic compounds that contain tin compounds may lead to chronic toxicity in marine life and have the possibility of entering into food chain. It is thus vital for chemists to identify these difficult chemical products and improve the design processes in early stages before such products are released for commercial and application purposes (Fig. 16.2).

1.5 Safer Solvents and Auxiliaries

This principle highlights that supplementary substances (e.g., separation agents and solvents) ought to be either sidestepped or as safe as possible. This can be achieved through solvent substitution, maximizing the use of preferable solvents (such as 2-butanol, 1-butanol, 2-propanol, methyl ethyl ketone, methanol, isopropyl acetate, ethyl acetate, 1-propanol, ethanol, acetone, and water), and using new solvents. Examples of such solvents include supercritical fluids and ionic liquids. Examples of undesirable solvents which ought to be substituted because of their toxicity, carcinogenicity, and mutagenicity at low threshold levels include benzene, dimethoxyethane dioxane, pyridine, N-methylpyrrolidone, chloroform, dichloroethane, dichloromethane, diethyl ether, di-isopropyl ether, hexane(s), and pentane.

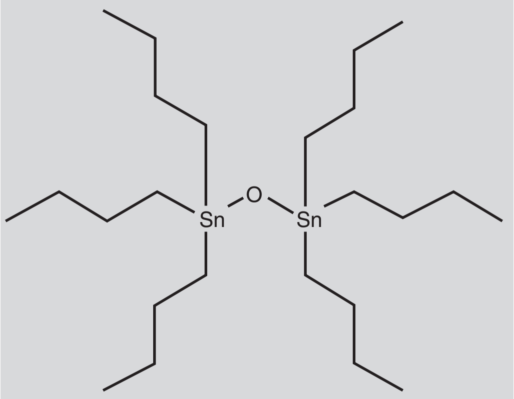


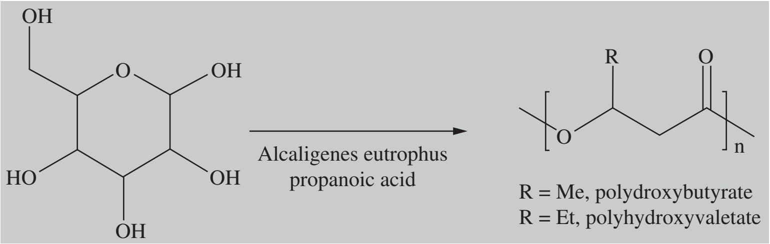
Fig. 16.2 Structure of tributyltin oxide

1.6 Design for Energy Efficiency

This principle highlights on the need to recognize and minimize the economic and environmental effects of energy requirements and to perform synthetic methods at ambient pressure and temperature. Energy in chemical processes is through distillation, cooling, heating, boiling, running laboratory equipment, photochemical, and microwave. In case of power plants, energy comes from coal, oil, and natural gas. In most cases, energy is not only costly but also the power generating plants contribute to pollution of the environment. Solar energy and microwave energy offer alternative energy-efficient sources. Microwave energy sources fall within the 1 mm and 1 m wavelengths with frequency fixed at 2.45 GHz. It offers more directed source of energy capable of achieving a heating rate of 10 °C per second. With microwave energy, solvent-free conditions are possible and penetration depth can be used to determine interaction with matter.

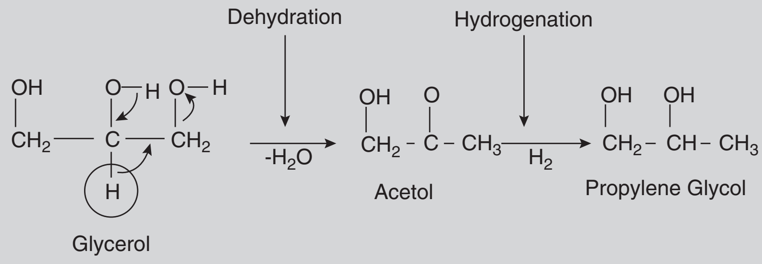
1.7 Use of Renewable Feedstocks

This principle stipulates that wherever economically and technically practical, the feedstocks or raw materials ought to be renewable instead of exhaustible nature. Renewable feedstocks usually come from agricultural products (e.g., biomass, potatoes, and corn) and waste materials from other anthropogenic activities. Examples of exhaustible feedstocks are those from fossil fuels (coal, natural gas, and petroleum) as well as those from the mining industry. Combustion of fossil fuels generates carbon dioxide which aggravates global warming. Renewable feedstocks are used to produce several products such as fuels (biodiesel and ethanol) as well as plastics just to mention but a few. Examples of polymers from renewable feedstocks include polyhydroxyalkanoates as shown in Eq. 16.4 (from the breaking down of glucose by bacteria under the influence of propanoic acid and the product also contains 5–20% polyhydroxyvalerate), poly (lactic acid), and levulinic acid (a platform to produce biodegradable biocides).



(16.4)

Another example for the use of renewable feedstocks is the production of propylene glycol from waste glycerol, a vegetable oil feedstock (Eq. 16.5). This process is a more efficient and economically viable option than production of the same propylene glycol from petroleum. Use of the renewable glycerol also greatly improves the biodiesel market by adding value to the glycerine coproduct in addition to providing a less costly and less hazardous antifreeze alternative to ethylene glycol.



(16.5)

1.8 Reduce Derivatives

This principle stipulates that chemical processes should minimize or avoid unnecessary derivatization processes as much as possible such as temporary alteration of chemical and physical processes, protection as well as deprotection, and blocking group. This is because such derivatization steps need extra reagents and have the potential to generate a variety of wastes, such of which lead to environmental pollution. One way of reducing use of derivatives is through employment of enzymes, which have high levels of specificity and reacts with target sites of molecules without affecting the rest of the molecule thereby eliminating the need for protecting groups. For example, in the synthesis of penicillin G in industries, the R=H group is protected as its silyl ester [R = Si(Me)$_3$] before reacting with phosphorus pentachloride at −40 °C to form the chlorimidate **1.** Subsequent hydrolysis leads to the production of the required 6-APA, which is used to produce semisynthetic penicillin (Fig. 16.3)

Note:

1. TMSCl then PCl_5, $PhNMe_2$, CH_2Cl_2, −40 °C
2. n-BuOH, −40 °C, then H_2O, 0 °C
3. Pen-acylase, water

This method of synthesizing penicillin has been replaced by use of enzymes, pen-acylase, a process that takes place in water at temperatures slightly above room temperature. In this process, use of the silyl protecting group is not required.

1.9 Catalysis

This principle advocates that highly selective catalytic reagents are grander to stoichiometric reagents. Catalysis is one of the central pillars of green analytical techniques. The approaches, strategies, and applications of new highly selective catalytic

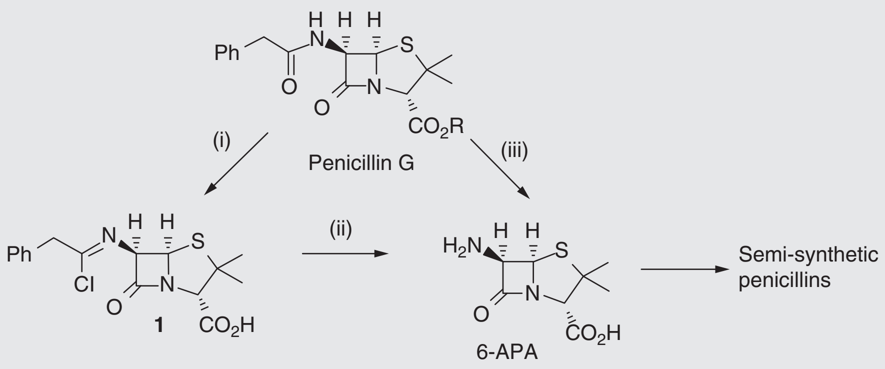


Fig. 16.3 Synthesis of Semisynthetic penicillin

reagents and catalytic systems aim at achieving both environmental protection and economic benefit goals simultaneously. For example, application of supercritical (sc) CO_2 in place of the traditional catalysts such as aluminum chloride can eliminate hazardous by-products, such as chloro-propane and create closed-loop processes by producing propanol (Fig. 16.4).

Use of enzymes and whole-cell microorganisms are also utilized in the principle of catalysis. Enzymes and whole-cell microorganisms have the virtuality of achieving fast reactions as a result of correct orientations. High substrate and stereo specificity could be achievable in water soluble, naturally available, and moderate reaction conditions. These conditions have the possibility of tandem reactions.

1.10 Design for Degradation

This principle for green analytical techniques states that chemical products ought to be formulated in such a way that they do not persist in environmental segments after their utility, rather they should break down into harmless products of degradation. Chemical products such as sulfonated detergents, chlorofluorocarbons, and DDT bioaccumulate and persist in the environment where they contribute to various adverse effects. Environmental persistency is an undesirable feature of several consumer products such as plastics and this can be overcome by coming up with products that can degrade in a short time.

1.11 Instantaneous Analysis for Prevention of Environmental Pollution

This stresses on the necessity to further develop analytical methodologies that allow analytical chemists to check the progress of chemical reactions as they happen in control and monitoring of processes prior to the production of harmful products.

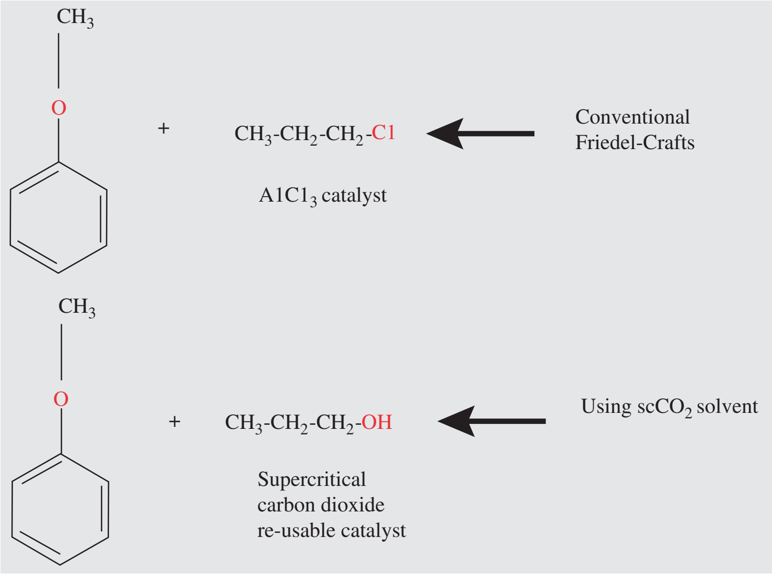


Fig. 16.4 Application of supercritical CO_2 in the place of traditional catalysts

Knowledge about the products forming time could help reduce the amount of waste, time, and energy.

1.12 Intrinsically Harmless Chemistry for Prevention of Accidents

This insists on prior selection of substances and the nature of materials utilized in chemical processes. This selection is in order to reduce the possibility for accidents involving chemicals as well as discharges, blasts, and fires. A good example is phosgene, which is known to be an insidious poison, corrosive, and highly toxic with its odor detection threshold being 0.4 ppm. The toxicity of phosgene emanates from its action on proteins in the site of gas exchange, i.e., the pulmonary alveoli, where it can cause suffocation due to the disruption of the blood–air barrier. As far as possible this must be avoided to minimize the occurrence of adverse as well as detrimental effects to man and the environment.

2 Green Separation Techniques

Materials and or chemicals in our daily life are available in an impure state as found to be mixed with other substances. A phenomenon that makes the direct utilization of chemical substances of interest is rather impossible. Various separation techniques therefore have to be applied in order to isolate useful substances from natural mixtures for particular applications. For example, different compounds of medicinal values occur naturally in different plants coexisting with other inactive compounds that are sometimes toxic. Chemists are able to extract desired compounds from mixtures of different substances by applying appropriate separation techniques. As though solvents play a significant role in separation techniques, it is quite unfortunate that most of conventional solvents are associated with harmful impacts to human and or to the environment. Traditional organic solvents are toxic, volatile, and flammable and (sometimes carcinogenic) pose serious threat to the environment and health of human beings. For sustainability of human kind and economic development, chemical processes including separation techniques need to be performed in a benign way to human and the environment. This subsection therefore discusses the trends and progresses in development of green separation techniques for sustainable development.

For more than two decades, chemists have been working to come up with greener alternative separation methods that can substitute the traditional hazardous separation techniques with improved efficiencies and reduced costs. Various avenues have been explored and useful green separation techniques have been developed and applied, with some upscaled to industrial level. The most promising areas of green separation techniques include the use of ionic liquids, supercritical fluids, aqueous phase separation, solvent-less separation systems, and most recently the use of green biosolvents. The following is a brief overview of each of the alternative separation methods.

(a) Ionic liquids

Ionic liquids are salts, mainly organics with melting points less than 100 °C. In their liquid form they consist of mainly ions than molecules. The domain of ionic liquids receives attention in a variety of chemistry uses such as synthesis, polymer fabrication, analytical and separation chemistry since its discovery in 1914 (Berthod and Cara-Broch 2004). This section discusses use of ionic liquids in chemical separation techniques.

This class of solvents presents superior characteristics as far as green separation techniques are concerned. Ionic liquids have negligible vapor pressure, are nonflammable, and have a wide range of thermal stability with good dissolving power for both inorganic and organic substances (Flieger et al. 2014; Ho et al. 2014). Their physicochemical properties could be appropriately designed to suit tailor-made applications. In separation chemistry, ionic liquids are used in a wide range of techniques which include liquid–liquid extraction, gas and liquid chromatography, and capillary electrophoresis. In most cases, separation is intended to recover pure compounds, but sometimes chemical separation is a prerequisite following a particular analysis. Ionic liquids are effectively utilized in liquid–liquid extraction techniques replacing hazardous organic solvents which were used traditionally. In liquid–liquid extraction, different analytes form metal cations to small organic and large macro molecules such as proteins can be separated by using ionic liquids as partitioning solvents (Flieger et al. 2014; Huddleston et al. 1998). Their liquids to form water insoluble solvents offer important avenues to be applied in liquid–liquid extraction techniques.

High performance liquid chromatography (HPLC) and gas chromatography (GC) are some of the areas of separation techniques where ionic liquids have gained a wide application. In these techniques ionic liquids have been mostly used to prepared stationary phases owing to the tunability of ionic liquids which alter the properties of stationary phase to the desired application. A comprehensive analysis on the advanced uses of ionic liquids in HPLC and GC stationary phases is given by Ho et al. (2014). Capillary electrophoresis is a separation technique that separates ionic substances depending on their differences in electrophoretic mobility upon applied voltage. A number of ionic liquids are being utilized as electrolytes in capillary electrophoresis backed by their high conductivity and tailored miscibility in aqueous solutions. For instance, poly(1-vinyl-3-butylimidazolium) bromide has been used as deposits in an electrophoresis silica capillary with high tolerance to organic solvents (Li et al. 2011). Yanes et al. (2000) discussed the application of tetraethylammonium tetrafluoroborate (TEA-BF4) as an electrolyte in the separation of polyphenols from grape with excellent productivity.

(b) Aqueous phase separation

Water is the most environmental friendly and cheapest natural solvent which can dissolve most of inorganic compounds but with limited solubility to organic compounds at ambient temperature. However, at elevated temperatures water can similarly work as organic solvents like methanol. At a temperature range of 100–374 °C water is referred to as superheated water or subcritical water. Within this temperature region water can be an effective nonorganic solvent to be used in separation processes. Extraction of supercritical water is a new separation technique that uses water at superheated temperature range (as well as elevated pressure enough to retain water in liquid state) as an extraction solvent. At this state the polarity of water is reduced and hence the solubility of organic molecules is enhanced than at ambient temperature (Shabkhiz et al. 2016). Superheated water extraction technique presents several advantages over the conventional extraction methods. Apart from being environmental friendly, this separation technique has reduced extraction time, high extract quality, and reduced costs. It is also possible to regulate the strength of the extraction solvent by varying temperature and/or pressure of the superheated water (Herrero et al. 2006).

In the last 10 years, superheated water separation technique has been widely used in research and industrial laboratories. The use of superheated water for organic compounds extraction from a variety of plant sources has been reported by substituting hazardous organic solvents (Shabkhiz et al. 2016; Smith 2006). Superheated water is also gaining acceptance in chromatographic techniques where water is recently used as mobile phase substituting the commonly used organic solvents (Kayan et al. 2016). Green chromatography after being extensively tested and optimized at a laboratory scale is currently tested for its usefulness at industrial level. Yang et al. (2011, 2012) investigated the industrial application of superheated water using chromatographic techniques for the separation of niacinamide and preservatives in skincare products where superheated water was successfully substituted (as green mobile phase) for hazardous organic solvents.

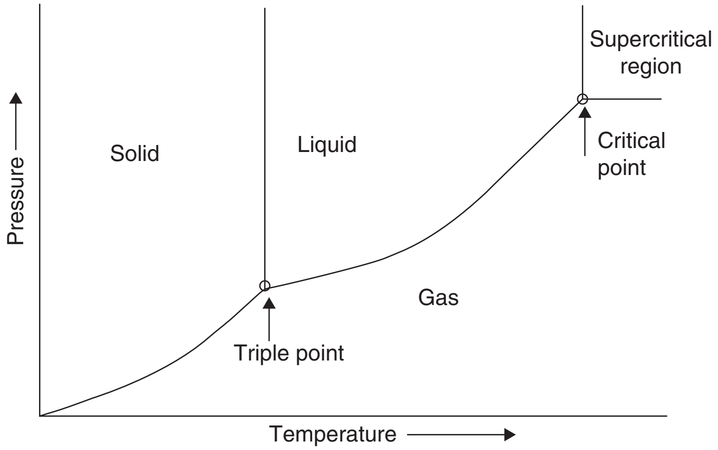


Fig. 16.5 Phase diagram of CO_2

(c) Supercritical fluids

The term 'supercritical fluids' refers to fluids in a state where it is difficult to distinguish a liquid from a gas. This happens when fluids are enforced to a temperature and pressure higher than that for their critical point (Fig. 16.5). The properties of the fluid under these conditions are the intermediate between a liquid and gas and these fluids have densities similar to their liquid state and viscosity of gaseous state. However, their diffusion behavior is intermediate of liquid and gaseous states. Extraction of supercritical fluid (SFE) has been extensively premeditated in the extraction of vital components from various plant sources for human nutrition and health application. The widely used SFE solvent is supercritical carbon dioxide (SC-CO_2) owing to its versatile properties. The critical pressure and temperature of CO_2 are relatively low which enables extraction of vital compounds without being degraded by high temperatures. SC-CO_2 is nonflammable, nontoxic, with increased purity, available at comparatively lower cost and easy removal from the extract. The polarity of SC-CO_2 is similar to that of organic solvent traditionally used in extraction of plant vital compounds. Furthermore, the properties of SC-CO_2 can be tuned and tailored for the recovery of specific extract by altering pressure and temperature.

The numerous scientific studies on SFE with CO_2 have enabled scientists to explore extensively the suitability of SC-CO_2 in separation industry. At present, the status has been technologically updated by the optimization of pressure, temperature, and flow rate. SC-CO_2 extraction selectivity and the use of cosolvents in the process is also well established. Several up-scaling studies of SC-CO_2 extraction have been conducted which include its economic feasibility at the industrial level (de Melo et al. 2014). There are expectations that SC-CO_2 extraction technology will be used in industry as green separation technology and substitute traditional hazardous extraction techniques in the near future. In addition to the above, SC-CO_2 has been extensively investigated and applied in chromatographic separations. In particular, supercritical fluid chromatography (SFC) presents a novel environmental friendly technique in chromatography where SC-CO_2 is used as a mobile phase to replace traditionally used organic solvents which are detrimental to man and the environment.

The ability to optimize solubility and diffusion by controlling temperature and pressure enables supercritical carbon dioxide to be used as a mobile phase in chromatography to get better and fast separations than in conventional liquid chromatography. SFC has been extensively employed in fractionate extracts of vital compounds from natural plant products to more useful fractions. Examples are many, but it is worth to mention that the first application of SFC was achieved by Sugiyama et al. (1985), who focused on the separation of tocopherols from wheat germ. Since then studies on the use of SFC have significantly been increased.

(d) Other techniques of green separation

Other techniques of green separation which have been extensively explored to replace conventional detrimental techniques include the use of solvent-less separation methods, substitution with green solvents of biological origin, and more recently the use of deep eutectic solvents. Solvent-less separation techniques are the most green and environmental friendly approach because no harmful substances like organic solvents are introduced to the environment or the products. This technique has long been used in petroleum industry to recover various useful fractions of crude oil by distillation. New developments have been reported in solvent-free techniques and exemplified by the application of extraction procedures which are assisted by the ultrasound in the recovery of lipids from algal cells by Adam et al. (2012).

The utilization of biosolvents in essential oils extraction from natural feedstocks has gained interest in the search for replacement of harmful organic solvents. Chemat et al. (2012) demonstrated successful substitution of dichloromethane with d-limonene extracted from citrus fruit peels. d-Limonene is environmental friendly and can be applied in extraction of a wide range of products by soxhlet process.

Deep eutectic solvents (DES) are formed when high melting point salts are mixed to form solutions with deeper decrease in freezing point (Francisco et al. 2013). A good example of DES is choline chloride, a quaternary ammonium salt and urea which could form a natural and drinkable solution. The governing principle behind the formation is the ability to form hydrogen bonds between the mixing compounds. DES have similar application as ionic liquids, nevertheless, it overcomes certain limitations reported for ionic liquids. The existence of DES as molecules of the starting materials in the solution makes it easy to recover after being used as solvents.

3 Spectrochemical Methods

Spectrochemical methods are based on the determination of the interaction of electromagnetic radiation with atoms or molecules of analytes. Spectrochemical determination of analytes may be made in the infrared (780 nm-300 μm), ultraviolet

(200–380 nm), and visible (380–780 nm) sections of the spectrum. The choice of the wavelength region is dependent on a number of factors including availability of instrument, colored nature of analytes, or the ability of analytes to form colored derivatives, availability of analytes' functional groups which absorb in the ultraviolet or infrared region, and the availability of absorbing species in solution. The spectrochemical methods, especially in the visible portion of the electromagnetic continuum, are the most widely applied methods of chemical analyses. In spectrochemical methods, the sample in liquid form absorbs the radiation from the sun from a proper source and the amount absorbed is correlated to the analyte concentration in solution. Absorption is the manner in which energy from the electromagnetic spectrum is transmitted to the molecules, ions, and atoms constituting a sample. Mathematically, absorbance (A) of an absorbing species is defined using the following equation:

$$A = -\log_{10} T = \log_{10} \frac{P_0}{p} = \varepsilon bc$$

Where P_0 (also presented as I_0) is the incident radiant energy passing through the solution of an absorbing species or optical density of the solution at concentration (c) and path length (b) also presented as (l)

P (also presented as I) is the transmitted radiant energy.

ε is the product of the absorptivity and molecular weight of the absorbing species commonly referred to as the molar absorptivity.

Absorbance can thus also be presented as follows:

$$A = \log_{10} \frac{I_0}{I} = \varepsilon bc$$

Qualitatively, the color of an object we see in the visible spectrum is as a result of the wavelengths transmitted or reflected. The other wavelengths are absorbed and the unabsorbed wavelengths are either transmitted or sometimes reflected. The color is complementary to the absorbed colors. The following table gives typical colors associated with different wavelengths.

The absorbed radiant energy promotes particles from ground state as represented in Fig. 16.6 (i.e., the state at normal room temperature) to excited state (the state at one or more higher energy levels). It is worth noting that ions, molecules, and atoms possess a limited number of distinct energy levels. It can be understood that for any absorption to take place, the energy of an exciting photon must be exactly equal to the energy difference between the excited and ground states of the absorbing species.

