**Clean Energy Production Technologies**
*Series Editors:* Neha Srivastava · P. K. Mishra

Manish Srivastava
Neha Srivastava · P. K. Mishra
Vijai Kumar Gupta *Editors*

# Nano-materials in Biofuels Research

Springer

# Clean Energy Production Technologies

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The consumption of fossil fuels has been continuously increasing around the globe and simultaneously becoming the primary cause of global warming as well as environmental pollution. Due to limited life span of fossil fuels and limited alternate energy options, energy crises is important concern faced by the world. Amidst these complex environmental and economic scenarios, renewable energy alternates such as biodiesel, hydrogen, wind, solar and bioenergy sources, which can produce energy with zero carbon residue are emerging as excellent clean energy source. For maximizing the efficiency and productivity of clean fuels via green & renewable methods, it's crucial to understand the configuration, sustainability and techno-economic feasibility of these promising energy alternates. The book series presents a comprehensive coverage combining the domains of exploring clean sources of energy and ensuring its production in an economical as well as ecologically feasible fashion. Series involves renowned experts and academicians as volume-editors and authors, from all the regions of the world. Series brings forth latest research, approaches and perspectives on clean energy production from both developed and developing parts of world under one umbrella. It is curated and developed by authoritative institutions and experts to serves global readership on this theme.

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# Nanomaterials in Biofuels Research

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ISSN 2662-6861 ISSN 2662-687X (electronic)
Clean Energy Production Technologies
ISBN 978-981-13-9332-7 ISBN 978-981-13-9333-4 (eBook)
https://doi.org/10.1007/978-981-13-9333-4

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# Foreword

Renewable energy is a potential and bright replacement of fossil fuels in the form of bioenergy. Bioenergy options, like biohydrogen, biogas, biomethane, bioethanol, biomethanol, biobutenol, algal biofuels, and biodiesel, are the promising alternative source of renewable energy which can contribute towards green and sustainable environment by vomiting pollution. Numbers of tremendous efforts have been taken worldwide for the development of innovative technologies which can evenly distribute bioenergy options. Nevertheless, in spite of continuous practice towards the development of low-cost technology for easy commercialization of biofuels, practical viability is still far away and unequally distributed. The application of nanotechnology is emerging as a new area in the field of biofuels production via various means such as catalyst, enzyme, and microbial immobilizer. Nanomaterials have enough potential for commercial exploitation, and their industrial market is expected to become