Typical examples of spectrochemical techniques include:

1. Infrared spectrometry (Dispersive and Fourier transform)
2. Raman spectrometry
3. Nuclear magnetic resonance

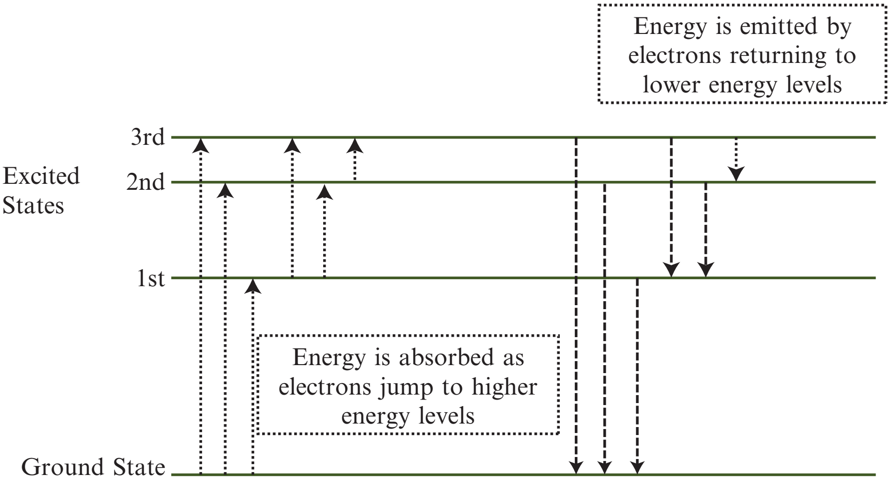


Fig. 16.6 Energy transformations between ground and excited states

4. X-ray spectrometry
5. Atomic fluorescence spectrometry
6. Inductively coupled plasma mass spectrometry
7. Inductively coupled plasma atomic emission spectrometry
8. Atomic absorption spectrometry
9. Ultraviolet/visible spectrometry
10. Molecular fluorescence spectrometry
11. Chemiluminescence spectrometry
12. X-Ray Fluorescence spectrometry

Conventional spectrochemical methods among others are known by (1) applying offline systems; (2) high energy, reagent, and time and energy consumption; (3) use of persistent, unsafe, and hazardous chemicals and reagents; (4) wet chemistry application; and (5) employment of methods that produce large quantities of waste.

(a) Greening of spectrochemical methods

It is difficult sometimes to minimize the use of reagents in the majority of spectrochemical methods. Nevertheless, there are approaches that can be used to make these methods greener. The aim of greening spectrochemical methods is to develop greener analytical techniques in elucidating the molecular structure. This is better achievable through minimization of the following such as sample preparation, sample handling, solvents/reagents consumption, energy, and waste consumption. These green techniques decrease adverse environmental side effects by chemical analyses when precision, selectivity, sensitivity, and accuracy of analytical methodology are absolutely executed. One way of achieving this is through the application of instrumental techniques which are largely automated and miniaturized as opposed to the predominantly wet chemical approaches. It is worth noting that every analytical technique comprises of several steps which largely depend on the properties of sample and the method selected. Generally, the larger the number of stages in a particular method, the less green the method is owing to higher quantities of energy used and volumes of waste generated. Consequently, an ideal green spectrochemical approach may involve reducing the steps of stages in a particular procedure. The role of green spectroscopy would be to explore more environmentally benign spectrochemical methodology.

Spectrochemical methods have been used in monitoring of environmental pollutants from a variety of sources through decreasing the application of preparation of samples and the solvent and reduction of accessories. Nevertheless, green spectrochemical methods warrant the enhanced greenness of analytical methodologies in addition to monitoring of environmental pollutants. The following are the better outcome of the green spectrochemical methods. They are

- Prevention of accidents such as explosions and fires through practicing of safer chemistry
- Better applicability of in situ analyses than the offline analyses
- Evasion of using other auxiliary substances, derivatization reagents, and chemical catalysts
- Designing analytical systems and approaches that are energy efficient
- Utilization of less toxic reagents and solvents, and
- Prevention or reduction of waste formation from spectrochemical processes

There have been attempts focused on the replacement of safer reagents for toxic ones and also amelioration of alternative reagents. Online waste decontamination processes, viz., passivation, degradation, and recycling have also been of a serious concern to achieve the greening process.

4 Green Analytical Chemistry and Associated Problems in the Existing Analytical Laboratories

With strategic focus of green analytical chemistry, different methods have been employed to decrease the risk of environmental impact in analytical techniques and methodologies (Armenta et al. 2008). However, introduction of the ideologies of the green analytical chemistry onto existing analytical laboratories seems to be challenging due to the huge number and variety of substances to be analyzed, methods of analysis, and the complication of matrices of sample (Gałuszka et al. 2012). Many efforts are made by researchers to adopt the existing methods in a greener way and also developing new techniques to ensure compliance with green analytical chemistry principles. However, following are some of the methodological problems associated with the formulation of ideologies of green analytical chemistry to existing analytical laboratories.

(a) Searching for new direct analytical techniques

Searching for direct analytical methods has been difficult as these processes involve multisteps in the processes of analytical techniques. Therefore, every step ought to be taken into consideration with respect to the formulation of greener analytical techniques (Tobiszewski et al. 2009; Gałuszka et al. 2012). The choice of method to be used in a chemical analysis ought to be based on limits of detection, selectivity, precision, and accuracy. It is worth noting that it is difficult to find a direct analytical method for the analysis of many analytes.

(b) Solvent-less sample preparation techniques

Sometimes it becomes difficult to meet some criterion of green chemistry and analytical laboratories. For example, substantial decrease in the amount or complete removal of reagents as well as solvents that are applied in sample preparation and measurements may be difficult. Due to this, more efforts are required to improve the methodologies. Such improvements may include the use of either solvent-less sample preparation procedures or miniaturizing analytical systems (Ramos et al. 2005). If the methodology on adopting the solvent-free technique is failed, the option for green alternative is to replace organic solvents using green solvents.

(c) New extraction media

The search for alternative solvents/new extracting media is another difficulty. This is a very vital step in chemical analyses. As discussed earlier on, subcritical water and ionic liquids (ILs) are some of the most popular green solvents employed as alternatives for organic solvents. Subcritical water has several advantages because it is nonflammable, easily available, and cheap. However, methods utilizing subcritical water are infrequently utilized because of their technical complexity. However, the great challenge available is their production in smaller amounts, which makes them costly.

(d) Miniaturization and amalgamation of analytical systems

Another challenge is miniaturization and amalgamation of analytical systems. It is a promising technology involving chemical analysis in the direction of green analytical chemistry. Miniaturization sets out to come up with tools that are more efficient, more flexible, and cheaper than the conventional ones. This system also includes sensor systems such as electronic nose, electronic tongue, and also microsystems such as lab on chip (LOC), lab on valve (LOV), and total chemical analysis system (TAS) (Tobiszewski et al. 2010). The remission of environmental hazards through analytical techniques and methods is possible as a result of the developed miniaturization process.

(e) Agents mediating operations and activities in chemical laboratories

Agents mediating operations and activities in chemical laboratories are another challenge which includes the application of the microwave radiation and the ultrasound energy. Microwave radiation is used in extracting analytes, fixing biological material samples, determining water content, sample drying and heating (Tobiszewski et al. 2010). Ultrasonic energy is mainly used in sample dissolution, homogenization, emulsion formation, filtration, analyte extraction, filtration, sample degassing, glassware cleaning, and reagent generation (He et al. 2007). The major benefits of microwave and ultrasound energy from the green chemistry point of view are the substantial decrease in the quantity of solvents used, reduction in waste generation, shortening the time of extraction, reducing the quantity of sample needed and energy input and, hence reduction in the cost (Tobiszewski et al. 2009). However, the application of microwave and ultrasound in sample preparation is still challenging. Upgrading of analytical procedures, particularly the extraction step is the need of the present context (Zlotorzynski 1995). In ultrasound applications, an insufficient sonication power in ultrasound baths inhibits fast extractions and thus heating/higher acid concentration to carry out digestion is recommended to accelerate the reaction. But this recommendation is against the criteria of GAC. In order to achieve the sonication process, powerful cup and probe sonication schemes are recommended to establish complete extractions with maximum efficiency (Bendicho et al. 2012).

(f) Environmental Impact Assessment of laboratory and analytical procedures

According to green chemistry, the consideration of environmental health impacts is most significant. Challenges on environmental impact assessment due to laboratories and analytical procedures are still unclear. In support of green analytical chemistry, there are no established methods available at this moment. In spite of the claims from the authors in the literature (Van der Vorst et al. 2009) regarding the green analytical procedures, it seems that such self-claimed discoveries are not backed by any metric proof or comparison with standard or analytical procedures developed earlier.

5 Evaluation of Green Analytical Techniques

In green chemistry, a variety of methods are utilized in the assessment of product life cycles, processes, and chemical species. Nevertheless, the most widely used tools are the LCA and twelve ideologies of green chemistry (Mulvihill et al. 2011). The way in which the LCA is utilized as a tool for describing processes, products, and making a comparison between different parts of the life cycle has been widely reported. In addition, the execution of green chemistry techniques leads to improvements on environmental conditions and can be transmitted throughout the life cycle. Therefore, assessing the greenness of green analytical chemistry is a crucial approach since it helps to understand how green the system is (the degree of its sustainability) in comparison with the current situation in terms of consumption and efficiency (Anastas and Lankey 2000).

(a) Evaluation of greenness of green analytical techniques in terms of energy

In green analytical techniques, energy is considerably important in revealing the economic aspects and also has a vital environmental effect when utilizing the impact assessment strategy toward the LCA. As analytical procedures are enriched, advantages by way of energy remission seem rather possible and become one of the objectives to achieve 100% sustainability. For example, in analytical procedures, requirements for energy reduction could be accredited to reducing temperature of reactions, which could be as a result of enhanced catalytic activity or incorporation of a cocatalyst (Gonzalez and Smith 2003).

With regard to energy, analytical procedures should operate at lower or ambient pressure and temperature. Such procedures should also employ minimum recyclable as well as separation steps that are energy intensive and lead to a decreased energy input. The energy economy is worth mentioning when the green analytical technique avoids the need to accelerate chemical processes with ultrafiltration, crystallization, purification of products through distillation, control reactivity through cooling, and heat. However, alternative analytical techniques such as sono-, photo-, or microwave-assisted chemistry have been applied not only for the energy saving but also for reducing the prolonged reaction time, simplifying experimental conditions, and increasing the effectiveness of catalysts (Beach et al. 2009).

(b) Evaluation of greenness of green analytical techniques in terms of reagent consumption efficiency

Consumption of reagents during reactions which ultimately lead to waste production at the end is one of the decisive factors on accounting the technique as green. Many of the reagents utilized may be extremely hazardous which can lead to air pollution, flammability hazards, and risks of exposure to workers and the surrounding community. It is imperative that such reagents need to be planned to lessen the human and environmental hazards. However, recycle of the reagents may possibly be achieved by processes of distillation that are energy intensive. Green chemistry has made a significant headway in decreasing the undesirable impacts of the reagents in use. In the present situation, every researcher has no way to admit the term "no solvent/reagent" is green as the toxicology and environmental impact of such solvent determines its greenness based on its entire life cycle (Stark et al. 2010). Even though, it has been suggested that "the best solvent is no solvent," the guidelines for the alternative green reagents (water, ionic liquid, and supercritical CO_2) instead of problematic reagents are recommended for the process assisted by reagents (Sheldon 2007; Jiménez-González et al. 2004). Moreover, a better selection of reagents that functions well for reactions/processes intend to save energy and minimize waste.

Through green chemistry techniques, miniaturization and solvent-less techniques using ultrasound and microwave instruments, remission in the utilization of reagents and hence the operational costs, hazards and wastes an appreciably be achieved. Moreover, the advancement in techniques such as flow injection analysis, membrane separations, and solid phase extraction has significantly minimized the reagent consumption and impact of analytical procedures on the environment (Licence et al. 2003). In addition, the advancement of spectroscopy has contributed to using research-based instruments in-process or on-site. In attribution to the advancement of spectroscopy, NMR spectroscopy is utilized in monitoring flow of materials with lower field NMR procedures fabricated to execute different measurements (such as quantity of fluorine, quantity of fat, and quantity of moisture in solid materials) in the absence of purification steps, separation, and pretreatment (Nordon et al. 2001) which helps for the lesser use of hazardous solvents.

6 Impact of Green Analytical Techniques on Man as well as the Environment

The impact of green analytical chemistry on man and the environment is discussed as follows:

- The environmental impact of green analytical procedures,
- Life cycle assessment (LCA),
- Eco-scale, and
- Eco-campus

It is necessary to assess the environmental impact of green analytical procedures. This could be achieved by environmental impact assessment, environmental impact indices, environmental risk assessment, and cost–benefit analysis of the green analytical approaches.

6.1 Environmental Impact Indices and Environmental Impact Assessments

The environmental impact assessment (EIA) is widely used to detect the effect of economic activities such as green analytical procedure on the environmental segment at a particular time (UNEP 1996). In green analytical techniques, the EIA is usually aimed at (1) quantitative and qualitative determination of the possible socio-economic and environmental issues associated with a proposed analytical process, (2) furnishing decision makers with the results from the EIA process in order to make them informed about the choice. This is very vital because the deleterious effects of the proposed analytical activity on the environment can be mitigated and the benefits of the activity in the environment can be identified. Formulation and application of environmental impact indices is one of the aspects of the EIA. Particularly, checklist methodologies that provide a list of categories of environmental impacts associated with a particular analytical approach are adopted. Although this aspect of EIA is able to include a larger number of relevant environmental issues, there is a need to make a sense of greater amounts of data generated from the quantitative as well as rigorous analyses. In addition, little has been done to combine the findings of EIAs with economic, market, and technical analyses (Golonka 1996).

6.2 Assessment of Environmental Risks

Assessment of environmental risks encompasses approximation as well as valuation of environmental risks arising from a specific exposure or action. The exposure may be connected to any segment of the products' life cycle, for instance, in product application or dumping, in addition to storage, transportation, and processing of substances during the course of distribution. Parameters such as equipment failure, anomalies in control mechanisms of instruments, plant operation errors by humans may contribute to a number of irregularities in practices and processing. Assessment of environmental risks and safety must interact in some specific circumstances as can be exemplified by the loss of contaminant materials that are toxic and might result into human and environmental damages. The concept of environmental risk assessment plays a vital role in the environmental risk management which makes an emphasis on the occurrences and the reducing probability of risk factors.

6.3 Cost–Benefit Analysis

This is a method used to estimate environmental impacts of processes including green analytical techniques. In the cost–benefit analysis, an estimation on the economic value of environmental quality losses (externalities) which is nonaccounted within the regular structure of market pricing is conducted. In the determination of the environmental impacts of green analytical techniques, the total net present value for the techniques is acquired from the total market, environmental costs and benefits. Although cost–benefit analysis is an effective and reliable tool, the issue of factoring in externalities is very complex since it takes into consideration a number of complexities as well as uncertainties. The capacity to put a feasible monetary value of impacts on the environment emanating from numerous mechanisms of controlling discharges is not likely to be attainable in the background of the majority of present-day decisions in engineering designs.

(a) Life cycle assessment (LCA)

This is a means employed to evaluate and enhance performance of analytical processes on the environment. LCA is a pillar of sustainability by giving influential insights into the entire value chain (ISO 2006a, b; ILCD 2010). LCA is executed to evaluate a priori reaction pathways and impacts of analytical processes on the environment (Hafizan et al. 2016). As a result of LCA's performance, it is known to

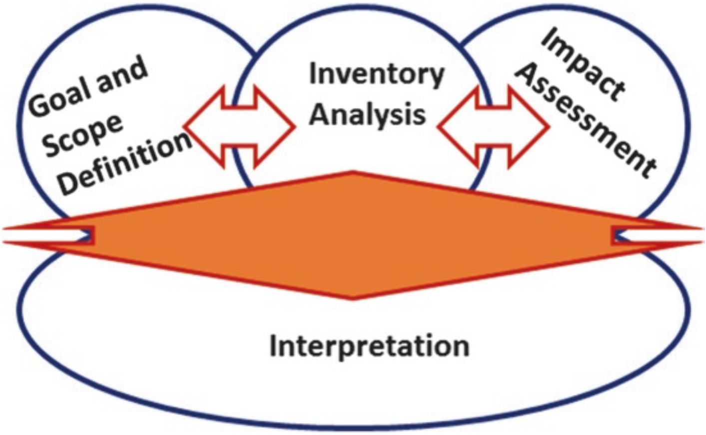


Fig. 16.7 Stages in the LCA approach

circumvent impacts as a consequence of places, categories, and analytical processes. In green analytical techniques, the LCA approach provides support by making various decisions such as supply chain reagents and selection of ideal and, effective analytical processes. Hence, LCA has its appreciation as a vital approach in the environmental proficiency of an analytical product or process and a valuable technique for evaluating the likely environmental impacts of an analytical processes or products throughout the life cycle of process.

The four stages (Fig. 16.7) of LCA approach are (1) goal and scope definition stage, (2) analysis of inventory stage, (3) assessment of impact stage, and (4) interpretation stage.

The definition of goal and scope stages consists of the system boundary, research aims, and objectives. It basically identifies an analytical product, process, and methodology in addition to the identification of the context as well as system boundaries. The analysis of inventory stage gives an outline of the input and output information regarding the analytical process under study. This stage identifies and quantifies inputs such as reagents, water and energy, and throughputs released into the environment. The assessment of impact stage, also known as the life cycle impact assessment (LCIA) stage utilizes inventory stage data analysis to assess probable effects of analytical process on men and the environment. During the interpretation stage, the data from the analysis of inventory, assessment of impact to make goal- and scope-based interpretations are performed to draw conclusions and make recommendations on a preferred analytical process and the product.

For the LCA approach to be successful, the available and collected data ought to be accurate and reliable. This means that a clear comprehension of assumptions and uncertainties is very critical in the LCA approach. LCA in green analytical techniques must combine the analysis of costs, evaluation of technical aspects, and social metrics for a holistic analysis of its sustainability.

(b) Eco-scale

Eco-scale (Van-Aken et al. 2006) is one of the approaches to evaluate environmental impacts on green analytical techniques. It is one of the most effective tools because it offers a simply comprehensible classification on the basis of ranking scale with a 0 to 100 range fixed by the attribution of various factors such as waste, hazards, and amount of reagents (Gałuszka et al. 2012). An ideal chemical process that takes place at room temperature with nonexpensive reagents and acknowledged harmless both to the environment and the user is justified with a typical score of 100. Each parameter is assigned penalty points that diverges from the green analytical techniques ideality by reducing the total score (Gałuszka et al. 2012). The higher the score on the Eco-Scale, the greener and more economical the analytical technique is. Since the impact of hazardous compounds is a function of their quantity, the summation of penalty points is computed based on the product of subtotal penalty points for a particular hazard. For the whole analytical process, all penalty points are included in the Eco-Scale computation, as per the equation as follows:

$$\text{Eco} - \text{Scale} = 100 - \text{the sum penalty points}$$

The resulting analytical Eco-Scale value from the above equation is ranked on a scale and interpreted as presented in Table 16.1.

It is worth noting that some of analytical procedures may not be capable of achieving the plausible analytical Eco-Scale ranking in spite of having certain benefits for determining certain groups of analytes (Gałuszka et al. 2012). For instance, analysis of samples for organic compounds is generally complex and takes place in multiple stages as opposed to analysis of samples for trace elements. The analysis of samples for organic compounds increases the penalty points and reduced rankings on the Eco-Scale. This means that the use of the Eco-Scale is particularly significant while trying to find novel, greener analytical techniques since it undoubtedly and markedly signifies the points in analytical procedures that are weaker (Gałuszka et al. 2012).

(c) Eco-Compass

The Eco-Compass technique is also one of the tools used to evaluate environmental impacts of green analytical techniques (Yan et al. 2001). The Eco-Compass works by comparing an existing analytical process or product with another or by comparing a current analytical technique or product with a newly developed option. The Eco-Compass approach is based on the ISO 14001 standard on environmental management system but is more centered on environmental protection. Eco-Compass is aimed at encouraging real actions that have the capacity to both minimize adverse

Table 16.1 The explanation of Eco-Scale values

Eco-Scale value	Meaning
<50	Inadequate green analytical technique
51–74	Satisfactory green analytical technique
>75	Excellent green analytical technique

Table 16.2. Six Eco-Campus dimensions

Eco-Campus dimension	Description
Mass intensity	Represents the transformation in consumption of material and mass burdens associated with the whole life cycle of analytical products.
Energy intensity	Accounts for energy consumption changes with respect to analytical products life cycle.
Health and environmental potential risk	Identifies environmental burden changes associated with the whole life cycle of analytical products.
Revalorization	Assesses how easy it is to carry out recycling, reuse, and reprocessing of analytical products.
Resource conservation	Determines changes in material conservation as well as energy related to the life cycle of analytical products.
Service extension	Evaluates the degree of service delivery in the entire life cycle of analytical products.

effects of analytical processes and maximize operations. The Eco-Compass approach is particularly suited for laboratory-based operations and it has six dimensions. These dimensions are service extension, resource conservation, revalorization, health and environmental potential risk, energy intensity, and mass intensity (Yan et al. 2001). Table 16.2. explains each of the six dimensions as follows.

In the Eco-Compass approach, a particular analytical product to be linked is selected as the baseline case for comparison and the alternate produce is awarded a notch relative to the baseline case. The base case is given a score of 2 in each of the 6 dimensions of the Eco-Compass. The alternate products of analytical techniques are then allocated a value comparative to the base case on a 0–5 range of score on every dimension of the 6 Eco-Compass. The exact score is dependent on the percent increase or decrease in efficiency of the method. The 6 dimensions of the Eco-Compass are utilized to assess important environmental factors related to an entire life cycle of an analytical product. The 6 Eco-Scale dimensions plus the cost and benefit of analytical processes are used to determine the index vector '*c*' which is utilized to determine the proficiency of life phases, analytical procedures, and dissimilar life loci of analytical products. The quantity '*c*' is calculated using the following equation:

$$\begin{aligned} \mathrm{c} = {} & \text{benefit} + \text{cost} + \text{service extension} + \text{resource conservation} \\ & + \text{revalorisation} + \text{health and environmental potential risk} \\ & + \text{energy intensity} + \text{mass intensity} \end{aligned}$$

7 Green Analytical Chemistry and Sustainable Development

It is an important concept in the implementation of green chemistry principles and objectives by making analytical methods green. In the process of making analytical procedure greener, sample preparation presents a major concern because of the large quantities of organic solvents used compared to other stages in the analytical process. A number of solutions as alternatives have been put forward to overcome the challenge of solvents in sample preparation. Novel sample preparation techniques that require no or less solvents have been developed. For example, the use of solid-phase micro extraction technique (SPME) which uses sorptive fiber rather than solvent (Turner 2013). The SPME technique has further been developed to introduce selectivity of analytes such as in molecularly imprinted SPE. In this procedure, a sorptive polymer is synthesized with a template molecule that introduces binding sites specific to the analyte molecule.

Chromatography plays an important role in analytical techniques for the separation of different compounds present in less complex matrices before detection stage. In the process of making chromatographic techniques greener, the use of miniaturized separation systems and greener solvents such as subcritical water is adopted. The use of gas chromatographic techniques as well as capillary electrophoresis is regarded greener than normal liquid chromatography. Supercritical carbon dioxide has been introduced as mobile phase in chromatography to substitute traditionally used solvents which poses harmful impacts to human and the environment. Another important area in analytical methods is the analyte detection stage. In greening, the detection part of analytical techniques emphasis is made on the in situ analytical techniques. In situ sample analysis reduces many sample preparation steps which then avoids the use of lots of chemicals. For example, Casadio et al. (2010) used Raman spectroscopy for in situ detection in monitoring procedures for analyzing dyes and paints.

Analytical techniques need be evaluated to assess the degree of greenness offered by different analytical procedures. Several researchers have used the term green analytical techniques without indicating the greenness of the procedures carried out in analytical chemistry. In order to obtain data on environmental impact of an analytical method, a few universal green metric tools are used to compare various parameters of analytical processes. A detailed account of the green metric tools used in analytical chemistry is given by Tobiszewski (2016). Some of these tools are briefly described herein.

The first introduced approach is the National Environmental Methods Index (NEMI) developed in US, which considers things like persistent, bioaccumulative, and toxicity of chemicals used. NEMI also considers hazardous criteria, corrosive nature during analysis ($pH < 2$ or > 12), as well as waste generated by the analytical procedure. However, NEMI does not take into account amount of energy used to prepare chemicals used in the analytical process. Another green metric tool used for the environmental impact assessment is the globally harmonized system of classification and labeling of chemicals (GHS) (Gałuszka et al. 2012). This method uses physical, environmental, and health hazard information to create an analytical eco-scale system utilizing penalty points for analytical processes that do not conform to the principles of green analytical chemistry.

The LCA tool is a very powerful green metric system since it takes into consideration the total environmental impact of chemicals from the production point to its disposal. One example is available where LCA has been applied in analytical chemistry. In a study by Van der Vorst et al. (2009), a comparison of preparative HPLC and SFC revealed that preparative HPLC is 26% more demanding in terms of resources than SFC separation. However, in terms of energy requirement the results were opposite in which preparative SFC required more energy for heating and cooling. So far, the green analytical method assessment tools described earlier consider only one component of the sustainable development, the environment. Other components, the economy, and the society also ought to be considered for green analytical techniques to be sustainable.

8 Conclusions

The guidelines of green chemistry facilitated toward syntheses of new materials and associated scientific process in preventing wastes. Owing to the hazardous nature of hydrocarbon solvents, the replacement of green solvents could lessen the usage of the former up to 85%. The possible solvent-free conditions substituting microwave energy could be achievable for the determination of interaction with matter. Renewable fuels such as biodiesel and ethanol are the outcome of imperishable green feedstocks. Enzymes capable of targeting specific sites without affecting the rest could eliminate the need for protecting groups. Identification of ionic liquids (as partitioning solvents) instead of conventional hazardous solvents in liquid–liquid extraction for different analytes seems quite meritorious. The LCA is appreciated as a prominent instrument for the likely effects on the analytical process or product.

9 Future Trends

The significance of sustainable processes in research and development is at its nascent stage which is anticipated for a greater establishment in the future years. The syntheses of oxygenated organic solvents like 2-methyl tetrahydrofuran, tetrahydrofuran, ethyl acetate, isopropyl alcohol, ethanol, methanol, methyl ethyl ketone, and acetone from renewable feedstocks rather than petroleum are presently viewed with much efforts by the researchers and this would have a great breakthrough to a sustainable trend possibly in the near future. However, the above accomplishments thus far achieved are a prelude to the envisaged challenges in the future. A systematic transformative innovation through sustainable development rather than incremental improvement is possible only when mutually reinforcing aspects of green principles are coerced. Multifunctional catalytic system for carrying out entire synthesis in one pot will scrupulously bring synthetic chemistry into a new level with higher material and energy efficiency. As of now, several approaches have lately been emerged to exclude the utilization of organic solvents in ionization techniques for mass spectrometry. Current and future explorations based on alternative green solvents, electrode materials, and the design of electro-analytical methods with miniaturized systems offer novel and exciting working strategies in pertinent to green chemistry principles. As per the statement of the Nobel Laureate Ryoji Noyori "Green Chemistry is not just a mere catch phrase; it is the key to the survival of mankind."

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Chapter 17
Emerging Aspects of Bioremediation of Arsenic

Anurakti Shukla and Sudhakar Srivastava

Abstract Arsenic is a toxic element whose widespread contamination in highly populated regions of world has led to environmental and human health concerns. Millions of people residing in contaminated areas are forced to drink water and eat food containing arsenic beyond maximum permissible limits. As the extent of problem is huge, there is need to devise cost-effective measures to tackle the problem. Physicochemical methods available presently are costly and are not easily operable by the poor people. Bioremediation comprises application of biological organisms and/or components in the removal/stabilisation of the contaminant. This review will focus on arsenic removal aspects of bioremediation and will also discuss prospects of utilising biological components for restricting arsenic entry into crop plants specifically rice.

1 Introduction

Arsenic (As) is a crystalline metalloid that is the 20th most abundant trace element in the earth's crust (Mandal and Suzuki 2002). It mostly occurs in four oxidation states: arsenate [As(V)], arsenite [As(III)], arsenic [As(0)], arsine [As(III)] and among these, As(V) and As(III) are the most prevalent inorganic forms (Abedin et al. 2002). There are some methylated As compounds, e.g. monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO) that also exist in nature (Huang and Matzner 2006). MMA and DMA are widely used in pesticides and herbicides. Arsenobetaine, arsenolipid and arsenocholine are other compounds of As which are mainly found in marine animals or sea foods (Huang and Matzner 2006; Sele et al. 2012). Thus, As contamination is now becoming a growing menace as it can be fatal to the human health. This high concentration of As also causes imbalance in soil diversity and even has negative impacts on plant growth. So the need of the hour is to reduce As pollution from both soil and water and one of the suitable and cost-effective options for this is bioremediation.

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Bioremediation is a biological process where living organisms are used to degrade and detoxify harmful chemicals and compounds. Moreover, now these organisms are also used to detect and monitor harmful compounds in the environment (Vidali 2001). It is a slow process but an eco-friendly approach (Valls and Lorenzo 2002). This process can be characterised in several other categories depending upon the organisms used, viz. microbe, plant or both. Apart from this, biological by-products may also be used in bioremediation and then this is known as by-product-based remediation. There are a number of mechanisms through which bioremediation is achieved. These include biotransformation, bioaccumulation, biosorption, phytosequestration, phytovolatilisation, phytoextraction, phytofilteration and phytoaccumulation and further the metals can be retrieved by phytomining process. The basic principle behind bioremediation is alteration in the redox reaction, pH fluctuations and enhancement/reduction of the As solubility using complexation reactions (Smith et al. 1994). It is believed that hyperaccumulation of As is a function of interaction between As and high-affinity chelating molecules present in the cytoplasm of plant cells (Tangahu et al. 2011).

2 Sources of Arsenic Pollution

Arsenic contamination in the environment (hydrosphere, lithosphere and atmosphere) occurs either by natural or anthropogenic processes (Table 17.1). Natural processes include dissolution of As compounds onto pyrite ores by geochemical factors, during atmospheric emissions, groundwater contamination through arsenic traps (As adsorbed authigenic secondary oxide/hydroxide precipitates) (Bhattacharya et al. 2003), volcanic eruption, wind deposition and weathering of As-bearing minerals. Anthropogenic sources for As contamination are mining, coal combustion, semiconductor industries, smelting, insecticides, herbicides, phosphate fertilisers and timber preservatives (Mondal et al. 2006; Bundschuh et al. 2011). Arsine, a toxic gas containing As is also liberated from marshy land, swampy surfaces along with MMA and DMA (Duker et al. 2005).

Table 17.1 Sources of arsenic pollution

Natural sources	Anthropogenic sources
Dissolution of arsenic compounds onto pyrite ores	Mining and smelting activities
Volcanic eruptions	Semiconductor industries
Wind mobilisation	Insecticides
Arsenic traps	Herbicides
Weathering of As-bearing minerals	Phosphate fertilisers
Marshy lands and swampy surfaces	Coal combustion
Arsenic-rich sediments	Timber preservatives
Marine aerosols	Use of roxarsone as additive in feeds

3 Bioremediation as a Solution for Arsenic Menace

Bioremediation is a process where living organisms or biological interventions are used to eliminate the deleterious effects of pollutants from environment. Unlike physical and chemical processes, biological process is little slow but its effects are long lasting and eco-friendly. It has been successfully applied on soil, surface water, ground water, sediments and is even used in eco-restoration. On the basis of organisms used, bioremediation has been categorised in several categories like microbial remediation (microorganisms), phytoremediation (plants), phytobial remediation (endophytic organisms), mycoremediation (fungi) and phycoremediation (algae). Nowadays, even agriculture waste, whole biomass and by-products from living organisms are also utilised in this process. This is gaining momentum due to its low maintenance and high efficiency property. Input-to-output ratio is very high, i.e. the cost involved in selecting biomass as a source of bioremediation is very low in comparison to its efficiency. Thus now each and every part of living entities can be used in bioremediation. Table 17.2 lists various approaches that have been used for the bioremediation of arsenic.

3.1 Microbial Remediation

Microorganism-assisted remediation process is the most exploited process for eco-restoration of metal-contaminated sites. The organisms are tiny in size and have very rapid life cycle because of their acute doubling time. They have very small DNA with lots of genes responsible for metal detoxification. Even, some of them have plasmid DNA which can be further used for gene modification and gene amplification. These microorganisms can tolerate extreme polluted environment through their innate adaptation process. Hence, they serve as suitable candidates for bioremediation of heavy metals. A number of studies have concentrated on the screening and identification of potential arsenic (As) tolerant and/or remediator bacteria from diverse As-contaminated areas and from various rhizospheric environments. These microorganisms sequester heavy metals through various mechanisms like bioaccumulation, biotransformation and biosorption.

Bioaccumulation is the most common among all and this property of microorganisms is widely exploited for industrial processes. It is energy-consuming process of metal uptake. Bioaccumulation potential candidates must have a tolerance for multiple contaminants. The possible mechanism used by microorganism in this process is the production of metal-binding proteins such as metallothioneins (MTs) in order to increase the metal-binding capacity (Krishnaswamy and Wilson 2000). *Brevibacillus brevis* is an arsenic-tolerant bacterium that can tolerate up to 1000 mg/L. of arsenite and 500 mg/L. of As(V) (Banerjee et al. 2013). In another study, a novel As(III)-oxidising bacterium (As7325) was isolated from Taiwan that could oxidise 2300 mg/L. As(III) in As(III)-contaminated groundwater under aerobic conditions

Table 17.2 Examples of bioremediation of arsenic by the use of various approaches

Bioremediation	Mechanisms used	References
Microbial remediation		
Brevibacillus brevis	Bioaccumulation	Banerjee et al. (2013)
Marinomonas communis	Bioaccumulation	Takeuchi et al. (2007)
Corynebacterium glutamicum	Bioaccumulation	Mateos et al. (2006)
Arthrobacter sp.	Biosorption	Prasad et al. (2013)
Bacillus cereus	Biosorption	Giri et al. (2013)
Acidithiobacillus ferrooxidans	Biosorption	Yan et al. (2010)
Staphylococcus xylosus	Biosorption	Aryal et al. (2010)
Pseudomonas sp.	Biotransformation	Lampis et al. (2015)
Brevibacillus brevis	Biotransformation	Banerjee et al. (2013)
Bacillus selenitireducens	Biotransformation	Lloyd and Oremland (2006)
Pseudomonas stutzeri	Biotransformation	Maity et al. (2011)
Phytoremediation		
Pteris vitatta	Phytoaccumulation	Ye et al. (2011)
Modified Arabidopsis thaliana	Phytoaccumulation	Doucleff and Terry (2002)
Ceratophyllum demersum	Phytoaccumulation	Weis and Weis (2004)
Hydrilla verticillata	Phytoaccumulation	Nigam et al. (2013)
Retama sphaerocarpa	Phytostabilisation	Moreno-Jiménez et al. (2011)
Viola baoshanensis	Phytoextraction	Macek et al. (2000)
Sedum alfredi	Phytoextraction	Zhuang et al. (2007)
Rumex crispus	Phytoextraction	Zhuang et al. (2007)
Arundo donax	Phytovolatilisation	Mirza et al. (2011)
Mycoremediation		
Penicillium sp.	Biovolatilisation	Edvantoro et al. (2004)
Ulocladium sp.	Biovolatilisation	Edvantoro et al. (2004)
Glomusmosseae	Bioaccumulation	Wu et al. (2009)
Saccharomyces cerevisiae	Bioaccumulation	Roy et al. (2013)
Phycoremediation		
Chlorella sp.	Biotransformation	Levy et al. (2005)
Monoraphidium arcuatum	Biotransformation	Levy et al. (2005)
Scytonema sp.	Biosorption	Prasad et al. (2006)
Chlamydomonas reinhardtii	Biotransformation	Miyashita et al. (2011)
Fucus serratus	Biotransformation	Geiszinger et al. (2001)
Phytobial remediation		
Lysinibacillus sp.+ *Pteris vittata*	Bioaccumulation	Singh et al. (2015)
Agrobacterium radiobacter + Populus deltoides	Bioaccumulation	Wang et al. (2011)
Glomusmosseae + Pteris vittata	Bioaccumulation	Trota et al. (2006)
Hymenoschyphusericae + Calluna vulgaris	Bioaccumulation	Sharples et al. (2000)

in 1 day. Further, these cell pellets of As7325 could efficiently adsorb As(V) (Kao et al. 2013). Van et al. (2010) utilised the services of bacteria to reverse the bacterial As reduction through the creation of oxidised zone in groundwater by injection of aerated water having DO of more than 4 mg/L. This stimulated the growth of Fe-oxidising bacteria and As-oxidising chemoautotrophic bacteria while inhibited that of As-reducing ones. *Marinomonas communis* are the potential candidates for bioremediation of As from contaminated water as it can accumulate around 2290 μg/g (dry weight) (Takeuchi et al. 2007). Another bacterial strain, *Corynebacterium glutamicum* has very high resistance to As stress [up to12 mmol L^{-1} for As(III) and 40 mmol L^{-1} for As(V)] and has good As removal potential (Mateos et al. 2006).

Biosorption is a passive uptake of metals by microbial cell which lead to the formation of metal–organic complexes with capsules or other extracellular polymers synthesised by microorganisms. It is a fast and reversible process which either work on the principle of adsorption or ion exchange (Gavrilescu 2004). The mechanisms that are utilised in this process are chelation, Van der Waal forces, electrostatic interaction, redox interaction and extracellular precipitation (Dhankhar and Hooda 2010). *Bacillus* sp., *Pseudomonas* sp., *Thiothrix* sp., and *Thiobacillus* sp. are commonly used for biosorption. *Arthrobacter* sp. cell biomass can be used as biosorbant for removal of As from As-contaminated site. Experimentally the biosorption efficiency for As(III) was found to be 74.91 mg/g (pH 7.0) and for As(V), it was found to be 81.63 mg/g (pH 3.0), when 1 g/L. of biomass was used and exposed for a contact time of 30 min at 28 °C (Prasad et al. 2013).

Biotransformation is another mechanism present in microorganisms where they convert one form into other which is less toxic. They are basically based on oxidation and reduction reactions (Oremland and Stolz 2003). This is commonly a detoxification mechanism in microorganisms. It includes transformation of highly toxic As(III) into As(V) (oxidation) and methylation of As to form MetAs compounds (Levy et al. 2005). A strain of *Pseudomonas* sp. was found to produce siderophores, which reduces As(V) to As(III) (Lampis et al. 2015). *Brevibacillus brevis* isolated from West Bengal arsenic-contaminated site is another example that can transform arsenite to arsenate. The strain can transform 90% of arsenite into arsenate (Banerjee et al. 2013). Members of Enterobacteriaceae were found to be most effective in biotransformation as the strains showed 92% efficiency in transforming arsenite to arsenate (Nagvenkar and Ramaiah 2010).

3.2 Phytoremediation

Plant-based remediation which has been studied over the years has been considered the cost-effective and easy operable approach. This is a slow process and often hindered by phytotoxicity, therefore the plants used are either of tolerant species or of hyperaccumulator variety. These plants have efficient detoxification method which is exploited in this approach. These plants have tremendous ability to complex and/

or sequester metal(loid)s in the non-reactive compartments of cells, viz. vacuole (Song et al. 2010). Cell walls and apoplastic spaces too play important role in metal(loid) tolerance (Srivastava et al. 2016). Various aspects of phytoremediation include phytoextraction, phytoaccumulation, phytostabilisation, phytovolatilisation and phytofiltration.

Phytoaccumulation is a process of taking up and concentrating metal(loids) within the root or above ground part. Mostly the plants used in this process are weeds or non-edible plants because these plants have high translocation factor, i.e. the above ground parts also come in picture for accumulating toxic compounds. The best example of phytoaccumulation for As is *Pteris vittata.* Ye et al. (2011) studied co-cultivation of *P. vittata* with rice and found that As removal by *Pteris* reduced As level in rice with significant decline in DMA content. Another example is modified *Arabidopsis thaliana* which has two bacterial genes, *arsC* and γ-*ECS*1. Gene *arsC* codes for arsenate reductase that help in arsenate conversion into arsenite, and γ-*ECS*1 codes for glutamylcysteine synthase that forms thiols which help in As(III) detoxification by forming As-protein thiates that are then stored in vacuoles (Doucleff and Terry 2002). Aquatic plants like *Ceratophyllum demersum* can also be used in phytoaccumulation process which may show up to 20,000-fold arsenic concentration (Weis and Weis 2004). Aquatic plant *Hydrilla verticillata* has been reported as a plant with good biosorption efficacy. This plant was used for arsenic uptake from drinking water where it showed maximum As adsorption of 96.4% at 100 ppb with half a gram of biomass applied in 100 mL for a period of 5 h and the pH was maintained at 6.0 (Nigam et al. 2013).

Phytostabilisation is another important mechanism used in arsenic removal. The plant roots hold the contaminated soils and sediments in a particular place and even immobilise the toxic contaminants in rhizosphere. Metals are non-degradable substances so capturing them in situ is one of the best solutions for low contamination sites. Moreno-Jiménez et al. (2011) conducted a study in Spain for the phytostabilisation of heavy metals (As, Zn, Cu, Cd and Al) and selected *Retama sphaerocarpa* as a potential shrub.

Phytoextraction is a very useful mechanism used for metal recovery. In this the metals get concentrated in the harvestable parts of hyperaccumulator plants. A hyperaccumulator plant is characterised with a very high translocation factor and bioaccumulation coefficient for a particular metal. *Viola baoshanensis, Sedum alfredi* and *Rumex crispus* are few examples of plants that have been used for As phytoextraction (Macek et al. 2000; Zhuang et al. 2007).

Another approach of phytoremediation is phytovolatilisation. In this, volatilisation of contaminants occurs from plants either through leaf surface or from stem surface. Phytovolatilisation of As has been successfully demonstrated by a member of poaceae family, *Arundo donax.* This grass can volatilise up to 7.2–22% As at 300–1000 μg/L. (Mirza et al. 2011). Another example for phytovolatilisation is transgenic rice with incorporation of foreign gene from *Rhodopseudomonas palustris*, arsM. This gene encodes for enzyme arsenic methyltransferase, which transforms As to trimethylarsine [TMA(III)]. This rice was found to volatilise good amounts of As (Meng et al. 2011). Even fungi and algae are extensively used in bioremediation. They are commonly used because they have short life cycle, huge biomass and can propagate asexually and sexually both. The remediation process carried out with the help of fungi is known as mycoremediation and bioremediation process through algae is known as phycoremediation.

3.3 Mycoremediation

There are various fungi that are used in mycoremediation. Fungal strains belonging to the taxonomic group of Zygomycetes are basically exploited for remediation process due to the presence of different biomolecules in their cell wall like chitin, chitosan and glucan. These molecules act like a metal biosorbent. *Aspergillus niger* and *Mucor rouxii,* two representative soil fungi, which have been extensively studied for the adsorption of several metals. Fungi like *Penicillium* sp. and *Ulocladium* sp. helps in As volatilisation as they can methylate As and thus produce MetAs compound which is volatile in nature (Edvantoro et al. 2004). For As remediation arbuscular mycorrhizal fungi (*Glomus mosseae)* play a very important role as it decreases the As content in plants (Wu et al. 2009). *Saccharomyces cerevisiae* is another example of fungi that can remove As up to 90% and is highly effective method for bioremediation of As(III) from the contaminated water (Roy et al. 2013). Even, dead fungi biomass is used in bioremediation. This kind of biomass is readily available and is inexpensive too. Further, the dead biomass does not show any kind of metabolic toxicity as there is no metabolic activity and needs no nutritional supply (Baldrian 2003).

3.4 Phycoremediation

Bioremediation based on microalgae and algae is known as phycoremediation. They are widely exploited due to their minimum growth requirements (sunlight and carbon dioxide) in comparison to higher plants and further, they are potential candidates to tolerate metal toxicity (Kumar et al. 2015). The advantages in using microalgae are rapid metal uptake, time and energy saving, year-round occurrence, low cost, faster growth rate, large surface-to-volume ratio, applicability to water having high metal concentrations or even at low contaminant level (Monteiro et al. 2012). The efficiency of microalgae varies from genera and species, the growth characteristics of alga and its tolerance and accumulation potential for metal (Kumar et al. 2015). *Chlorella* sp. and *Monoraphidium arcuatum* are two microalgae which have been studied for biotransformation of As. These microalgae reduces As(V) to As(III) in the cell, which further transform into methylated species (Levy et al. 2005). Immobilised algal biomass, *Scytonema*, has been utilised effectively to remove As(III) from contaminated water. The tentative mechanism involved in this process was complex formation between arsenous acid and sulfhydryl groups of *Scytonema* surface proteins (Prasad et al. 2006). Several microalgae belonging to genera *Chlorella, Scenedesmus, Chlamydomonas, Phaeodactylum, Spirogyra, Spirulina, Stichococcus, Planothidium, Oscillatoria,* etc. have been used in As bioremediation (Doshi et al. 2007). *Chlamydomonas reinhardtii,* a freshwater green alga is another example for As bioremediation. It can rapidly transform As(V) into oxo-arsenosugar-glycerol and then oxo-arsenosugar phosphate which are less toxic in nature (Miyashita et al. 2011). *Fucus serratus,* a marine water brown alga has been studied for biotransformation of As(V) that leads to the formation of methylated arsenic compounds (Geiszinger et al. 2001).

3.5 Phytobial Remediation

Endophyte-assisted phytoremediation is a recent approach for restoration of metal-contaminated sites. Here microorganisms and fungi together with higher plants are utilised for remediation. This association is beneficial for metal sequestration as it can improve extracellular metal uptake, chelation, ion exchange potential, redox balance and thus help in enhancing metal accumulation. *Lysinibacillus* sp., which has been found to be associated with *Pteris vittata* rhizosphere, is a potential candidate for As bioremediation as it can tolerate As up to 3256 mg/L. for As(V) and 1136 mg/L. for As(III) and also accumulated 23.43 mg/L. of As(V) and 5.65 mg/L. of As(III) (Singh et al. 2015). *Agrobacterium radiobacter* is a highly resistant strain to arsenic. This strain was used with *Populus deltoides.* The endophytic bacteria have enhanced the As accumulation of Poplar (Wang et al. 2011). *Pteris vittata* when associated with AM fungi *Glomus mosseae* showed high accumulation efficiency for arsenic (Trotta et al. 2006). Endophytic strain of fungi *Hymenoscyphus ericae* which forms ericoid mycorrhizal association with *Calluna vulgaris* helps the plant in colonisation of arsenic-contaminated sites. This is also an example of co-evolution as this association is obligate for plants as it helps plants in obtaining phosphate and removing arsenate (Sharples et al. 2000). Hence this type of plant–microbes interaction plays a vital role in bioremediation process as this enhances the speed of the process which is generally high for phytoremediation.

3.6 Natural by-Products Used in Bioremediation of Arsenic

Agricultural wastes or natural by-products obtained from living organisms or even entire biomass can also be used in bioremediation. These materials possess functional groups including amido, alcoholic, acetate, carbonyl, amido, amino, sulfhydryl and hence can serve the purpose of metal removal. Immobilised biomass has been found to be more effective due to its suitable size and porosity for continuous operation. As the biomass remains immobilised, it can be used for metal recovery at a later stage. Biomass can be immobilised with introduction of magnetic properties also may allow better separation of biomass. The life of immobilised biomass is higher and usage is easy. Sud et al. (2008) tested cellulosic agricultural waste for metal biosorption and suggested that processes like adsorption, chemisorptions, complexation, ion exchange and diffusion take place during biosorption. Fox et al. (2012) explored the prospects of using cactus mucilage for As removal. Lab-made solutions were treated with gelling extract (GE) or non-gelling extract (NE) and the treatment resulted in 14 and 9% increase in As concentration at the air–water surface. This was attributed to bonding and transport of As by the mucilage. Baig et al. (2010) reported As removal from water with the use of biomass of stem of *Acacia nilotica.* A company named Bio-recovery Systems Inc., Cincinnati, OH has done some variations of AlgaSORB that are prepared for specific purpose. For As removal, a variant, AlgaSORB-scy with the use of cyanobacteria, *Scytonema*, has been developed (Prasad et al. 2006). Kim et al. (2009) innovatively used single-stranded DNA aptamers for As removal from groundwater in Vietnam. The DNA aptamer, Ars-3 had highest affinity for both As(V) and As(III) and can remove As completely in a short time of 5 min from water containing As ranging from 28 to 739 μg/L. As. Copper–Chromium–Arsenate (CCA) treated waste wood wafers were treated with two-step remediation process. This method was found to be 93% efficient for arsenic removal (Clausen 2000).

4 Case Studies

Sang-E-Noghreh area in Iran has polluted spring water problem. Mitra et al. (2012) tried to remediate the problem by analysing naturally occurring four algal divisions (Cynaphyta, Chlorophyta, Euglenophyta and Heterokontophyta). The study included three sampling sites where Algae–As concentration was found to be in the range of 16,000–75,000 ppb and the As concentration in water was found to be in the range of 2–23 ppb; this showed that bioaccumulation factor (BAF) of those four groups of algae was in the range of 700–12,000. According to the result, the four groups of algae were suggested to be effective against arsenic concentration.

Another study was carried out to investigate As-removal efficiency of an aquatic plant *Hydrilla verticillata* from simulated As-contaminated water in field condition. These aquatic plants (100 g fresh weight) were grown in 8 L. of contaminated water which contain 1500 μg/L. of As(V) for 45 days. After every 15-day interval, As accumulation and biochemical changes were analysed. On 45th day of experiment, As concentration was found to be 388 μg/g dry weight. After approximate estimation of total biomass, the total As removed from the contaminated water was 8546 μg which is 72% of total As supplied. Even, with this huge accumulation these plants were found to be normal and no toxic effects were observed in terms of reactive oxygen species (ROS) production. Hence, this study demonstrated that *Hydrilla,* an aquatic plant can be used for As bioremediation even in field condition (Srivastava et al. 2011).

In 2005, a study was conducted in City of Albuquerque, New Mexico to reduce As load from drinking water. In this pilot-scale model, arsenic hyperaccumulating ferns of genus *Pteris* were used to minimise As concentration from drinking water. The system employed in this process was continuous flow phytofilteration system. The experiment was carried out for a period of 3 months. The system used in this set up produced water having As concentration as low as 2 μg/L. which is even less than the detection limit. The flow rate was as high as 1900 L/day. The total treated water volume was approximately 60,000 L. Fronds were chosen for measurement of As concentration. Prior to the treatment, As concentration in harvested fronds was nearly 20 mg/kg whereas after treatment, the concentration level was found to be in the range of 66–407 mg/kg. As was not detected in roots, indicating high translocation factor and thus this plant can also be used in phytoextraction or metal recovery process. In addition, no significant effect on As uptake was observed from variation in day length, light intensity and humidity and therefore this system was found to be more effective in removing both As(III) and As(V) (Elless et al. 2005).

5 Conclusions

With increase in global pollution, the problem of As contamination is also increasing. So the need of the hour is to elucidate some solutions which are effective and eco-friendly. The true answer to this problem is bioremediation. This process in fact is beneficial in destroying or rendering harmful and toxic metal(loids) using biodiversity and biological interventions. These methods range from utilising single cell organisms to multiple cell organisms. The effectiveness of these strategies entirely depends on the feasibility of organisms used and their detoxification mechanism. Organisms used can either be hyperaccumulators or can be hypertolerant in both ways they are useful in bioremediation. Moreover, this process does not require expensive equipment or highly specialised and sophisticated mechanisms, and therefore, it is relatively easy to implement. It might turn out to be permanent remedy for As contamination.

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Chapter 18

Distillery Wastewater: A Major Source of Environmental Pollution and Its Biological Treatment for Environmental Safety

Pankaj Chowdhary, Ashutosh Yadav, Gaurav Kaithwas, and Ram Naresh Bharagava

Abstract Distillery industries are one of the major sources of environmental pollution because these industries discharge a huge volume of dark-colored wastewater into the environment. The wastewater discharged contains high biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), sulfate, phosphate, phenolics, and toxic heavy metals. On terrestrial region, distillery wastewater at higher concentration inhibits seed germination, growth and depletion of vegetation by reducing the soil alkalinity and Mn availability, whereas in aquatic region, it reduces sunlight penetration and decreases both photosynthetic activity and dissolved oxygen content damaging the aquatic ecosystem. The large volume of dark-colored wastewater acts as a major source of soil and water pollution and thus requires adequate treatment for its safe discharge into the environment. Therefore, the removal of pollutants and color from distillery wastewater is becoming increasingly important for the environment and sustainable development. Thus, this chapter provides the detailed information on the generation, characteristic, toxicity as well as various biological methods employing bacteria, fungi, microalgae, etc. for the treatment of distillery wastewater. In biological treatment approaches microalgae have a number of applications over the conventional approaches as it is useful in wastewater treatment, CO_2 sequestration, cost-effective, sanitation and also in the production of renewable energy sources such as methane gas, biodiesel, biofuel, glycerol, hydrogen gas, biofertilizers, etc. Furthermore, the merits and demerits of existing processes have been also summarized in this chapter.

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1 Introduction

Distillery industry is among the major sources of environmental pollution like water and soil pollution (Satyawali and Balakrishnan 2008). In India, the number of distilleries has gone up to 319 with annual production of 3.25×10^9 L. of alcohol and 40.4×10^{10} L of wastewater (Uppal 2004). The worldwide production of bioethanol was above 50 billion liters in 2007 and over 60 billion liters in 2008 which represents almost 4% of world gasoline consumption (Sanchez and Cardona 2008; Balat and Balat 2009; Mussatto et al. 2010). Mainly four steps are involved in alcohol production such as feed preparation, fermentation, distillation, and packaging (Satyawali and Balakrishnan 2008). The distillery wastewater has dark brown color due to the presence of melanoidins and some other coloring compounds. Molasses-based distillery generates an average 15 L. of spent wash in one alcohol production (Beltran et al. 2001).

In distillery wastewater (DWW), melanoidin is one of the major pollutants causing serious environmental and health problem (Tamanna and Mahmood 2015). Melanoidins are the dark brown to black-colored natural condensation product of sugar and amino acids, recalcitrant compounds (Plavsic et al. 2006; Mohana et al. 2007; Wang et al. 2011; Arimi et al. 2014, 2015). DWW is hazardous for the aquatic fauna and flora because its colored component reduces photosynthetic activity and depletes dissolved oxygen in water bodies (Kumar et al. 1997a, b; Pal and Yadav 2012). The physical and chemical methods suggested for treatment of wastewater are not much effective for decolorization (Jain et al. 2002). Biological methods like anaerobic digestion treatment reduce BOD load of the spent wash, but the substantial amount of organic components and dark brown color left behind requires secondary treatment (Gadre and Godbole 1986). However, fate and extent of toxicity of anaerobically treated DWW remains unknown in the environment (Chandra 2004; Yadav and Chandra 2012).

Treatment through biological way is an incredible alternate for DWW pollutants due to their low cost, environmental friendly, and publicly acceptable treatment (Moosvi et al. 2005; Mohana et al. 2007; Wang et al. 2011). There are various biological processes such as bioadsorption and biodegradation that have been reported having prospective application in color removal from spent wash by fungi such as *Coriolus, Aspergillus, Phanerochaete* and certain bacterial sp. as *Bacillus, Alkaligenes*, and *Lactobacillus* (Kumar and Chandra 2006; Plavsic et al. 2006; Pant and Adholeya 2007; Nwuche and Ugoji 2008; Yadav and Chandra 2012) and yeast (Rajor et al. 2002), etc., for the bioremediation of spent wash. Microbially treated effluent may be less toxic and safe device for effluent management (Chandra 2004).

However, the biological treatment of DWW containing melanoidin largely depends on pH, temperature, concentration of nutrients, oxygen and inoculums size as well as there are some enzymatic systems responsible for the degradation of melanoidin that consists mainly sugar oxidases and peroxidases as sarbos oxidase, glucose oxidase, etc. and also ligninolytic enzyme (Laccase, MnP and LiP) (Wang et al. 2011). Since ligninolytic enzyme (MnP and MIP) showed melanoidin decolorizing activity in the presence of H_2O_2, the decolorizing activity of both sugar oxidases and peroxidases was found to be optimum at a particular pH, temperature and substrate-specific (Boer et al. 2006).

Hence, this chapter contributes and focuses on the DWW characteristics, major pollutants, a toxicological effect on the environment, health hazards, enzymatic biodegradation, and challenges for DWW pollutants degradation have been emphasized. The overall objective of this chapter is to present background information, related to DWW and address the issues requiring further research for sustainable development.

2 Generation of Distillery Wastewater and Its Characteristics

DWW is highly colored and acidic with strong and objectionable odor that presents significant disposal or treatment problem (Arimi et al. 2014). The wastewater generation and its characteristic depend upon the use of raw materials and used chemicals in the process of alcohol production (Fig. 18.1) (Mall and Kumar 1997; Arimi et al. 2014). Alcohol is mainly produced from cellulosic materials. Raw materials,

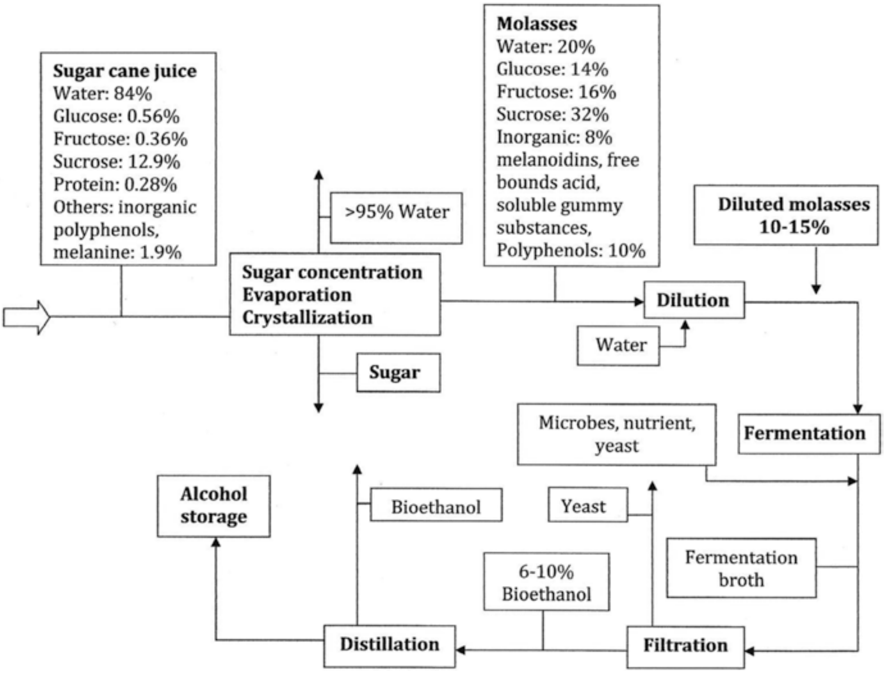

Fig. 18.1 Molasses-based distillery wastewater characteristics and generation from raw cane reeds (Adapted from Arimi et al. 2014)

mainly used in distilleries, are sugarcane molasses, grains, grapes, sugarcane juice, and barley malt. Diluted sugarcane molasses is inoculated with yeast and fermented in either batch or continuous mode to yield a broth containing 6–8% ethanol. In the continuous process, the cellulosic materials first delignified and hemi-cellulose and cellulose are subsequently acid hydrolyzed into simple sugars. In this process, yeasts are mainly responsible for the fermentation of sugar and produced ethanol and CO_2. The alcohol vapor is removed from the fermentation solution under reduced pressure and subsequently distilled. CO_2 gas may be sparged throughout the fermenting solution in order to aid in the removal of the alcohol from the fermenting solution (Tewari et al. 2007; Satyawali and Balakrishnan 2008). The gaseous CO_2 is captured and utilized for the manufacture of additional quantities of ethanol or other basic chemicals.

The spent wash generated from distilleries has high COD (80,000–100,000 mg/L) and BOD (40,000–50,000 mg/L), high temperature, and is dark brown in color having low pH (5.4–4.5) (CPCB 1994). There are various types of DWW having very high levels of BOD, COD, TDS, TS, TSS, as well as high K, PO_4^- and SO_4^-, etc. (Table 18.1). COD and BOD values of this spent wash are due to the presence of a number of organic compounds, such as polysaccharides, reduced sugars, lignin, proteins, melanoidin, waxes, etc. The amount of inorganic substances such as N, K, PO_4^-, Ca, and SO_4^- is also very high in DWW (Jain et al. 2002; Melamane et al. 2007).

In addition, cane molasses spent wash contains low molecular weight compounds such as lactic acid, glycerol, ethanol, and acetic acid (Wilkie et al. 2000; Bharagava and Chandra 2010). Spent wash contains about 2% melanoidin which has an empirical formula of $C_{17-18}H_{26-27}O_{10}N$ and molecular weight between 5000 and 40,000 kDa (Kalavathi et al. 2001; Martin et al. 2002; Manisankar et al. 2004). These compounds have antioxidant properties, which render them toxic to many microorganisms such as those typically present in wastewater treatment processes (Kumar et al. 1997a, b; Kharayat 2012).

The distilleries and fermentation industries wastewater are the major source of aquatic and terrestrial pollution due to the presence of water-soluble recalcitrant coloring compounds called melanoidin (Tiwari et al. 2012). The characteristics of the DWW generated by various processes differ considerably. This is because the final DWW is a function of the feedstock used as well as the waste management philosophies adopted by individual distillery plants.

3 Environmental Pollution and Toxicity of Distillery Wastewater

Distilleries industry produces on an average of 15 L. of effluent per liter of alcohol which has the characteristics of BOD as 40,000–50,000 ppm and COD as 80,000–100,000 ppm (Sankaran et al., 2014). Whereas a medium-sized alcohol distillery can produce approximately 1 × 10^6 L. of ethanol per year (Wilkie et al. 2000), this

Table 18.1 Physico-chemical characteristics of distillery wastewaters

	Wastewater types					
Parameter	Distillery wastewater	Wine distillery wastewater	Vinasse	Raw spent wash	Lees stillage	Molasses wastewater
BOD_5 (g/L)	30	0.21–8.0	42.23	–	20	–
COD_T (mg/L)	100–120	3.1–40		37.5	–	80.5
COD_S (mg/L)	–	7.6–16	97.5	–	–	–
TOC (mg/L)	–	2.5–6.0	36.28	–	–	–
Ph	3.0–4.1	3.53–5.4	4.4	4–5	3.8	5.2
EC	346	–	–	2530	–	–
Alkalinity (meq/L)	–	30.8–62.4	–	2	9.86	6000
Phenol (mg/L)	–	29–474	477	–	–	450
VFA (g/L)	1.6	1.01–6	–	–	0.248	8.5
VS (g/L)	50	7.340–25.4	–	–	–	79
VSS (g/L)	2.8	1.2–2.8	–	–	0.086	2.5
TDS mg/L	–	–	51,500	–	–	51,500
TS (g/L)	51.5–100	11.4–32	1.5–3.7	2.82	68	109
TSS (g/L)	–	2.4–5.0	–	–	–	–
MS (g/L)	–	6.6	–	–	–	30
MSS (g/L)	–	900	100	–	–	1100
TN (g/L)	–	0.1–64	–	2.02	1.53	1.8
NH_4^+ (mg/L)	–	140	–	125–400	45.1	–
NO_3^- (mg/L)	4900	–	–	–	–	–
TP (g/L)	–	0.24–65.7	–	0.24	4.28	–
PO_4^{3-} (mg/L)	–	130–350	–	139	–	–

Adapted from: Nataraj et al. (2006), Bustamante et al. (2005), Martin et al. (2002), Melamane et al. (2007), Yadav and Chandra (2012), Arimi et al. (2014), and Prajapati and Chaudhari (2015)

approximates to ten million liters of wastewater production annually. DWW contains high amount of organic and inorganic pollutants which is generated from different process during alcohol production. The DWW also contains major natural colorant products that are of a wide variety and have been well documented including carotenoids, chlorophyll, heme pigments, anthocyanins, betalains, riboflavins, quinones pigments, caramels, melanoidins, alkaline degradation of hexoses (ADPH), and melanins (Dai and Mumper 2010).

Moreover, polyphenolic compounds (colorant) are important antioxidants, antimicrobial and anticarcinogenic activities with mechanisms involving both free radical scavenging and metal chelation (Silvan et al. 2006; Borrelli et al. 2003). The occurrence of polyphenols in molasses wastewater is dependent on both the sources of molasses and the sugar concentrations in the feed flow. In general, polyphenols in most molasses wastewater are in the range of dozens to hundreds of mg/L (Martin et al. 2003; Jimenez et al. 2004; Bustamante et al. 2005). They are categorized into three broad classes: phenolic acids, flavonoids, and tannins. Phenolic acids have been detected in molasses DWW, including benzoic acid and its derivatives (e.g., gallic acid), cinnamic acid and its derivatives (e.g., coumaric acid, caffeic acid, chlorogenic acid, and ferulic acid) (Payet et al. 2005, 2006; Incedayi et al. 2010). Pollutants which are found in DWW have high toxic effect for sounding environment, in which melanoidins play a crucial role.

3.1 Melanoidins as a Major Pollutant

The formation of melanoidins is the result of polymerization reactions of highly reactive intermediates formed during Maillard reaction. In the formation of melanoidins compounds several reactions occur including cyclizations, dehydrations, retroaldolizations, rearrangements, isomerizations, and further condensations. The molecular weight of colored compounds increases as browning proceeds (Kim and Lee 2009). The complexity of Maillard reaction has been extensively studied during recent years and new important pathways and key intermediates have been established (Martins et al. 2001).

Although melanoidins are found to be chemically diverse such as in coffee, they were determined to be negatively charged but heterogeneous with respect to their polyanionic behavior (Bekedam et al. 2006, 2007); high molecular weight (HMW) melanoidins in coffee were found to be more negatively charged than low molecular weight (LMW) melanoidins (Bekedam et al. 2008). Chlorogenic acids have been hypothesized to be the source of the negative charges in coffee melanoidins (Bekedam et al. 2008; Wang et al. 2011), though melanoidins obtained from sugar-amino acid model systems also showed anionic characteristics in the absence of chlorogenic acids (Cosovic et al. 2010; Morales 2002). Melanoidins prepared by refluxing glucose and lysine could be separated into 14 bands over a pI range of 3.5–4.85, indicating that melanoidins were negatively charged at neutral pH (Kwak et al. 2005). Under these conditions, the type of amino acid present during the reaction determined the anionic properties of the melanoidins (Morales 2002). Some researchers have attributed molasses DWW color and its antimicrobial effects to melanoidins (Mohana et al. 2007; Chandra et al. 2008; Naik et al. 2010).

The structure and biological properties of melanoidins have been reviewed and documented elsewhere (Chandra et al. 2008; Wang et al. 2011). There is no exact structure of melanoidins and their size may range from small molecules to very large polymers (le et al. 1998; Yaylayan and Kaminsky 1998; Wagner et al. 2002; Wang et al. 2011). The basic structure of glucose-derived melanoidin was suggested as in Fig. 18.2.

LMW brown MRPs isolated from dry heated glucose-glycine systems (125 °C, 2 h) (Glosl et al. 2004) and HMW brown MRPs from aqueous sugar-lysine model systems (121 °C, 1 h, pH 9.0) (Jing and Kitts 2000) have genotoxic and cytotoxic effects on cells in high doses. Bread crust and coffee melanoidins were also shown

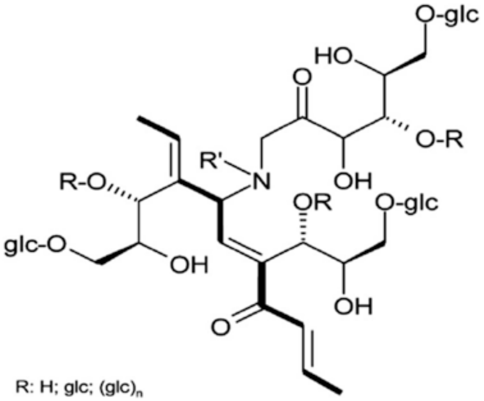

Fig. 18.2 Basic structure of glycine-glucose melanoidin as proposed by Cammerer et al. (2002)

to impair the viability of lung epithelial H358 cells in high doses (Bartling et al. 2005). However, low concentration of melanoidins in foods does not pose a health risk (Wang et al. 2011).

The wastewaters released from distilleries and fermentation industries are the major source of environmental pollution which mainly affect soil and aquatic region due to the presence of water-soluble recalcitrant coloring organic and inorganic compounds (melanoidin) (Tiwari et al. 2012; Evershed et al. 1997); melanoidins have high resistant power for microbial attack, and conventional biological processes such as activated sludge treatment process are insufficient to treat these melanoidins containing wastewater released from distilleries and fermentation industries. Hence, due to these adverse effects of DWW on environment it requires pretreatment before its safe disposal (Mohana et al. 2007; Kumar and Chandra 2006; Bharagava and Chandra 2011).

3.2 Toxicological Effects of Distillery Wastewater Pollutants in Environment

DWW-polluted areas are affected by skin allergies, headache, vomiting sensation, irritating eyes, fever, and stomach pain. These kinds of water have dissolved impurities like carbonate, bicarbonate, sulfate, chloride of calcium, magnesium, iron, sodium & potassium and colloidal impurities like coloring matter, organic waste, finely divided, silica & clay (Chaudhary and Arora 2011). In addition, DWW poses a serious threat to the water quality in several regions of the country. Lowering the pH value of stream, increase in organic load, depletion of oxygen content, discoloration, destruction of aquatic life and bad smell are major problems of DWW (Pal and Yadav 2012). High concentration of organic content, coloring agents and salts concentration in river are responsible for low dissolve oxygen content, pH and increase in BOD, COD values and total dissolve solid of water. Due to this, large scale fish mortality was observed in fresh water resources (Mahimaraja and Bolan 2004; Ramakritinan et al. 2005).

The industrial wastes generated by various distillery units are posing serious threat to the adjoining aquatic and terrestrial habitats due to the practice of discharging them into nearby wastewater courses and lands (Agarwal and Pandey 1994). The DWW has high BOD, COD, lignin derivatives, phenols, heavy metals and has high load of organic matter (Chaudhary and Arora 2011). The color of the wastewater persists even after the anaerobic treatment and poses a serious threat to environment. The water bodies receiving color wastes got colored and affect the penetration of light in aquatic ecosystems, which in turn affect the aquatic fauna and flora (Kumar and Chandra 2004; Bharagava and Chandra 2010; Yadav and Chandra 2012). The molasses spent wash is a potential water pollutant in two ways. First, the highly colored nature of molasses spent wash can block out sunlight from rivers and streams, thus reducing oxygenation of the water by photosynthesis and hence becomes detrimental to aquatic life (Mahimaraja and Bolan 2004; Ramakritinan et al. 2005). Secondly, it has a high pollution load which would result in eutrophication of contaminated water courses (Fitz Gibbon et al. 1998).

DWW also leads to significant levels of soil pollution and acidification in the cases of inappropriate land discharge. It is reported to inhibit seed germination, reduce soil alkalinity, cause soil manganese deficiency, and damage agricultural crops (Mohana et al. 2009). Further, Bharagava and Chandra (2010) found that untreated PMDE was highly toxic in nature carrying high BOD, COD values along with high metals content and it causes deleterious effects on seed germination (*Phaseolus mungo* L.) and seedling growth parameters whereas treated PMDE has significantly reduced the toxicity. The colored compound in DWW has antioxidant properties and become more toxic to all living systems including microorganisms, therefore, must be treated before disposal into environment (Dahiya et al. 2001; Chandra et al. 2008).

3.3 Health Hazardous from Melanoidins

The chemical characterization of melanoidins and the health effects of chemically differed melanoidins are emerging scope in food science research. The formation of toxic Maillard reaction products (MRPs) has been reported frequently and in vitro studies have revealed some toxic effects such as mutagenic, carcinogenic and cytotoxic effects of MRPs (Somoza 2005; Silvan et al. 2006). Excessive glycation has also resulted in the destruction of essential amino acids, inactivation of enzymes, inhibition of regulatory molecule binding, cross-linking of glycated extracellular matrix, abnormalities of nucleic acid function, altered macromolecular recognition, endocytosis and increased immunogenicity (Silvan et al. 2006; Plavsic et al. 2006).

Genotoxic compounds can act at various levels in the cell (causing gene, chromosome, or genome mutations), necessitating the use of a range of genotoxicity assays designed to detect these different types of mutations (Taylor et al. 2004;

Bartling et al. 2005). Sugar-casein model melanoidins consisting variable sugars exhibit different mutagenic activity. For example, ketose sugars (fructose and tagatose) showed a remarkably higher mutagenicity compared with their aldose isomers (glucose and galactose) and generated active oxygen species resulting in DNA strand breaking and mutagenesis. Some other MRPs were also reported to induce chromosome aberrations in Chinese hamster ovary cells and gene conversion in yeast. Mutagenicity and DNA strand breaking activity of melanoidins from a glucose-glycine model was demonstrated by Hiramoto et al. (1997) who reported that the LMW fractions act as lipid sink (Larter and Douglas 1980) and induced DNA damage, where the effect increased with the concentration added. High concentrations (1%) seem to be cytotoxic for the cells, but also lower concentrations between 0.05 and 0.2% reduced cell proliferation and cell viability.

Furthermore, health hazards from melanoidins have been investigated in the context of their ability to promote glycation reactions in vivo, which are involved in the progression of various types of diseases, such as cardiovascular complications, diabetes mellitus, and Alzheimer's disease (Somoza 2005). Toxicological studies were performed showing that melanoidin structures cannot be classified as potent dietary mutagens or genotoxins. Thus, further studies are needed to distinguish between chemically identified harmful and health-beneficial melanoidins.

4 Biological Approaches for the Treatment of Distillery Wastewater

4.1 Aerobic Process

In aerobic process, various types of microorganisms such as bacteria, fungi, actinomycetes, etc. are used due to their inherent capacity to breakdown a variety of complex compounds for degradation/decolorization of toxic and recalcitrant compounds present in various industrial wastes for environmental safety. The second factor hampering aerobic wastewater biotechnology is the relatively low density of the microbial biomass in the reactor. Aerobic treatment of DWW was also performed in activated sludge process and biokinetics coefficients were evaluated (Shah et al. 1998).

4.1.1 Bacterial Treatment

Microbial treatments employing pure bacterial culture have been reported frequently in past and recent years. Bacterial degradation/decolorization of industrial wastes is an eco-friendly and cost-effective alternative to chemical decomposition process of wastes minimization (Mohana et al. 2007; Pant and Adholeya 2007). Treatment of DWW by the use of *Pseudomonas putida* followed by *Aeromonas* sp. in a two-stage bioreactor resulted in COD as well as color reduction (Ghosh et al. 2002).

Dahiya et al. (2001) isolated *Pseudomonas fluorescence* from reactor liquid and found that these bacterial strains are capable of decolorizing melanoidin wastewater up to 76% under non-sterile condition and up to 90% in sterile condition. Further, Yadav and Chandra (2012) isolated bacterial consortium comprising *Proteus mirabilis* (IITRM5; FJ581028), *Bacillus* sp. (IITRM7; FJ581030), *Raoultella planticola* (IITRM15; GU329705) and *Enterobacter sakazakii* (IITRM16, FJ581031) in the ratio of 4:3:2:1 resulted in 75% color reduction and 71% COD removal within 10 days at optimized nutrient. A detailed list of bacteria tried by different researchers for decolorization of DWW is given in Table 18.2.

4.1.2 Fungal Treatment

There are several fungal species, which are used in the treatment of DWW such as basidiomycetes and ascomycetes as well as used in the decolorization of natural and synthetic melanoidins in connection with color reduction of wastewaters from distilleries. Fungal treatment is use to purify the wastewater by consumption of organic compounds, thus, reducing its COD and BOD, and at the same time to obtain some valuable product, such as fungal biomass for protein-rich animal feed or some specific fungal metabolite. Filamentous fungi have lower sensitivity to variations in temperature, pH, nutrients and aeration and have lower nucleic acid content in the biomass (Knapp et al. 2001; Satyawali and Balakrishnan 2008).

Some white rot fungal species, which secrets ligninolytic enzymes (LiP, MnP & Laccase) are capable of degrading xenobiotics and organo-pollutants. *Phanerochaete chrysosporium* and *Trametes versicolor* are the most widely studied among these. *P. chrysosporium* JAG 40 resulted in 80% decolorization of diluted synthetic melanoidin (absorbance unit of 3.5 at 475 nm), as well as with 6.25% anaerobically digested spent wash (Kumar et al. 1998; Gonzalez et al. 2000; Dahiya et al. 2001; Satyawali and Balakrishnan 2008). *T. versicolor* produces a 47 kDa extracellular enzyme identified as peroxidase, which is involved in mineralization of melanoidins. The fungus resulted in 82% decolorization of 12.5% anaerobically–aerobically treated effluent. Of this, 90% color was removed biologically and the rest by adsorption on the mycelium (Dehorter and Blondeau 1993; Benito et al. 1997). In addition, treatment by Trametes species I-62 (CECT 20197) detoxifies the effluent by degrading furan derivatives as observed by gas chromatography analysis (Gonzalez et al. 2000).

Several researchers evaluated the white rot fungus for degradation/decolorization of DWW. Kumar et al. found that four white rot fungi were screened for their ability to decolorize and bioremediate anaerobically digested molasses spent wash (DMSW) (Kumar et al. 1998). Coriolus versicolor and Phanerochaete chrysosporium showed the ability to decolorize and reduce COD of diluted DMSW (12.5% v/v). There are several fungal species that have been investigated for their ability to decolorize/removal of melanoidin from DWW as mentioned in Table 18.2.

Table 18.2 Microorganisms capable for decolorization of distillery wastewater

Reported microorganisms	Comments	Color removal (%)
Bacterial species		
Pseudomonas putida	The organism needed glucose as a carbon source, to produce hydrogen peroxide which reduced the color	60
Pseudomonas fluorescens	This decolorization was obtained with cellulose carrier coated with collagen. Reuse of decolorized cells reduced the decolorization efficiency	94
Pseudomonas aeruginosa	The three strains were part of a consortium which decolorized the anaerobically digested spent wash in presence of basal salts and glucose	67
Pseudomonas sturzeri	The organism required sugar especially, glucose for decolorization of DWW	Up to 60.00
Pseudomonas sp.	The organism required sugar especially, glucose and fructose for decolorization	56.00
Bacillus thuringiensis	Addition of 1% glucose as a supplementary carbon source was necessary	22
Xanthomonas fragariae	All the three strains needed glucose as carbon source and NH4Cl as nitrogen source.	76
Acinetobacter sp.	All these organisms were isolated from an air bubble column reactor treating winery wastewater after 6 months of operation. Most isolates from the colonized carriers belonged to species of the genus *Bacillus*	–
Acetobacter acetii	The organism required sugar especially, glucose and fructose for decolorization of MWWs	76.4
Fungal species		
Trametes versicolor	COD and N–NH_4 removal observed in presence of sucrose and KH_2PO_4 as nutrient source	82
Geotrichum candidum	Fungus immobilized on polyurethane foam showed stable decolorization of molasses in repeated-batch cultivation	80
Aspergillus niger UM2	Decolorization was more by immobilized fungus and it was able to decolorize up to 50% of initial effluent concentrations	80
Mycelia sterilia	Organism required glucose for the decolorizing activity	93
Flavodon flavus	MSW was decolorized using a marine basidiomycetes fungus. It also removed 68% benzo(a)pyrene, a PAH found in MSW	80
Phanerochaete chrysosporium JAG-40	This organism decolorized synthetic and natural melanoidins when the medium was supplemented with glucose and peptone	80
Coriolus hirsutus IFO4917	Melanoidins present in heat treatment liquor were subjected to sequencing batch decolorization by the immobilized fungal cells	45

(continued)

Table 18.2 (continued)

Reported microorganisms	Comments	Color removal (%)
Aspergillus niveus	The fungus could use sugarcane bagasse as carbon source and required other nutrients for decolorization	56
Williopsis saturnus strain CBS 5761	Yeast isolates from a rotating biological contactor (RBC) treating winery wastewater. Only 43% COD removal could be achieved	
Coriolus versicolor sp. no.20	10% diluted spent wash was used with glucose @ 2% added as carbon source	34.5
Phanerochaete chrysosporium	Sugar refinery effluent was treated in a RBC using polyurethane foam and scouring web as support	55
Marine basidiomycete NIOCC #2a	Experiment was carried out at 10% diluted spent wash	100
Citeromyces sp. WR-43-6	Organism required glucose, Sodium nitrate and KH_2PO_4 for maximal decolorization	68.91
Yeast		
Citeromyces sp.	The organism required sugar especially, glucose and fructose for decolorization	75.00
Cyanobacteria		
Oscillotoria boryana	The organism required sugar especially, glucose and fructose for decolorization	60.00

Adapted from: Agarwal et al. (2010)

4.1.3 Algal Treatment

Treatment of DWW through microalgae is more effective in today's era. Algal treatment attracts researchers not only by treating waste but also by its products/byproducts, which are in high demands for social welfare (Sankaran et al. 2014). Microalgal treatment is commonly suggested after the anaerobic digestion of spent wash since the process is energy efficient and also microalgae have the ability to take up its nutrients (majorly inorganic compounds) requirement from biomethanated spent wash and energy requirement from the sun. Additionally, it has the mechanism of taking carbon dioxide and converting them into oxygenase electron donor there by reducing the energy need of the aerobic treatment (Sankaran et al. 2014). The treatment of anaerobically treated 10% DWW using the microalgae *Chlorella vulgaris* followed by *Lemna minuscula* resulted in 52% reduction in color (Valderrama et al. 2002). Further, Kalavathi et al. (2001) examined the degradation of 5% melanoidin by the marine cyanobacterium *Oscillatoria boryana* BDU 92181.

Further, Boer et al. (2006) isolated and purified the melanoidin decolorizing enzymes (MnP) from *L. edodes.* Two peaks of MnP as MnP1 and MnP2 were obtained by gel filtration. They revealed that the purified enzyme yielded a single band after denaturing SDS-PAGE. A molecular mass of 46 kDa was estimated after SDS-PAGE, and this molecular mass was confirmed by Sephadex G-100 gel filtration. *Oscillatoria willei*, when grown under conditions of nitrogen limitation but

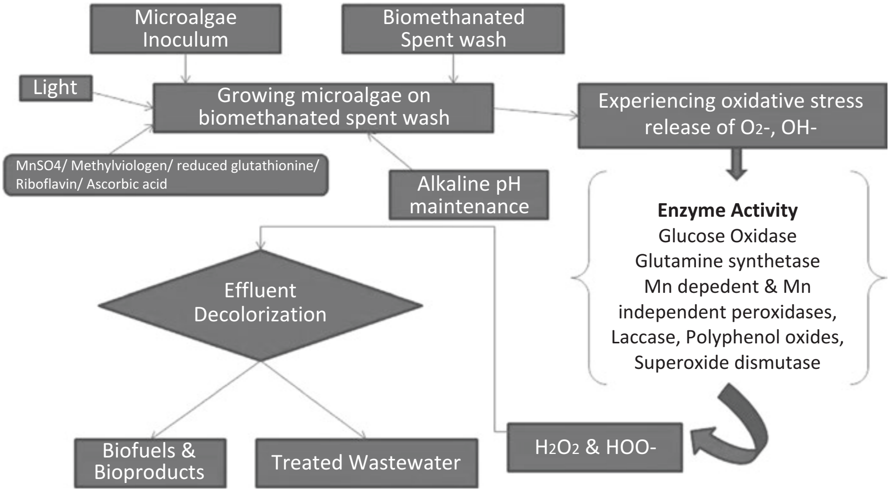

Fig. 18.3 The conceptual mechanism of melanoidin containing biomethanated spent wash treatment using microalgae (Adapted from Sankaran et al. 2014)

supplemented with phenolic compound, showed enhanced oxidation. The organism was found to release H_2O_2, OH^- and molecular oxygen during photosynthesis resulting in 60% of DWW. In addition, this study suggested that cyanobacteria could use melanoidin as a better nitrogen source than carbon.

The toxic active oxygen species, namely superoxide anion O_2^-, hydroxylradical OH^-, and hydrogen peroxide H_2O_2, are generated in all photosynthetic organisms through electron transport systems (Patel et al. 2001). Hydrogen peroxide, a strong oxidizing agent produced by cyanobacteria during the photosynthetic c process, is regarded to be fundamentally involved in melanoidin degradation. Evidence for this comes from the fact that in-vitro addition of H_2O_2 to melanoidin containing effluent had shown a color reduction of 97% (Patil and Kapadnis 1995). The conceptual degradation mechanism has been given in Fig. 18.3 (Sankaran et al. 2014). Thus coupling microalgae biomass production with nutrient removal/pollutant degradation may represent an important milestone in the bioenergy goals since the wastewater market is immense (Sankaran et al. 2014). However, an appropriate technology for biomass harvesting must be developed to bridge all together.

Anaerobic digestion is followed to produce biogas by treating the spent wash. Anaerobic digestion is the most suitable method for the treatment of effluents containing high concentration of organic carbon such as DWW. The high organic content of molasses spent wash makes anaerobic treatment more attractive in comparison to direct aerobic treatment (Mohana et al. 2007; Satyawali and Balakrishnan 2008). Anaerobic digestion is a process where organic compound in spent wash is digested by the microorganism to produce biogas (CH_4 60% and CO_2 40% roughly).

On an average 1 m^3 of spent wash produces 38–40 m^3 of biogas. Other products of the anaerobic digester are treated spent wash and digested sludge, both are rich in nutrients. The sludge can be used as manure since it contains more nutrient contents (Nandy et al. 2002). The most widely used anaerobic process is the up-flow anaerobic sludge blanket (UASB) (Wilkie et al. 2000; Mohana et al. 2007; Satyawali and Balakrishnan 2008). The major challenges of this anaerobically digested DWW are the removal of color and treatment of inorganic compounds.

Despite the high COD removal with diluted DWW, the inhibiting chemicals still remain in effluent after anaerobic digestion. Therefore, a further treatment step is required to remove the remaining dark color and COD, BOD, etc. However, another strategy is to pre-treat DWW before aerobic digestion, e.g., with ozonation, UV light plus titanium dioxide in order to improve the efficiency of DWW anaerobic digestion (Martin et al. 2002; Jimenez et al. 2004; Arimi et al. 2014). However, most of the input energy in these processes goes to eliminate the organic COD, which could be recovered as biogas. This makes the pretreatment of DWW untenable. It is thus preferable to treat the DWW anaerobically first and then with further treatment steps. If another biological treatment could treat the distiller wastewater for color and inorganic compounds, it will solve the effluent problem. Further, Arimi et al. (2015) reported the anaerobic digestion for the colorant elimination from melanoidin-rich DWW using natural manganese oxides (MnOx). Anaerobically digested melanoidins-rich DWW was used to test the removal of organic contents and color at different pH values.

4.2 Anaerobic Process

More detail information about the anaerobic processes can be found in other reviews (Wilkie et al. 2000; Melamane et al. 2007; Satyawali and Balakrishnan 2008).

4.3 Green Aspects of Biological Treatment

DWW wastewater treatment by biological treatment such as using microalgae shows its potential ability toward the greenery of environment and its suitability for applying it in green technical methods. Microalgae are among the most productive organisms on earth. The cellular organism, microalgae also possesses a number of advantages over higher plants as a food source (Fig. 18.4) and it has a very high potential market value (Table 18.3). Beside this, other advantages are CO_2 sequestration and treating the waste through biochemical oxidation. The green applications of this technique are listed below.

- Biological treatment are more effective for removal of nutrient pollutants and heavy metals
- Low cost requirement

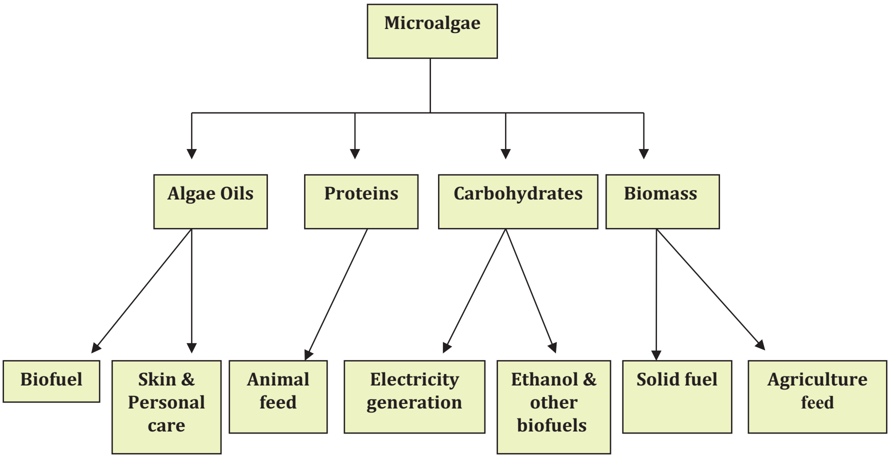

Fig. 18.4 Useful products of microalgae (Adapted from Sankaran et al. 2014)

Table 18.3 Power generation and revenue generation by carbon credit (market characterization report-productive use of methane in Indian distilleries)

Distillery capacity (kLPD)	Power generation potential (MW)	Carbon credit due to power generation	Carbon credit due to methane avoidance	Total carbon credit potential per annum	Total carbon revenue (INR)
10	0.38	6	5	3283	1,969,553
20	0.76	12	20	9565	5,739,107
30	1.14	18	43	18,248	10,948,660
40	1.52	24	77	30,230	18,138,214
50	1.90	30	120	44,913	26,947,767
60	2.28	36	172	62,296	37,377,321
70	2.65	42	234	82,678	49,606,874
80	3.03	48	305	105,761	63,456,248
90	3.41	54	306	131,843	79,105,981
100	3.79	60	477	160,926	96,555,535

Adapted from: Sankaran et al. (2014)

Note: *kLPD* kilo liters per day, *MW* mega watt, *INR* Indian rupees for a typical capacity of 40 kLPD, power generation potential is 1.52 MW, cumulative carbon credit for a day is 101; for a year (appx. 300 days) is 30,230. A carbon credit point earns Rs. 600/−(appx.) therefore for 30,230 points generate total revenue of Rs. 18,138,214/−

- Low energy requirement
- Useful biomass production
- Reduction in sludge formation
- Algae contain more than 50% of oil in its biomass
- They provide much higher yields of biomass and fuel
- 10–100 times higher than comparable energy crops
- They can be grown under condition, which is unsuitable for conventional crop production.

5 Other Emerging Eco-Friendly Treatment Approaches

5.1 Constructed Wetlands (CWs)

Constructed wetlands for treating DWW is that wetlands are naturally among the most biological active ecosystem on earth. Mulidzi (2010) study showed the impact of shorter retention time on the performance of constructed wetland in terms of Chemical Oxygen Demand (COD) and other elements removal. The results had shown an overall average COD removal of 60% throughout the year. Results also showed reasonable removal of other elements, namely potassium, pH, N, EC, Ca, Na, Mg and boron from the wastewater by constructed wetlands.

Billore et al. (2001) have demonstrated a four-celled horizontal subsurface flow (HSF) CW for the treatment of DWW after anaerobic treatment. The post-anaerobic treated effluent had a BOD of about 2500 mg/L and a COD of nearly 14,000 mg/L. A pre-treatment chamber filled with gravel was used to capture the suspended solids. All the cells were filled with gravel up to varying heights and cells three and four supported the plants *Typha latipholia* and *Phragmites karka* respectively. The overall retention time was 14.4 days and the treatment resulted in 64% COD, 85% BOD, 42% total solids and 79% phosphorus content reduction.

5.2 Biocomposting

Industrial wastewaters are also treated by various biological processes for the mitigation of their harmful properties. Biocomposting is one of the most valuable thermophilic processes resulting in a product rich in humus used as fertilizer in agriculture fields. The spent wash, either directly, or after biomethanation is sprayed in a controlled manner on sugarcane pressmud. The latter is the filter cake obtained during juice clarification in the manufacture of sugar. Jimenez and Borja (1997) Brja reported that both beet spent wash and molasses, aerobic pretreatment of beet spent wash with *Penicillium decumbens* resulted in about 74% reduction in phenolics content and 40% reduction in color. This is a popular option adopted by several Indian distilleries attached to sugar mills with adequate land availability.

5.3 Enzymatic Mechanism for Distillery Wastewater Decolorization

Various enzymes (e.g., peroxidases, oxidoreductases, cellulolytic enzymes, cyanidase, proteases, amylases, etc.) and ligninolytic group (Laccase, MnP, and LiP) from a variety of different sources have been reported to play an important role with broad range substrate specificity in waste treatment applications (Dec and Bollag 1994;

Chandra and Chowdhary 2015; Chowdhary et al. 2016). The demand for these enzymes has increased in the recent year due to their potential applications in the diverse biotechnological areas. Among these enzymes, the laccases have the most potential, with a broad range of specificity, and well-studied ligninolytic enzymatic activities, which is highly versatile in nature with wide variety of industrial applications (Chandra and Chowdhary 2015).

The bacterial ligninolytic enzyme laccases are most important roles in the pollutant bioremediation of industrial waste because they oxidize both toxic and nontoxic substrates. Laccases are also included in the cleaning of industrial effluents, mostly from paper and pulp, textile, tannery, and distillery industries. Among the biological agents, laccases represent an interesting group of ubiquitous oxidoreductase enzymes that show great potential for biotechnological applications (Sangave and Pandit 2006; Chandra and Chowdhary 2015).

Several studies regarding degradation of melanoidins, humic acids, and related compounds using basidiomycetes have also suggested a participation of at least one laccase enzyme in fungi belonging to Trametes (*Coriolus*) genus. The role of enzymes other than laccase or peroxidases in the decolorization of melanoidins by Trametes (*Coriolus*) strain was reported during the 1980s.

Furthermore, a laccase-producing white-rot fungus *Trametes* sp. has been tested for bioremediation. The maximum effluent decolorization of 73.3% and chemical oxygen demand (COD) reduction of 61.7% were achieved after 7 days of fungal treatment to 20% v/v of distillery waste in culture medium. Under these conditions, a 35-fold increase in laccase production by this fungus was observed (Gonzalez et al. 2000). Therefore, the knowledge role of enzymes in bioremediation applications for various industrial wastes will open ample opportunities for large-scale application.

The advantages and disadvantages of physico-chemical and biological treatment are mention in Table 18.4. This may be helpful to select suitable treatment process for the treatment of industrial wastewater.

6 Challenges for Bioremediation of Distillery Wastewater

Distillery industries facing many challenges for the bioremediation and disposal of wastewater generated during alcohol production. There are many problems, which are described below:

(a) The distillery industries produce only 7–9% of alcohol (depends on quality) from the cane molasses after fermentation. The rest 91–93% leave as wastewater causes severe environmental problems (Sankaran et al. 2014).
(b) Lack of advanced processing techniques and waste treatment technologies in developing countries.
(c) High percentage of total dissolved solid (TDS) value of DWW.
(d) Government should provide financial support.

Table 18.4 Various treatment approaches/technologies with their merits and demerits used for the treatment of distillery wastewaters

Treatment technology	Advantages	Disadvantages	References
Physico-chemical treatment			
Adsorption	Adsorption and biodegradation of melanoidin via the biofilm on activated carbon can largely improve the color removal in comparison to the biofilm or activated carbon alone	Temperature and pH sensitive, biomass concentration in solution seems to influence the specific uptake	Venkat et al. (2000)
		High cost of commercial adsorbents is their main drawback for application	
Coagulation/flocculation	Simple, proven technology and cost-effectiveness	pH sensitive, As^{+3} and As^{+5} must be fully oxidized coagulation/flocculation is accompanied by high removal of organic matters and, therefore, may be problematic as a pretreatment for anaerobic digestion because a high quantity of energy is lost in the process	Pandey et al. (2003) and Migo et al. (1993)
	Widely accepted	Excess use of chemicals and sludge generation with subsequent disposal problems	
	Separates many kind of particles from wastewater		
	Enhances wastewater filtration process		
	Uses abundant and low cost chemicals		
Oxidation process	Oxidize a broad range of organic compounds with the aid of free radicals	Ozone can selectively attack the double bonds (e.g., C=C, N=C) and functional groups (e.g. $–OCH_3$, $–OH$, and $–CH_3$) in acid or neutral conditions with limited concentrations	Coca et al. (2005), Afify et al. (2009), and Battimelli et al. (2010)
	The method has advantages over AOP since it can be used in either the pretreatment step or in the final treatment step	High cost	

Membrane treatment	Membrane processes like nanofiltration and RO can result in significant color removal thereby permitting reuse of the treated effluent, removal of multiple contaminants	Membrane treatment facing problems such as membrane fouling, clogging, scaling and cleaning, Poor production efficiency, Requires pretreatment	Satyawali and Balakrishnan (2008)
Evaporation and combustion	Combustion of vinasse disposal is an effective method as it contains potassium-rich ash that can be used for land application	Poor efficiency	Gulati (2004)
Biological treatment			
Aerobic treatment/anaerobic treatment reactors	Biological treatments are good approaches to treat the wastewaters having high organic load	Anaerobic treatment is the requirements of high dilution due the antimicrobial component	Farhadian, et al. (2007), Travieso et al. (2006), Bharagava and Chandra (2010), and Prajapati and Chaudhari (2015)
Bacterial treatment	Eco-friendly in nature	Slow growing process	
Fungal treatment		It acquires large space for treatment	
Treatment by other microorganisms			
Micro alga	Nutrients are available in the effluent itself used, and there is no further water is added for the micro algal growth and also the biomass yield further can be used for production of biogas or biodiesel or fertilizer.	Micro alga required sufficient light energy for photosynthesis	Sankaran et al. (2014)

(continued)

Table 18.4 (continued)

Treatment technology	Advantages	Disadvantages	References
Cyanobacteria	Energy obtain from photosynthesis	Slow growth rate	Kalavathi et al. (2001)
Yeast	Produced ethanol for biofuel industry	Slow growth rate	Sirianuntapiboon et al. (2004)
Enzymatic treatment			
Laccases	Enzyme are naturally produced by microorganism which is ecofriendly	Slow growing process and also not applicable at large scale application	Duff et al. (1994), Dec and Bollag (1994), and Chandra and Chowdhary (2015)
Peroxidases	Reusable in nature		
Oxidoreductases	Enzymes play important roles in the biotransformation of recalcitrant compounds, in which laccases hold large potential for the economical treatment of wastewater containing large amount of toxic compounds		
Cellulolytic enzymes			
Cyanidase			
Proteases			
Amylases			

The removal/remediation of DWW pollutants from contaminated water resources is a thrust need for environmental safety. Further, the financial support from government is also required for the upgradation of distillery industries, especially small-scale industries. Thus, there is urgent need for more research attention in this area.

7 Conclusion

This chapter concluded that distillery industries use huge amount of water, various types of chemicals and raw materials in the production of alcohol, which generate high strength of wastewater. With their high BOD and COD, this wastewater creates various problems in environmental. When released in water bodies they cause oxygen depletion and associated problems, and/or if released in soil they reduce the soil alkalinity and manganese availability, inhibit seed germination and affect vegetation. Besides causing unaesthetic discoloration of water and soil, melanoidin pigments are also toxic to microorganisms present in soil and water.

Biological method of decolorizing the DWW is gaining its momentum in the arena of wastewater treatment methods. The recalcitrant coloring compound melanoidins and their chemical structure transforming nature during treatment process are not well known. Biological treatments are mostly preferred because this is the only treatment technique that produces very useful byproducts, which may be in large demand and also it is beneficial for the sustainable development of society.

Physico-chemical methods are capable of both color and organic load reduction; consequently, in spite of the cost, even high-end options like membrane filtration are being field tested. On the basis of available literature on DWW treatment, it seems that there is a need of attention to address the limitation in existing treatment process and provide an effective solution on it. Thus, this chapter covers all the DWW problems and treatment technology for the sustainable development of environment.

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Chapter 19
Ecological Restoration Techniques for Management of Degraded, Mined-Out Areas and the Role Played by Rhizospheric Microbial Communities

Prashant Pant and Pratibha Pant

Abstract Restoration of degraded lands is an ecological, socio-economic, legal and national prerogative. Rebuilding healthy and resilient soils in such environments along with complex above- and below-ground biota for maintenance of ecosystem is required for establishment, growth, productivity and desired trajectories of succession of native plant communities at restoration sites. Complex networks that connect above- and below-ground ecosystems involve the rhizosphere in processes of mineralization and nutrient cycling. Such massive efforts need to be monitored by studying changes over time in native vegetation cover using on ground and remote sensing-based methods, changes in soil conditions and succession in bulk and rhizospheric microbial communities. These communities respond to the plant and soil types in which they occur and their interactions likely involve utilization of plant exudates, carbon sequestration, and available matter through detritus, etc. This chapter provides a brief insight into need for ecologically restoring sites, factors influencing them, the choice or selection of species for undertaking such a work, indicators of ecological restoration that can be applied for monitoring purposes and some of the popular models of ecological restoration in India that have been successfully established, and the techniques used in these models. In the end, it briefly summarizes the importance of soil microbial diversity as a driver of above- and below-ground biodiversity and the linkages between them.

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1 Introduction

Deforestation, mining, land use change, rapid industrialization, overgrazing, non-sustainable extraction of forest produce for fodder and fuel wood and simultaneous loss of biodiversity due to direct or indirect factors lead to degradation of environment and ecosystems (Daily 1995). These activities bring major environmental challenges which mankind is facing at the moment. Situation is bound to worsen going with current rate of blind, ecologically disturbing, anthropogenic activities in the name of development. India is among the major mining hubs in the world. Mining is one of the biggest cause for large-scale deterioration (that too, in a very short span) of forest ecosystems, wild life and also of the healthy soil layers which harbour life sustaining properties (Walker and Willig 1999). Large areas get deforested, denuded, and the soil is lost or polluted with mined-out ore waste. Such environmentally poor, degraded, mined-out areas subsequently become derelict and are of little benefit for local inhabitants. Apart from ecosystem imbalance, and loss of biodiversity, open cast mining activities also harm a country's natural beauty (Ehrlich 1993). Such area when seen in a satellite imagery or using applications such as Google Earth appears as a blot on the country's geographical and vegetational continuity. After the commercial extraction or land use change, these areas are left on the mercy of nature resulting in weed infested areas which further degrade the surrounding habitats being invasive, only increasing the total area under degradation. Consequently, there is loss of native biodiversity, ecological services, affecting the locals, aboriginals, and tribals who are compelled to mass migrate to urban areas in search of livelihood, thus extinguishing hopes of preservation and maintenance of cultural diversity, language diversity. To avert such changes, *Ecological Restoration* is the only feasible human induced effort which can be taken up to treat the degraded site to prevent it from getting infested with weeds and also to undo the damages caused to certain extents (Holl et al. 2003; Doren et al. 2009; Feng et al. 2013). This chapter provides a succinct introduction on the basics of Ecological Restoration as a concept and as a technological tool with which it can be practised on degraded environments to manage them effectively and minimize the long- and short-term damages by restoring the area.

2 Ecological Restoration

Though there are many definitions of ecological restoration, the most relevant one was defined by Society for Ecological Restoration (SER) in 2004. According to SER (2004), ecological restoration is defined as "*an intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability and attempts to accelerate the natural recovery process artificially to achieve the otherwise long term goals in a short time*" (SER 2004). It is an intentional activity to recover the ecosystem towards its original structure and function (SER 2004; Rajdeep et al. 2011). Eco-restoration also aims to increase soil fertility in terms of nutrients as well as richness in soil biota, and increased biotic control over biogeochemical fluxes faster than time period required for natural recovery (Parrotta 1992). Eco-restoration of mined-out areas and wastelands thus aims to return the degraded ecosystem to its natural, pre-mining state, thereby bringing stability and sustainability in associated human activity. Restoration Ecology provides clear concepts, models, tools, and methodologies to practice ecological restoration of degraded ecosystems.

2.1 Need to Ecologically Restore Sites

Most encroachments in natural forest areas and natural ecosystems are for some special yet peculiar land use such as denudation for industrialization, mining, extraction of oil, or other natural resources, etc. All these extractions/developmental activities are based on resources which are finite and so is the land use, i.e., the so-called developmental activity is one day going to shut down, but by that time the damage will be almost irreparable and irreversible, if left as it is. Therefore, it becomes imperative to undertake “social, environmental and legal” steps to ensure proper reclamation once the desired extraction is over. In India, such steps have been initiated. Restoration of wastelands, or degraded lands has become a national prerogative and part of Corporate Social Responsibility (CSR) to institute a dedicated department on Environmental Impact Assessment and mitigation cum protection from the possible damages as well as post-use reclamation/restoration. However, proper restoration tools and techniques have not yet been normalized. To meet the major goals of sustainable development, restoration is crucial as it is the restored land that remains available indefinitely for future generations. Ecological restoration of environmentally degraded areas is the only successful long-term approach to promote both sustainability and the maintenance of ecosystem biodiversity. The environmental problems arising out of large-scale developmental activities such as Open Cast Mining, Residential and Corporate Construction, Cement factories, etc., involve all three major types of pollution, i.e., air, water and soil pollution. For instance, suspended particulate matters (SPM) and gases released in these areas bring about air pollution (Gautam et al. 2012). Similarly, discharge of fine dust, organic compounds, oil and grease (and in some cases acidic water and chemicals) as well as exposure of the parent rock material to the surface water bodies makes it contaminated with toxic and heavy metals compounds causing pollution (Barbour 1994; Mishra et al. 2004). Soil gets polluted due to increasing concentration of parental rock in soil and also due to mixing of unused form of ore (fine dust or fine ore which is commercially not viable for meeting extraction cost) with the top layers, and forming overburdened dumps over top soil due to open cast mining activities with high concentrations of heavy metals and toxic elements coming on the surface. Open cast mining or quarrying activities cause change in the general topography of the terrain due to formation of overburdened dumps, depletion of top soil, destruction of woody vegetation inducing a high risk of erosion, drainage, and increasing failure of rehabilitation attempts (Gams et al. 1993; Sort and Alcañiz 1996).

2.2 Factors Influencing Ecological Restoration of Degraded Mined-Out Areas

Different sites and their site factors such as Climatic, Edaphic, Topographic and Biotic influence the vegetation at ecological and physiological levels. Climatic factors mainly include Light, Temperature, Precipitation, Wind, and atmospheric injuries. Soil physicochemical properties such as soil texture, structure, profile, soil depth, stoniness, pH, organic matter content, organic carbon, electrical conductivity, bioavailable cations, anions, etc., comprise the edaphic factors. Configuration of the physical relief, altitude, slope, aspect etc., determine the topographic factors while all the interactions between Plant-Plant, Plant-Animals, Plant-Microbes, Plant-Man and his animals constitute the Biotic factors within a site. The interactions are such that these factors affect the composition and distribution of the above and below ground biotic communities and in turn the above and below ground communities bring about a change in these factors. The influence of the factors is so supreme that the entire choice of species, methods of planting and the order in which different groups of plants are going to be planted is dependent on the site factors only.

To make any ecological restoration programme a successful one, whether it is a long term or a short term one, needs to be critically evaluated from the very beginning. Every site is different and so is the nature of techniques applied in the area for restoring it. The success mainly depends on scientific evaluation of the site factors entailing Climatic, Topographic, Edaphic and Biotic Factors and subsequent careful selection of plant species to be introduced in the site (Hassan et al. 2007). Some features considered for choosing a species for introduction to a site selected for restoration are: (1) species should be native to the area, (2) it should be of some socio-economic importance, viz. a multipurpose tree (MPTs e.g. *Leucaena leucocephala*) or nitrogen fixing tree (NFTs e.g. *Casuarina* sp.,), (3) better adapted to poor soils (ability to survive under stress conditions), and (4) the debris or leaf litter should be biodegradable (for increasing soil organic matter (SOM) content and to positively influence soil microbial biomass). Floral records from previous surveys for the vegetation type and structure, if available, provide valuable information for planning a restoration strategy as they undoubtedly indicate the pre-mining climax or seral communities (Chari et al. 1989). Native species and early colonizers (such as nitrogen fixing legumes) known to improve soil health and nutrient status have been used as part of various eco-restoration and rehabilitation programmes for degraded environments in the past. Such approaches constitute good initiation points for restoring degraded mined-out sites (Babu et al. 1985a, b; Nair et al. 1988; Babu et al. 1993).

The species composition, distribution, community structure, species richness, rate of growth of above- and below-ground biota at a site undergoing eco-restoration respond to changes in environmental variables. Changes in climate and season affecting conditions of light, temperature, precipitation, relative humidity, etc., are obviously important even at an un-restored setting. Shifts in edaphic factors like soil structure, soil moisture, pH, soil organic matter (SOM), cation exchange capacity (CEC), electrical conductivity (EC), levels of bio-available cations and anions, trace elements, heavy metals, are associated with growth of plants and rhizospheric biota. All or some of these factors are essential for monitoring eco-restoration efforts (Schoenholtz et al. 2000; Fierer and Jackson 2006; Andrew et al. 2012).

Among the many site factors, two factors are considered extremely important for the colonization and establishment of the biotic community. These are Soil pH and Soil Organic Matter (SOM). Soil pH determines acidity or basicity of the soil environment. Most plant species have a requirement of an optimum pH range for growth. For instance, tree species *Xylia xylocarpa*, *Shorea robusta* (pH 4.5–5.5) and *Tectona grandis* (6.5–7.6) prefer to grow in acidic soils, while *Acacia arabica*, *Azadirachta indica* and *Terminalia arjuna, Acacia catechu*, *Acacia nilotica*, *Adina cordifolia*, *Anogeissus latifolia*, *Dalbergia sissoo* and *Bauhinia variegata* prefer to grow in basic soils and can be found commonly growing in calcareous soils in natural as well as in ecologically restored areas (Soni et al. 1994; Singh et al. 2002; Verma 2003; Rajdeep et al. 2011). Soil pH determines the degree of maturity of soil, the successional stages and distribution of plant communities (Gianello et al. 1995; Favreto and Medeiros 2006; Meurer 2007; Sanchez-Azofeifa et al. 2014). In moist localities acidic pH is an indicator of soil maturity and climax vegetation, while basic pH indicates immaturity. Soil organic matter (SOM) is another very important and rather decisive factor for success of eco-restoration. Decomposition of leaf litter, dead decaying organisms, animal refuse, plant parts constitutes the organic matter content which increases the nutrient load and moisture holding capacity as well increases the cation exchange capacity, further enhancing the selective uptake of the cations in the plant systems. SOM releases nutrients to the soil environment, making it a useful resource for plant roots and soil microbes. SOM is important for bacterial and fungal decomposers, as well as for soil fauna that influences nutrient cycling and plant growth (Wardle 2002; Moore et al. 2004; Bardgett and Wardle 2010).

2.3 Indicators of Successful Ecological Restoration Effort

To monitor an ecological restoration programme for its progress, there are many ways with which one can establish the success of the human-induced restoration efforts. The methods of monitoring may consist of floristic composition analyses, species composition surveys, community structure changes over time (temporal) and space (spatial), species richness, rate of growth of above- and below-ground biota, change in above-ground plant biomass using various indices such as NDVI

(Normalized Vegetation Differential Index) depicted by Remote Sensing technologies using Geographical Information System (GIS) and also changes in environmental variables such as soil physicochemical properties and microclimatic profile such as soil and air temperature, relative humidity, spore concentration and the kind of bacteria and fungi above and below ground. A combination of the previously mentioned variables needs to be monitored in parallel over seasons and years to conclude success or failure of the effort. Apart from these indicators, reference sites should also be kept such as a native forest ecosystem or a degraded unrestored site to establish effects of human induced restoration to be real and not just a natural process, as one might argue.

2.4 Selection of Species to Be Introduced as Part of Restoration

There are no set rules of choice of tree or ground cover species (forbs, as some may refer to) to be planted/regenerated to restore sites. The site itself or the nearby semi-disturbed, native sites/habitats are the best guide as to what needs to be planted and in what proportion of distribution. However, some leads are taken from the general site profile using the microclimatic, water and soil profile of an area to sort out what kind of species and in what order should be planted to have a successful restoration. This part is decisive for any restoration effort and so needs to be validated by experts. An account of how a very basic site factor such as soil pH affects choice of the species is mentioned previously.

Similarly, there are evidences that soil microbial parameters may be useful as early and sensitive indicators for stressed soil and its alleviation. Composition of soil biota has been seen as a biological indicator of soil health. The treatment or management of soil with application of arbuscular mycorrhizal fungi (AMF), free living, associative and symbiotic nitrogen fixers and other plant growth promoting bacteria (PGPRs), Chitin amendments, green manure or agricultural waste, application of pesticides and the introduction of transgenic crops have been shown to affect rhizospheric soil microbial community structures. Soil type and cropping practices also influence the microbial community composition thus leading to sooner establishment of plant communities in a site undergoing eco-restoration.

3 The Ecological Restoration Work Done in India and the Techniques of Restoration Applied

There are some success stories of ecological restoration work done in India. A number of environmentally degraded sites were not only ecologically restored, but were also declared self-sustainable, having a number of ecological services at their disposal making the locals engage in their traditional occupational activities thus preventing their migration and protecting the cultural diversity of the tribal folk. Some of the recently successful ecological restoration works in India are Ecological Restoration of Chilika Lake (Odisha), Limestone quarries of Musoorie Hills (Uttarakhand), conversion of Asola Bhati mines area into Asola Bird Sanctuary on Delhi-Haryana border, Aravalli Biodiversity Park on the *Prosopis* infested Aravalli ranges bordering Delhi, and the restoration and creation of Yamuna Biodiversity Park on the Old Yamuna basin in Delhi (Burari, Delhi region). A brief description of the site, nature of restoration and techniques applied in some of the selected case studies is given here.

3.1 Chilika Lake, Odisha

Chilika lake is a brackish water lagoon with coordinates 19°43′N 85°19′E. This lake is on the eastern coast of India in the state of Odisha. It was declared India's first Ramsar Site on 1st October 1981 and a wetland of international importance. This lake is considered India's largest brackish water lagoon (1185 km^2) and World's second largest lagoon. The lake enjoys an estuarine ecosystem with tidal inundations and river opening into the lake. It has its importance in terms of ecological, economic and socio-cultural features. However, over time, with non-regulated use of the lake resources, illegal shrimp culture and due to anthropogenic alterations in the lake hydrology, the ecological services of the lake were severely affected and consequently the lake was added to the Montreux Record, the threatened list of Ramsar sites in 1993, merely 13 years after it was declared a wetland of international importance. Briefly, to ecologically restore the status of the lake, Chilika Development Authority (CDA) followed Ramsar guidelines and carried out establishment of a water inlet thereby having hydrological intervention administered to the lake. Finally, in 2000 the lake was re-monitored for its ecological services and was declared restored with its name removed from the Montreux Record. This was the first lake in Asia, to have its status retracted from threatened Ramsar site to a standard Ramsar site.

3.2 The Asola-Bhatti Wildlife Sanctuary

The Asola-Bhatti Wildlife Sanctuary (declared as wildlife sanctuary in 1991) is located between 28° 24.2′ N and 77° 13.6′ E, encompasses an area of 4000 acres in the south-eastern part of the Southern ridge near Tughlaqabad in Delhi. The Delhi Ridge represents the spur of Aravalli mountain range covering an area of 7000 acres and has performed several ecological functions in the past such as ground water recharge; an area for grazing activities, *Acacia* woodland and 3–5 storeyed tropical dry deciduous forests; provide fodder and firewood for the local communities; regulated local weather patterns and served as CO_2 sink.

Prior to ecological restoration, the Bhatti Mines areas was known for its open cast morrum mines having quartzite soil with hard red crust due to oxidation of iron which has poor moisture retention capacity and little organic matter. The landscape of the area ranges from gentle undulating terrains with deep gorges and gullies, steep cut slopes to overburden dumps. The entire area was dominated by *Prosopis juliflora* – an exotic invasive species with scattered xerophytic vegetation, weedy species and lacked characteristic (native) Aravalli vegetation type. The environmentally challenged site was taken up to restore degraded lands to their pristine ecosystems and make it sustainable over the years. The exemplary work of ecologically restoring the site was done under the aegis of Prof. C.R. Babu and his team at Center for Environmental Management of Degraded Ecosystems, University of Delhi.

The main restoration techniques applied in this area were:

- Removal of weeds and other invasive species (woody and non-woody).
- Addition of farmyard manure (FYM) to the top layers of soil.
- Steep slopes were stabilized by planting grasses and legumes, as mentioned below. Approximately nine perennial grass species namely, *Cenchrus ciliaris, C. setigerus, Eremopogon sp., Eragrostis tenella, Dactyloctenium aegyptium, Chyrsopogon fulvus, Dichanthium annulatum, Sporobolus diander, Aristida* sp., nine tree legumes namely *Acacia senegal, A. leucopholea, A. nilotica, A. catechu, Albizzia lebbeck, Butea monosperma, Cassia siamea, Cassia fistula, Dalbergia sissoo*, three shrubby legumes (*Indigofera tinctoria, Tephrosia purpurea, Dichrostachys cineraria*), two herbaceous legumes (*Trigonella spp., Melilotus spp.*) and eight non-leguminous woody species (*Wrightia tinctoria, Grewia tenax, Ficus spp., Syzigium cumini, Balanites roxburghii, Maytenus, Fluggia, Holoptelia integrifolia*) were selected for processing the habitat leading to the development of ecosystem.
- AMF (arbuscular mycorrhizal fungi) and a number of plant growth-promoting bacteria isolated from the rhizospheric soils of grasses and legumes were also used as inoculants in the top soil for enhancing mineralization, decomposition and other useful soil processes.

3.3 Yamuna Biodiversity Park and Aravalli Biodiversity Park

Under the aegis of Professor C.R. Babu and his team at CEMDE, Delhi University, working on similar lines mentioned as above, two environmentally challenged sites in Delhi were taken up and ecologically restored. One was near Old Yamuna River Basin along the Burari region of North Delhi and one was near Vasant Kunj Area. The first area has been developed into Yamuna Biodiversity Park (https://dda.org.in/greens/biodiv/yamuna-biodiversity-park.html) and the second one is now developed into Aravalli Biodiversity Park (https://dda.org.in/greens/biodiv/aravalli-biodiversity-park.html). Post restoration these sites are now harbouring a number of forest communities such as (Source: Delhi Development Authority Database available at www.dda.org.in)

1. **Subtropical mixed evergreen forest ecosystem**
   Top canopy - *Toona ciliata, Dalbergia latifolia, Mitragyna parvifolia, Syzygium cumini*
   Middle storey - *Trewia nudiflora, Artocarpus lakoocha, Cinnamomum camphora*
   Shrub layer- *Dillenia indica, Coffea benghalensis, Murraya paniculata, Bauhinia malabarica*
   Herbs and Grasses- *Barleria cristata, Flemingia bracteata, Desmodium triflorum*
   Climbers- *Vigna capensis, Combretum decandrum, Vitis paniculatum*
2. **Moist tropical deciduous forest ecosystem with Teak as a dominant species**
   Top Canopy- *Tectona grandis, Pterocarpus marsupium, Diospyros melanoxylon*
   Middle storey- *Buchanania lanzan, Albizia lebbeck, Bauhinia variegata*
   Shrub layer- *Flemingia rugosus, Vitex negundo, Nyctanthus arbortristris, Zizyphus mauritiana*
   Herbs and grasses- *Desmodium triflorum, Crotolaria juncea, Bothriochloa pertusa*
   Climbers- *Pueraria phaseoloides, Asparagus racemosus*
3. **Tropical dry decuduous forest ecosystem with Sal as a dominant species**
   Top canopy – *Shorea robusta, Diospros melanoxylon, Putranjiva roxburghii*
   Middle storey – *Erythrina indica, Cassia fistula, Albizia sp., Sterculia urens*
   Shrub layer – *Carissa spinarum, Zizyphus oenoplea, Nyctanthus arbortristris*
   Herbs & Grasses – *Chloris, Eragrostis, Fimbristylis ferruginea, Indigofera tinctoria*
   Climbers – *Smilax zeylanica, , Clittoria turnatea, Marsidenia, Cocculus hirsutus*
4. **Tropical Dry Deciduous forest with Teak as a dominant species**
   Top Canopy – *Tectona grandis, Butea monosperma, Sterculia urens, Terminalia chebula*
   Middle storey – *Emblica officinalis, Bauhina variegata, Cochlospermum religiosum*
   Shrub layer – *Gardenia turgida, Randia dumetorum, Grewia asiatica*
   Herbs & Grasses – *Barleria prionitis, Bothriochloa pertusa, Dicanthium Hetropogo*
   Climbers – *Abrus pulchellus, Cocculus hirsutus*
5. **Tropical thorn forest**
   Top Canopy- *Acacia sp., Prosopis cineraria, Anogeissus pendula*
   Underwoods- *Zizyphus mauritiana, Maytenus emarginatus, Wrightia*
   Herbs and Grasses- *Vicovestata, Vico auriculata, Desmostachya bipinnata,*
   Climbers- *Valletia, Leptochloa fusca, Tinospora cordifolia*
6. **Scrub jungle**
   Top Canopy- *Acacia catechu, A. senegal, A. leucophloea*
   Underwoods- *Euphorbia neriifolia, Cassia auriculata, Maetenus emarginatus*
   Herbs- *Tephrosia purpurea, Justicia simplex, Cyperus rotundus, Eragrostis tenella*
   Climbers – *Cocculus laurifolius, Rhynchosia minima*

In most cases, the general techniques used in ecological restoration can be summed up by the following:

1. Identification and extensive surveys in the site/plot selected for ecological restoration followed by enlisting the floristic composition (if any) and also recording the degree of infestation by weeds, degree of degradation such as properties

of surface (with or without soil) and the levels of degradation of the general landscape. Also, in the process identified the key weed species which needed to be eradicated from the site using physical, chemical or biological means and for this gather pertinent information on the ecological and biological information on the said weed species, e.g. Phenological data, means of dispersal of the pollens and seeds, etc.

2. From the floristic documentation of the area done in past such as Forest Types for the area in past, such as the one from a flora record, or even an Environment Impact Assessment (EIA) Committee report list out the species that will be used to raise a forest/grassland community. This is important to resurrect a native community which will be ecologically sustainable and will help in augmenting biodiversity. With this data one can outline the kind of ecosystem one is aiming to develop effectively "restoring" the area. It will be also important to devise means to monitor the effects of the techniques applied. For this, a native forest ecosystem found in a similar site needs to be kept as a positive control or the reference site. Other means of monitoring the ecosystem could be by on ground vegetational surveys and monitoring changes in above-ground biomass from remote sensing techniques using geographical information system (GIS) and also correlating the same with the changes in ecological processes provided by changing composition such as changes in soil properties, moisture levels in soil, moisture rentention period per year etc. In this manner, a detailed monitoring program specifying the chosen indicators on which information will be recorded during the restoration period to assess the recovery of ecosystem over time needs to be done.
3. Initiate restoration by choosing a proper intervention method to eradicate weeds and other undesirable non-natives species. This needs to be followed by addition of biological inputs to the substratum with farmyard manure, microbial inoculum enrich in plant growth-promoting rhizobacteria (PGPRs) and other saprophytic microbes, decomposers, mineralizers, nitrogen fixers, mycorrhizal fungi, etc.
4. To begin with plantation inputs, an exhaustive planning needs to be made regarding digging of pits, the spacing between pits, time of plantation, establishment of a temporary nursery to generate a massive amount of planting material of trees, bamboos, ground cover species, etc., and planting material from other relevant sources such as a nearby natural forest area from where planting material could be brought in by "wilding".
5. Stabilization of the slopes should be the emphasis of the programme in the initial years to prevent erosion by wind and water. For this, grass species such as *Heteropogon, Bothriochloa and Tetrapogon* need to be planted during early monsoons.
6. Next step should be stabilization of the general surface by forbs comprising of annual and perennial grasses, legumes, and other herbaceous plants, so that litter formation and basic ecological processes can augment. This helps in building a microbial flora and fauna and creates a below-ground conducive environment for the tree roots to develop.

7. Planting of the woody species in well-spaced pits of 1 to 3 feet depth. The spacing should be optimum, not too close or wide, e.g. a spacing of 1.5 × 2 m is ideal. The planting material should be minimum 1 year old and if originating from a nursery should be properly hardened-off for at least 3 weeks before transplantation.
8. Regular monitoring after transplantation should be carried out to record success rate of seedling-sapling establishment and mortalities/casualties need to be replaced immediately from the nursery material.
9. Monitoring and mid-course correction, if and when required should be laid out explicitly for ensuring no failures in the efforts of the human-induced restoration.
10. Depending on the degree of degradation and the approximate time required, strategy needs to be made for long-term protection and maintenance of the restored ecosystem.

4 Soil inhabiting Microbial Communities and Ecological Restoration

4.1 Soil as a Microbial Habitat

Soil is one of the most diverse habitats on earth. It provides highly heterogeneous environments for organisms inhabiting it (Gans et al. 2005; Zwolinski 2007). *Microbial diversity* indicates the number and abundance of species comprising a community in a given habitat. At the molecular level it is the sequence diversity for a given gene family in a community DNA sample isolated from an environment (quintessentialy, the metagenomic approach)(Liesack et al. 1997; Garbeva et al. 2004). *Microbial community structure* is a qualitative term. It indicates the number of individuals of the diverse taxa which share a common habitat and perform key functions at a trophic level. The functions performed by different trophic levels of microbial communities (bacterial and fungal) include decomposition of organic matter, involved in mineralization and nutrient cycling (van Elsas and Trevors 1997; Garbeva et al. 2004). Below-ground soil communities also maintain essential ecosystem functions in both managed and unmanaged soils (Hairston et al. 1968; Doran et al. 1996; van Elsas and Trevors 1997; Garbeva et al. 2004), by participating in soil structure formation and toxin removal (van Elsas and Trevors 1997), by promoting plant growth, suppressing a number of soil borne pathogens and bringing about changes in above-ground vegetation (Nitta 1991; Abawi and Thurston 1992; Doran et al. 1996).

There are evidences that soil microbial parameters may be useful as early and sensitive indicators for stressed soil and its alleviation (Dick 1992, 1994; Dilly and Blume 1998). The composition of soil microbiota has been established as a biological indicator of soil health and quality (Dick 1994; Fauci and Dick 1994;

Filip 1998). The treatment or management of soil with application of pesticides (Heilmann et al. 1995), amendment with chitin (Hallmann et al. 1999), compost or manure (Schonfeld et al. 2002) and the introduction of genetically modified microorganisms (De Leij et al. 1994; Mahaffee and Kloepper 1997) have been shown to affect soil microbial community structures. Soil type and cropping practices also influence the microbial community composition (De Leij et al. 1994; Latour et al. 1996; Westover et al. 1997; Grayston et al. 1998; Horwath et al. 1998; Lupwayi et al. 1998).

4.2 Rhizospheric Microbial Biodiversity

In 1904, a German agronomist and plant physiologist, Lorenz Hiltner, coined the term "Rhizosphere". He defined a rhizosphere as a zone of soil close to the plant root in which the activity of the microbial community was enhanced, with consequent effects on nutrient (mainly nitrogen) availability to the plant (Hiltner 1904; Smalla et al. 2001; Sanguin et al. 2006; Hartmann et al. 2008). The definition is now slightly modified to mean the volume of soil directly in contact with plant roots, or the zone of influence of plant roots on the associated biota (Lynch 1998). The influence may be chemical, biological or physical (Jones and Schmitz 2009; Walker et al. 2003; McNear 2013). This zone supports an active microbial population distinct from the bulk soil and is mainly selected by the rhizospheric environment (Sørensen 1997; Marilley et al. 1998; Hawes et al. 1998, 2000; Hinsinger et al. 2009; Raaijmakers et al. 2009; Chaudhary et al. 2012). The bulk soil is that soil which is not associated with plant root-soil interaction zone. Bulk soil is remarkably diferent from rhizospheric soil in not one but many ways. The rhizosphere can cover large areas if the roots are extensive (Bolton et al. 1993). The rhizosphere is highly complex because of the numerous interactions co-existing within constituent biotic groups (van der Heijden et al. 1998, 2008). These interactions may be beneficial or harmful to the plant (Brimecombe et al. 2001).

5 Linkage of Above- and Below-Ground Biodiversity

Community structure, biomass and specific functions of soil microorganisms can indicate soil health and quality (Hooper et al. 2000; Wolters et al. 2000). Soil biota is assumed to be responsible for soil ecosystem processes, especially nutrient cycling and decomposition of soil organic matter (Wardle and Giller 1996; Brussaard et al. 2004; Coleman et al. 2004). Soil organic matter in turn is an important attribute that shows positive correlation with above-ground plant diversity (Le Houerou 1969; Lugo et al. 1986; Cesar 1989; Aronson et al. 1993). Organic matter and carbon is mainly contributed by crop refuse, leaf litter, animal refuse and detritus influencing microbial communities (Schaeffer and Whitford 1981; Bardgett 2005; Bardgett and Wardle 2010). In a nutshell, the composition of the soil microbial assemblage can (i) affect plant growth, (ii) alter species composition and distribution, and (iii) change the colonization pattern of plant species (Turkington et al. 1988; Bever 1994). Likewise, rhizospheric microbial communities are influenced by various plant processes viz. root exudates, respiration and absorption of chemical ions that affect rhizospheric chemistry (Grayston et al. 1998; Paul and Clark 1996a, 1996b; Marschner et al. 2001). In simpler words, it can be concluded that the success of establishing above-ground plant communities as part of an ecological restoration programme essentially depends on site and the taxonomic and functional diversity of native or introduced below-ground communities and therefore understanding microbial community structure and improving/enhancing the richness will bear fruits in terms of successful establishment and perpetuation of above ground communities, which is the very goal of restoration effort. (Zak et al. 1992; Wardle et al. 2002, 2004; Moore et al. 2004; Bardgett et al. 2005; Bardgett and Wardle 2010; Miki et al. 2010).

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Chapter 20
Biochar: An Emerging Panacea for Contaminated and Degraded Soils

Shilpi Jain and Puja Khare

Abstract Biochar is a black solid material derived from the thermo-chemical decomposition of solid organic material in an oxygen-deficit atmosphere. In recent years, biochar has been contributed as a technique that can provide several environmental benefits upon application to soil, including long-term storage of carbon (C) in soil. Because of their dominantly aromatic nature, biochars are advised as a resistant form of C with long mean residence times (MRTs) in the range of hundreds to thousands of years.

Different pyrolysis techniques (e.g., torrefaction (a pyrolysis process at low temperature), slow pyrolysis, gasification, fast pyrolysis, intermediate pyrolysis, hydrothermal carbonization (htc), or flash carbonization) are used for biochar production. Recently, research on torrefied biomass as soil ameliorant has started only. Biochar characteristics are governed by production variables such as feedstock, highest treatment temperature, holding time at HTT, pyrolysis conditions, etc. Feedstock properties (both physical and chemical) and HTT are considered to be the main factors influencing biochar physico-chemical characteristics. Currently, biochar is prepared at small scale to large scale. In some countries, it is used for kitchen garden and prepared from the domestic waste. Both traditional earthen charcoal kilns and modern charcoal retorts can be used for biochar production. The traditional earthen charcoal kilns and charcoal retorts can be used for the industrial production of biochar. In former technology, pyrolysis, gasification, and combustion processes occur in earthen kiln layer. In the modern charcoal retorts, a metal barrier is used for the separation of pyrolysis and combustion processes. A specific biochar according to its inherent physico-chemical properties can be utilized for particular application. For an example, high surface area biochar may be utilized as a sorbent, whereas high recalcitrance biochar may be used in carbon fixation. Biochars rich in nutrient and mineral contents with high water holding capacity could be more suitable for soil fertility enhancement.

The application of biochar as an organic amendment is favourable in terms of carbon capture and fertility of soil. Biochar accommodates a suitable habitat for

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microorganisms due to its high porosity, adsorption and cation exchange capacity and affecting different microbial processes involved in nutrient cycling, green house gas emission and organic matter (OM) decomposition, etc. Other than its agriculture benefit, there is increasing interest in the implementation of biochar as an alternative technique for many environmental issues such as amelioration of contaminated sites. In recent years the effectiveness of the combination of biochar and other organic materials, for example compost, has been reported widely with regard to the remediation of polluted soils and the improvement of soil resistance against erosion and nutrient retention.

The liming and sorptive properties of biochar make it suitable for reclamation of low pH and metal polluted soils such as acidic mine spoil. Biochar amendment in acidic and polluted soil can serve dual purpose: (a) improve soil health, (b) extenuate the risk of heavy metal pollution in various environmental surroundings. The combination of phyto-remediation in combination with biochar addition could be an excellent technology to improve the soil quality index in the coal mine area. In this chapter, the potential of biochar amendment for promoting the establishment of a plant cover and phyto-stabilization strategies on contaminated soils has been discussed.

1 Introduction

The concept of biochar came in existence after the discovery of rich Amazonian soils called terra preta which has 70 more times carbon, more phosphorous, nitrogen, calcium and potassium in comparison to surrounding soil. It is supposed that soils of significantly better quality were formed where society lived for a longer period over a long period of time. It may lead to enrich the soil with waste and charcoal, which, as a soil amendment, performed the functions of decontaminating waste and recovering the texture of soil (Schmidt 2012). Recently the focus is to meliorate the common agricultural soil, particularly polluted one with charcoal to acquire the quality of Amazon soil which has similar properties as in biochar according to PNW-Biochar and U.S.

Biochar is defined as inhomogeneous material rich in aromatic carbon and minerals. Sustainably obtained biomass is pyrolysed under controlled conditions for biochar production, an environmental friendly technology. The application of biochar in soil not only reduces CO_2 emission but also improve soil health and texture of degraded one. Biochar is a carbon-negative technology and can remove almost 0.25 Gt/year of carbon from the atmosphere by 2030 by stabilizing it. And this is only by using sustainable organic waste material for biochar production (IBI 2015). In terms of climate change the production and use of biochar in soil or as a fossil fuel not only help to prevent carbon addition to the atmosphere in the form of CO_2 but also maintain level of greenhouse gases in the atmosphere, as occurs in the case of burning biomass, which is carbon neutral. When biomass is pyrolysed and converted into biochar, carbon becomes more resistant, pollutants emission into the atmosphere is less than combustion. When biochar is used to ameliorate soil, the texture and productivity of the soil improves and soil becomes an effective carbon sink as a result stability of carbon enhanced and carbon can be sequester permanently from the atmosphere and this makes carbon negative technology. It is a modern concept according to its function and application (carbon sequestration). In terms of carbon sequestration the benefit of biochar is that carbon is transformed into less circular form (i.e., black carbon), which has the capacity to remain stable from hundreds to thousands of years. Stability of biochar in soil and change into its resistance is characterized by the H/C ratio. If the value of the H/C ratio is less than 0.7, the organic carbon will remain stable for 100s of years in biochar.

Sustainable` biochar is one of the modern, inexpensive, rapidly scalable and widely applicable technology that can be used for climate mitigation and soil health enhancement. There is an urgent need to recycle increasing amount of solid waste and sewage sledges in a sustainable way. Converting these wastes into biochar and biofuels is highly doable, sustainable and renewable. For each community, it is required to design processes to tailor for biochar production and application. Different biochars are produced with different physicochemical properties depending upon the biomass properties and pyrolysis conditions.

2 Current Technology Used in Biochar Production

Carbonization of wood is in practiced for biochar production as old as human history (Emrich 1985) and civilization (Brown 1917). All the processes of biochar production involve heating organic material in oxygen-deficit environment to drive off volatile gases, leaving carbon behind. This process is called pyrolysis or gasification.

2.1 Pyrolysis

Pyrolysis is a process which includes thermal decomposition of organic waste in less or no oxygen environment to obtain solid (biochar), gas (syngas used as biofuel), and liquid (bio-oil). The black solid material termed as biochar, charcoal, char consists of high carbon content. This process is simply represented in Fig. 20.1. All of the three products obtained through the pyrolysis can have properties and uses that provide value to the process.

Pyrolysis process occurs in three steps in commercial biochar production: (a) volatiles and moistures are released; (b) unreacted material is converted to biochar, gases and liquid; (c) chemical rearrangement of char (Demirbas 2004). Eco-friendly energy can be produced by this method in the form of oil or gas along with biochar. It is one of the best technologies that is low expensive, quickly scalable and widely applicable.

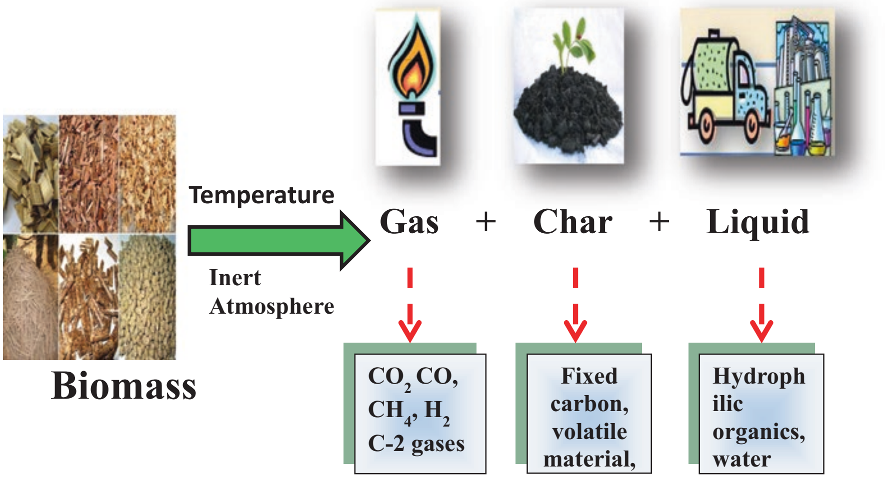

Fig. 20.1 Process of pyrolysis

Different pyrolysis reactors recommended by Emrich (1985) are given below:

1. Kiln: a traditional biochar maker, specially used to produce biochar.
2. Retorts and converters: used as an industrial reactor capable of producing not only biochar but also recovering volatile matter (oil and gas).
3. Retort: a reactor that has the capability to pyrolyse pile-wood, or wood log over 30 cm long and over 18 cm in diameter (Emrich 1985).
4. Converters: small particles of biomass such as chippes and pellets processed through carbonization to produce biochar in converters.

2.2 Pyrolysis Types

2.2.1 Slow Pyrolysis

Thermal decomposition of lignocellulosic biomass in the absence of oxygen by slow heating to temperature in excess of 400 °C producing almost equal amount of biochar, bio-oil and syngas. In conventional charcoal kilns the pyrolysis vapours and syngas are vented to the atmosphere causing serious air pollution threat (Kammen and Lew 2005). The modern slow pyrolyzers can be used in two different ways: (a) indirectly, they can capture volatiles from source which can be used for heat production after conversions. (b) Directly coupling with electricity generation (Fig. 20.2). However, the slow pyrolyzers are difficult for scaling due to slow heat transfer from coarse biomass. Hence, the relatively long residence time in the reaction chamber is required for the feedstock. Slow pyrolyzers produce lower amount of energy by-products hence used to produce high-quality biochar commercially.

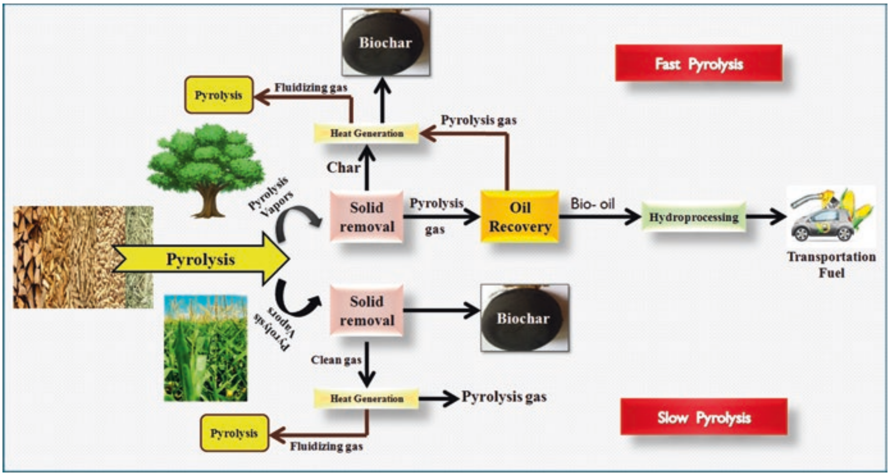

Fig. 20.2 Schematic diagram of fast and slow pyrolysis

Slow pyrolyzers have several benefits relative to other technologies. Slow pyrolyzers are small, inexpensive, and the feedstock does not need to be ground finely.

2.2.2 Fast Pyrolysis

In fast pyrolysis biomass is heated up to 400–700 °C rapidly in less or no oxygen environment. Finely ground biomass (<2 mm) is required to achieve rapid heating rates (Cummer and Brown 2002). Fast pyrolysis yields less biochar than slow pyrolysis. Fast pyrolyzers, i.e. fluidized and circulating bed reactors can be scalable. However, they can be operated at larger scale. The slow pyrolyzers are more economically viable as compared to fast pyrolyzers. Generally, the economic viability of fast pyrolyzers depends significantly on the market for products such as bio-oil. The sale of the biochar co-product will offer a comparatively minor source of profits. The lack of refineries which could be able to convert bio-oils into transportation fuels and other value-added products limits the growth of fast pyrolyzers (Jones et al. 2009; Garcia-Perez et al. 2009).

2.2.3 Laboratory Batch Pyrolysis Reactor

The laboratory batch pyrolysis reactor has a static bed reactor of quartz tube with 50 mm diameter and 200 mm sample bed depth. A12 kW infrared furnace with PID control is used in this type of reactor, which provide a broad range of heating rates with maximum temperature of 1300 °C. It has variable holding times from 5 to 180 min. A nitrogen carrier is used for sweeping of volatile and gaseous products of pyrolysis and carrying them into a sequence of condensers and receivers for the collection of liquid. A volumetric flow meter and an on-line, mobile mass spectrometer is used for measuring the cleaned syngas flow and gas composition, respectively. This type of system provides on-line data logging for temperature, pressure and gas volume flow. The 20 g of biochar per run can be produced in these types of apparatus; however, feedstock and pyrolysis conditions could effect the biochar yield.

2.2.4 Small-Scale Continuous Pyrolysis Reactor

Small-scale continuous pyrolysis reactor is a small continuous unit. It is able to produce up to 2 kg of biochar per hour. It can produce multiple samples of differently produced biochar from one feedstock by a series of different conditions in one run. It has electrically heated horizontal tubular reactor with dimensions 102 × 900 mm, residence time between 10 and 60 min. The maximum temperature in these reactors is 850 °C. After pyrolysis, the biochar product was collected in the vessel. The nitrogen carrier gas is used for the sweeping of vapours and gases into an afterburner chamber, where the complete combustion is ensured by propane-fuelled pilot flame. The sampled liquids are condensed and then collected. On-line mass spectrometer is used for syngas mixture analysis.

2.2.5 Large to Pilot-Scale Continuous Pyrolysis Reactor

The Pilot-scale unit, housed in a dedicated new building, is closely similar to the smaller scale pyrolyzer. However, it has rotating tube reactor with no internals. A feed hopper of capacity 600 L is mounted on load cells. It controls the feed rate. The tube is electrically heated in three independent zones at up to 850 °C each with PID control. Rotation speed range of 1–7 rpm allows variable residence times in the hot zone. An afterburner is used for disposal of the volatiles by combustion. The biochar product is collected into a sealed product drum on a weight-scale. These technologies provided production rates up to 20 kg h^{-1}, which can depend upon the feedstock and pyrolysis conditions.

3 Structural Properties of Biochar

Biochar is a black recalcitrant organic solid material derived from partial or complete decomposition of carbonaceous biomass as well as the mineral fraction. Biochars obtained from different feedstock materials have unique physicochemical properties according to their feedstock material, hence each biochar has unique characteristics (Spokas et al. 2011). Slow pyrolysis is best suited for biochar production due to decreasing yields of bio-oil. Biochar derived from woody feedstock has higher carbon contents than those derived from herbaceous biomass (Zabaniotou et al. 2008; Al Jeffrey 2013), but depleted in nitrogen (Cao and Harris 2010). Still these biochars showed positive effect on soil and promote their microbial colonization. Biochars produced from agricultural wastes are more enriched in phosphorous, nitrogen and micronutrients (Cantrell et al. 2012). It is important to know how feedstock type and pyrolysis conditions are influenced by physicochemical properties of biochars (Lehmann and Joseph 2009; Spokas et al. 2011). It is important to choose appropriate feedstock material. The contaminated feedstock may behave differently duringe pyrolysis. Their resulting concentration in biochar depends upon the process of enrichment, elimination or conversation in the final product. There is recently no consent regarding standardized analytical procedure for biochar characterization in the scientific community. Therefore, it is urgent to standardize a simple analytical protocol to acquire a "fingerprint" of each biochar. Fourier transform infrared (FT-IR) spectroscopy is a widely used technique to differentiate the main functional groups of biochar such as cellulose, carbohydrates, proteins and lignin. Different biochar properties can also be characterized by using proximate and ultimate analysis. Biochar is composed of graphite-like layers and when pyrolysis temperature increases the spacing between these layers also increases in slow pyrolysis (Cantrell et al. 2012). Aromatic carbon (aryl-C) with small proportions of alkyl-C and alkyl-O are dominant in biochar when produced at 350 °C. These alkyl-C and alkyl-O is totally converted to aryl-C when temperature increases up to 450 °C and H/C ratio becomes low.

Porosity, surface area and pH are important properties of biochar for its application to soil. Biochar is made up of micro- as well as macronutrients along with minerals in the ash fraction. Biochar physicochemical properties vary according to the nature of feedstock used and pyrolysis temperature and conditions. Thus a desirable biochar can be derived from both its proximate and ultimate analysis. The maximum value of molar H/C ratio prescribed by the International Biochar Initiative (IBI) is 0.7 (Wiedner et al. 2013) to differentiate biochar from its biomass that has been somewhat altered or not. Thus high-quality biochar can be produced by selected working conditions and technology.

3.1 Physical Characteristics of Biochar

It was reported that surface area increased from 120 to 460 m^2/g (Day et al. 2005) with increase in temperature from 400 to 900 °C. Low-temperature biochars may be best suited to control release of nutrients (Day et al. 2005), while high-temperature biochars have more activated carbon (Ogawa et al. 2006). It is observed that low-temperature biochars may have low capacity to store water in soil. High-temperature biochars are fragile and prone to scrape into fine fraction once added to the mineral soil, while low-temperature biochars are strong. Volatiles and nitrogen components are lost in biochar if temperature increases from 400 to 600 °C and ash and fixed carbon contents increased (Purakayastha 2012). C:N ratio is more in biochar when prepared at 60 °C and more stable in soil too. Due to negative surface charges, most of the biochars have high cation exchange capacity (CEC). Therefore, several research studies have been designed to investigate the adsorption of cations onto biochar (e.g., Cao et al. 2009; Jiang et al. 2012); this is the reason by which biochar can be used for reclamation of heavy metal contamination.

3.2 Chemical Characteristics of Biochar

The pH of biochar ranged from 8.2 to 13.0 according to the feedstock used to produce biochar and carbon content is also increased with the increase in pyrolysis temperature. Total carbon content of biochar varied from 33 to 82.4% according to the carbonaceous material used to produce biochar. N and S compounds volatilize at 200–375 °C, whereas K and P volatilize between 700 and 800 °C (DeLuca et al. 2006). Therefore, reduction in N and S is there in biochar prepared at high temperature.

Low-temperature biochars (350 °C) tend to have greater amount of extractable P, NH_4^+, and phenols, while high-temperature biochars have higher pH, EC and extractable NO_3^- (DeLuca et al. 2006). The ratio of O/C and H/C decreased at high temperature and the lower ratio was found good in terms of stability and aeromaticity (Baldock and Smernik 2002). In general, biochar had low nitrogen content as well as C/N ratio varied from 19 to 221. Ca, K, P and Mg are present in appreciable quantity in biochar. Biochar can be used as a liming agent in acid soil due to its high pH and appreciable quantity of Mg and Ca. Higher pH was found in biochar prepared from maize (10.7) and pearl millet (10.6) than that in wheat (8.8) and rice (8.6) and highest CEC was found in rice residues biochar. Total carbon content found higher in pearl millet biochar (61%) followed by wheat (52%) and rice biochar (49%), while lowest carbon content (37%) was found in maize. General physico-chemical properties of biochar samples prepared from different feed stocks are given in Table 20.1.

4 Biochar: An Adsorbent for Contaminants

Biochar is used as an adsorbent for pollutant reduction in soil and water. By removing contaminants from soil and water, biochar can help to alleviate environmental issues (Ahmad et al. 2014). Biochar can be used as a sorbent due to high number of oxygenated groups such as hydroxyl, carboxyl and phenolic surface functional groups present on biochar surface and have proven to be binding sites for soil pollutants (Uchimiya et al. 2011). Due to biochar amendment, retention of heavy metals in soil has been reported (Houben et al. 2013). Bioavailability and eco-toxicological impacts of these toxic elements can be sequester by biochar application in soil and water due to its microporocity and high surface area. High uptake of heavy metals (Cu, Cd, Pb and Ni) from soil with cottonseed hulls biochar has been reported by Uchimiya et al. (2011).

Table 20.1 Physico-chemical properties of biochars prepared at different pyrolytic temperatures

Pyrolysis type	Biomass	Pyrolysis temp (°C)	pH	EC ds/m	CEC (cmol + kg^{-})	Ash (g kg^{-1})	VM (g kg^{-1})	C %	H %	O %	Reference
HTT	Poultry shed waste Papermill residue	550	8.9 6.8	6.1 1.3	–	–	–	–	–	–	Van Zwieten et al. (2015)
Slow	Sugarcane straw	700	10.2	.05	14.3	13.4	–	–	–	–	Puga et al. (2015)
Slow	Conocarpus wood wastes	400	9.67	1.34	–	–	–	76.83	2.83	14.16	El-Naggar et al. (2015)
Slow	*Tectona grandis* (teak)	500	7.5	–	10.9	–	–	23.1	–	–	Partey et al. (2016)
Gasification	Kentucky bluegrass seed waste	650–750	10.2	2.98	43.1	50.6	–	32.7	–	–	Novak et al. (2016)
Slow	Poultry litter swine manure	400	9.5 10.0	–	30.2 52.2	25.3 27.5	44.9 29.9	52.1 54.9	–	–	Subedi et al. (2016)
Slow	Willow (*Salix* spp.) feedstock	400–550	9.7	–	20	10.6	13.6	81.3	1.9	3.9	Hangs et al. (2016)
Slow	Poultry litter	350 450 550	10.20 10.45 10.75	25.0 27.0 30.0	47 40 30	–	–	–	–	–	Song and Guo (2012)
	Poultry litter Wheat straw	450	7.7 8.4	5.2 9.2	18.1 23.7	–	–	38.3 53.1	–	–	Macdonald et al. (2014)
Slow	Sugarcane trash Begasse	550	9.6 8.4	4.8 .18	40 3.5	–	–	68 65	–	–	Quirk et al. (2012)
Slow	Variety of wood species	500–700	9.2	–	111.9	4.6		72.9			Major et al. (2012)

(continued)

Table 20.1 (continued)

Pyrolysis type	Biomass	Pyrolysis temp (°C)	pH	EC ds/m	CEC (cmol + kg^{-})	Ash (g kg^{-1})	VM (g kg^{-1})	C %	H %	O %	Reference
Slow	Giant reed (Arundo-donax L.) a perennial C3 grass	–	9.45		12.5	10	–	73.4	3.01	23.1	Zheng et al. (2013)
Slow	Rice straw Bamboo	600	10.2 9.81	18.1 .07	44.7 15.3	383.3 62.3	–	51.3 83.2	26.2 22.8	–	Dong et al. (2013)
Slow	Burcucumber (*Sicyos angulatus* L.)	300 700	10.54 12.56	–	–	31.24 54.29	–	68.37 78.07	6.00 2.8	20.39 14.66	Vithanage et al. (2014)
–	*C. vulgaris*	500	–	–	–	19.96	23.46	61.96	3.87	4.78	Wang et al. (2013)

4.1 Inorganic Pollutants

To test the ability of metal fixing by biochars, aqueous solution tests are often done. Kim et al. (2014) compared the ability of spent coffee ground (SCG) biochar to reclaim acid mine drainage contaminated with metallic elements such as Cd, Zn, Pb and Cu. The maximum sorption capacities of spent coffee ground (SCG) biochar for Cd (99%), Cu, Pb and Zn were 99%, 88%, >99% and 99%. The maximum sorption capacities of spent coffee ground (SCG) biochar for Cd (99%), Cu, Pb and Zn were 99%, 88%, >99% and 99%. Recently, Frišták et al. (2015) evaluated the application of beech wood chips biochar and garden green waste residue biochar as sorbent of Cu, Zn and Cd in aqueous solution. The maximum sorption capacities of beech biochar for Zn, Cd and Cu were 0.97, 1.99 and 2.50 mg/g, while 2.23, 7.80 and 3.65 mg/g for green waste biochar. Additionally authors reported that the metal sorption efficiency of biochar is affected by contact time, application rate, pH value and initial metal concentration. The efficiency of biochar for reclamation of soil was evaluated by tests with “natural” as well as artificial contamination. Biochars have potential to reduce the availability of inorganic pollutants and as a result to decrease their toxicity (Kim et al. 2014; Rodríguez-Vila et al. 2015). To conclude, metal availability and toxicity have been shown to reduce in amended soil. However, it is difficult to think that one type of biochar could be universally used to reclaim all contaminated soils. The remediation ability of biochar depends on the nature of metallic element and on the characteristics of biochar produced from different feedstock material and pyrolysis conditions (Zhang et al. 2013b).

4.2 Organic Pollutants

The ability of biochar to remove PAHs from soil and water has been reviewed in several studies. It has been reported by Oleszczuk et al. (2012) that biochar produced from maize stover and paper mill waste amended at different rates from 0.5 to 10% to sewage sludge decreased 13 dissolved PAHs tested depending on the dose. With the increase of contact time between biochar and sludge, reduction in PAH concentration also increases. Similarly, Chen and Yuan (2011) reported that the addition of pine needle biochar in soil also increases sorption of pyrene, naphthalene and phenanthrene. The ability of biochar to control the bioavailability of pollutants is influenced by the feedstock and pyrolysis conditions.

4.3 Multiple Contaminations

Sites polluted with different organic and inorganic contaminants are difficult to reclaim because different pollutants show different behaviour and require a specific treatment (Sneath et al. 2013). Several literatures reviewed the impacts of biochar

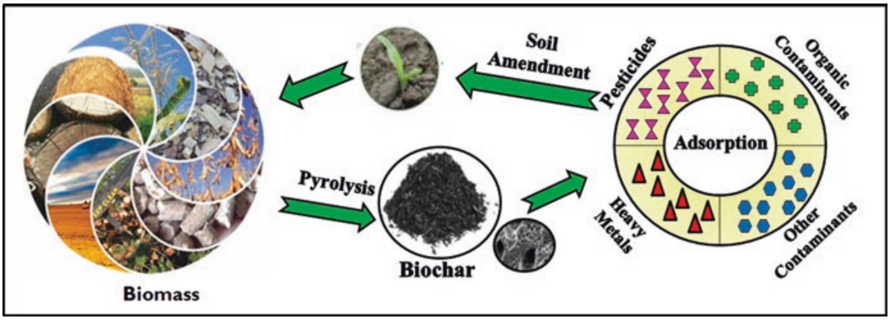

Fig. 20.3 Biochar as an adsorbent

on soil polluted with metallic and organic pollutants (i.e. PAHs and pesticides). Biochar produced from poultry litter and paper mill sludge increased the sorption of diuron and atrazine in soil by two- and five-folds (Martin et al. 2012). Brennan et al. (2014) reported the effect of two biochars prepared from pine wood chip and maize stubble produced at 450 °C and amended in a loam soil polluted with PAHs and metallic contaminants (Cu, As and Zn) and planted with maize. The authors found that maize stubble biochar had greater efficiency to remediate contaminated soil by decreasing the accumulation of PAHs and metallic compounds in shoots, due to difference in biochar characteristics (e.g. pH, CEC) (Fig. 20.3).

5 Biochar: A Soil Fertility Enhancer

Application of biochar for the soil fertility improvement and carbon sequestration will be discussed which include the studies carried out globally and feasibility of biochar as a soil enhancer.

There is a major necessity to develop more sustainable agriculture system to improve weak and rural economies. In global agriculture infertility, soil degradation and increased erosion is a major concern (Jianping 1999). Continuous cultivation of soil could result in organic matter depletion, soil erosion, degradation and containing soil acidification (De Meyer et al. 2011). Furthermore, the aggregate stability of soil is lost due to decrease in depletion in organic matter (Annabi et al. 2011). Hence, a simple and sustainable method is required to remediate the degraded soil.

Organic fertilizers and manures contain pharmaceuticals, pathogens and heavy metals which may cause long-term pollution of agricultural land. Along with global warming, serious groundwater and stream nutrient pollution caused by manures and fertilizers have the potential to release methane and ammonia. Biochar is a sustainable and promising resource for soil's fertility management due to its economic and environmental benefits. To enhance soil fertility biochar could also be proposed as a slow-release fertilizer if loaded with phosphate, ammonia and nitrate (Schmidt et al. 2015; Kammann et al. 2015; Xu et al. 2014). Generally nitrogen, carbon, hydrogen and some lower nutrient element such as Ca, Mg, Na and K are included in biochar composition (Zhang et al. 2015). Commonly, hydrogen and nitrogen contents decreased with increasing pyrolysis temperature from 300 to 800 °C, whereas the carbon content increased. Biochar has a strong affinity to inorganic ions such as nitrate, phosphate and heavy metal ions due to the presence of polar and nonpolar substances in biochar along with high surface area (Kammann et al. 2015; Schmidt et al. 2015). It is reported in several studies that biochar has the potential to improve microbial properties of soil along with chemical and physical properties. Many authors found that the biochar amendment in soil could improve soil structure, enhance aggregation and water retention and decrease bulk density (Baiamonte et al. 2015). In addition it is also reported that biochar can increase cation exchange capacity by 20% (Laird et al. 2010), electrical conductivity by 124.6% (Oguntunde et al. 2004) and reduce soil acidity by 31.9% (Oguntunde et al. 2004).

Many authors reported in field trials that biochar application is not only improved soil quality but also increased crop production and plant growth. Novak et al. (2016) reported that corn yield increased by 150 or 88% with applying 15 or 20 t ha^{-1} of biochar, when compared to control. Bhattacharjya et al. (2016) found that wheat grain yield increased by 6.2–24.2% over control with the application of Pine needle BC and Lantana BC.

5.1 The Effect of Biochar on Physico-Chemical Properties of Soils

Performances and mechanisms of biochar for soil fertility enhancement can be understood by its physical and chemical properties. Water holding capacity enhancement after biochar treatment is the main possible mechanism for yield improvement (Jeffery et al. 2011). Due to high porosity biochar could hold water in small pores and thus increase water holding capacity after heavy rain and assist water to infiltrate through large pores from the ground surface to the topsoil (Asai et al. 2009). Peake et al. (2014) reported that available water capacity increased 22% with biochar application and Nelissen et al. (2015) demonstrated increase in available water capacity from 0.12 to 0.13 m^3 m^{-3} with biochar treatment. After the application of rice husk biochar the capacity of soil aggregates increased from 8 to 36% and increase soil pore structure parameters by 20% and shear strength whereas decrease soil swelling by 11.1% (Lu et al. 2014). In addition, biochar could decrease bulk density from 1.47 to 1.44 mg m^{-3} and enhance porosity from 0.43 to 0.44 m^3 m^{-3} (Nelissen et al. 2015). Overall biochar has the potential to improve physical properties of soil such as porosity, surface area, water holding capacity, bulk density and aggregation ability, may enhance the retention of both nutrients and water, which promote to soil fertility directly. Many studies indicated that biochar application could increase soil pH and EC (electrical conductivity). Wang et al. (2014) found that tea garden soil (acidic soil) pH increased from 3.33 to 3.63 by rice husk biochar.

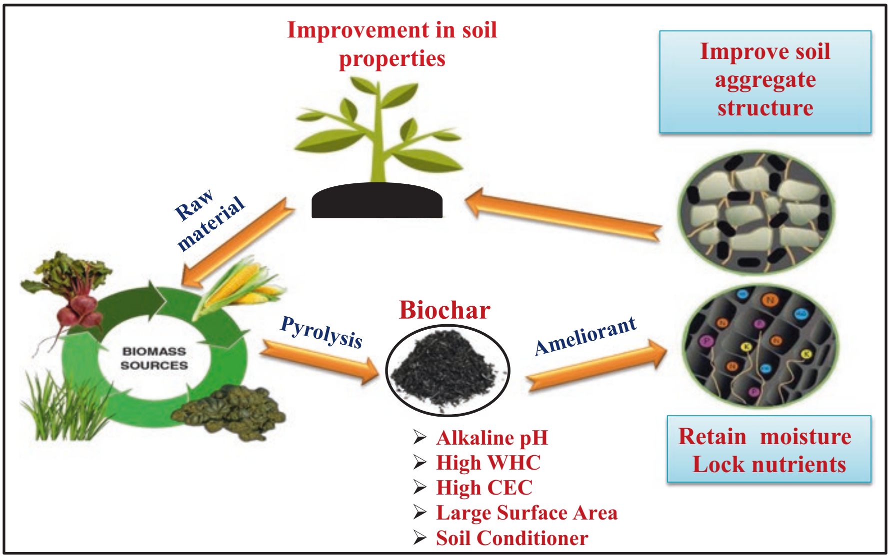

Fig. 20.4 Biochar as

Enhanced soil pH could facilitate some elements adsorption of the root. The high specific surface area and a number of carboxylic groups of biochar could increase the amount of exchangeable cations in the amended soils, facilitate improvement in soil fertility and nutrient retention (Cheng et al. 2006). It is also reported that biochar application could increase amount of extractable nutrients such as Ca, Mg, K and Na from 60 to 670% (Wang et al. 2014). In addition, biochar treatment could increase total carbon from 2.27 to 2.78, available phosphorous from 15.7 to 15.8 mg kg^{-1} and total nitrogen from 0.24 to 0.25% (Jones et al. 2012).

However, changes were not always detected due to biochar amendment. For instance, after 3 years of biochar application in a UK field trial, soil electrical conductivity (46–43 μS cm^{-1}) and bulk density (1.04–1.08 g cm^{-1}) were not considerably influenced (Jones et al. 2012). However, the difference between hydraulic conductivity and plant available water capacity was not observed after 2 years of biochar addition by Nelissen et al. (2015). These studies indicated that the influences of biochar on soil fertility enhancement varied with different application rate, pyrolysis conditions and feedstock material used to produce biochar (Fig. 20.4).

The effect of biochar application on soil microorganism has been widely studies. In general, it has been found that microbial abundance of soil increased with biochar addition (Fox et al. 2014; Luo et al. 2013) due to modification of soil pH, moisture, porosity, available nutrient contents and surface area (Steiner et al. 2007). Muhammad et al. (2014) found that biochar prepared from *P. australis*, *B. Rapa*, swine manure and fruit peels enahnced the total microbial PLFA concentrations except for *B. Rapa*. In this case, the change in microbial abundance and microbial community structure were correlated with pH and dissolved organic carbon in biochar added soil. 114% increase of the microbial biomass measured after 3 years input of corn stover biochar with the fumigation extraction method at an application rate of 30 t ha^1 (Domene et al. 2014). In addition, microbial communities were also modified along with microbial abundance in biochar amended soil (Muhammad et al. 2014; Hu et al. 2014).

Anyika et al. (2015) explained in their review how biochars affect microbial activity and microbes in soil. In the same study cited several reasons (a) provide suitable habitat to microbes due to high surface area and presence of pores, (b) increase not only water holding capacity but also oxygen and nutrient contents, (c) soil pH cations modification, (d) provide carbon and energy source. Two mesofauna species (enchytraeids and collembolan) were assessed with corn stove biochar addition to a loamy soil (Domene et al. 2015). High-temperature biochars have provided a suitable new habitat to favour microbial growth due to high surface area (Khodadad et al. 2011).

5.2 *Carbon Sequestration*

Soil carbon sequestration is the removal of CO_2 from atmosphere through photosynthesis to form organic matter, which is eventually stored in the soil as long lived and resistant form of carbon. The worldwide carbon cycle is comprised of streams and pools of carbon in the World's framework. The important pools of carbon are climatic, terrestrial, geological, and sea. The carbon inside these pools has fluctuating lifetimes, and streams take place between them all. Carbon in the dynamic carbon pool moves quickly between pools (Lehmann 2007b). In order to reduce carbon in the environment, it is important to move it into an inactive pool containing resistant or inert carbon. Controlled carbonization changes over significantly larger amounts of biomass organic matter into stable C pools which are accepted to persevere in the earth over hundreds of years (Schmidt and Noack 2000; Glaser et al. 2001). The transformation of biomass carbon to biochar leads to sequestration of about half of the initial carbon compared to the less amounts retained after burning (3%) and biological deterioration (under 10–20% following 5–10 years) (Lehmann et al. 2006). This efficiency of carbon transformation of biomass to biochar is very subject to the kind of feedstock, however is not affected by the pyrolysis temperature (within 350–500 common for pyrolysis).

Compared with other earthbound sequestration techniques, for example, afforestation or re-forestation, carbon sequestration in biochar expands its storage time (Sohi et al. 2010). The current slash-and-burn system causes release of greenhouse gases and degradation of soil. It additionally gives opportunity for improvement by transformation of the slash-and-burn system to the slash-and-char system. If the slash-and-burn system is replaced by the slash-and-char system, about 12% of the total anthropogenic carbon emission by land use change (0.21 Pg C) can be offset per year in the soil. Many global and domestic policy issues can be tackled by production and application of biochar to farm soil. Due to financial constraints, the application of biochar at the farm level is discouragingly slow. The quality and functionality of agricultural soil is always enhanced by adding soil C. Soil physical properties such as water retention, decrease in run-off as well as erosion and increase in aggregate stability is directly influenced by soil organic matter enhancement. These effects limit the requirement of nitrogen fertilizers for crop production and indirectly contribute to climate change mitigation (Khan et al. 2007). C storing ability of biochar has been confirmed by several field trial studies (Ogawa et al. 2006; Lehmann 2007). Using a wide range of biochar application program could be sequestered 9.5 billion tons of C in soil by the year 2100 (Lehmann et al. 2006). Globally 7.2 gigatons of C/year (GtC/year) emitted due to the use of fossil fuel and cement production (Glaser et al. 2002). Of that 3.1 GtC/year is absorbed by oceanic (2.1 GtC/year) and terrestrial ecosystem (1.0 GtC/year) and nearly 4.1 GtC/year remains in the atmosphere and increase green house gas concentration. Matovic (2011) expressed that, if 10% of the world biomass net essential production (60.6 GtC/year) is changed over into biochar at 50% yield and 30% energy from volatiles, it would sequester 4.8 GtC/year, which will be 20% more than the present yearly increment of environmental C at 4.1 GtC/year. Moreover, Woolf et al. (2010) demonstrated that biochar application could enlarge 12% of the yearly net emission of CO_2, CH_4 and N_2O (Table 20.2).

6 Future Prospective

The climate change, land deterioration, environment contamination, and water are the major scarcity global threats. They cause the damage of economic, society and environment. The biochar can become a solution to these global problems. It is capable to serve as carbon negative technology and used to improve soil fertility and sustainability in different ecosystems. It is a unique fertilizer which can be used directly or indirectly. It is flexible, because the diverse biomass materials can be applied for the biochar production at different temperatures. This provides us flexibility in choosing the biochar for various soils managements. The surface area, porous structure, high exchangeable cations and available nutrient and liming effect of biochar make it very effective and efficient for improbing the soil properties. The climate-negative carbon budget of biochar along with its unique properties for fertility improvements may cause to increase the biochar application to agricultural soils (Glaser et al. 2002; Lehmann 2007). The recalcitrant nature of biochar proved implications for the C sequestration potential in soils. The cost of the feedstock, collection and transportation cost, the processing method of the feedstock in use and the value of any co-products are responsible for the cost of biochar. Green waste and waste wood biochars cost between $150 and $260/ton. The biochar produced could have cost from bagasse at $50 to $200/ton. However, the US Biochar Initiative suggests the cost of biochar as $500/ton. The cost of chars from poultry litters and wastewater sludge is negligible, as compared to other wastes. The cost to commercially apply biochar to the field is not yet well established. The cost of biochar on application rate 25 tons/ha by the broadcast and disk method was approximately

Table 20.2 Effect of biochar on physical and chemical properties of soil

Biomass	Temp (°C)	Application rate	pH (units)	OC (Increase in folds)	EC	P	N	CEC	References
Poplar woodchips	1200	50 t ha^{-1}	↑1.2	–	–	↑1.43	↑1.4	–	Viger et al. (2015)
White clover residues	500	30 t ha^{-1}	↑0.9	↑2.03	–	–	↑1.61	–	Abbasi and Anwar (2015)
Poultry manure	500	30 t ha^{-1}	↑0.82	↑1.82	–	–	↑1.64	–	Abbasi and Anwar (2015)
Corn stover	650	52 mg ha^{-1}	↑0.73	–	↑1.21		–	↑1.87	Chintala et al. (2014)
		104 mg ha^{-1}	↑0.99	–	↑1.40	–	–	↑2.20	Chintala et al. (2014)
		156 mg ha^{-1}	↑1.36	–	↑2.58	–	–	↑2.42	Chintala et al. (2014)
Swichgrass	650	52 mg ha^{-1}	↑0.49	–	↑1.18	–	–	↑1.58	Chintala et al. (2014)
		104 mg ha^{-1}	↑0.74	–	↑1.50	–	–	↑1.89	Chintala et al. (2014)
		156 mg ha^{-1}	↑0.91	–	↑1.57	–	–	↑2.23	Chintala et al. (2014)
Oak	650	7.5 mg ha^{-1}	↑0.4	↑1.2	↓0.86	–	–	–	Mukherjee et al. (2014)
Poultry litter	550	10 t ha^{-1}	↑0.36	–	–	↑10.61	–	↑1.35	Bai et al. (2015)
Green waste	500	10 t ha^{-1}	↓0.02	–	–	↑2.13	–	↑1.06	Bai et al. (2015)
Vineyard Purning	525	3% w/w	↑1.3	2.68	↑2.33	↑1.61	–	↑1.28	Kloss et al. (2014)
Wheat straw	525	3% w/w	↑1.2	↑2.87	↑2.33	↑1.75	–	↑1.25	Kloss et al. (2014)
Mixed woodchips	525	3% w/w	↑1.6	↑2.18	↑2.33	↑1.69	–	↑1.34	Kloss et al. (2014)
Cattle feedlot manure	550	5 t ha^{-1}	↑0.2	–	–	↑1.88	–	↑1.25	Slavich et al. (2013)
Municipal green waste	550	5 t ha^{-1}	–	–	–	↓0.7	↑1.15	↑1.03	Slavich et al. (2013)

$63/ha and $70/ha using the trench and fill method. The field efficiency and economic feasibility of biochar application in different agro-climatic conditions is needed for the future prospects and its sustainable use.

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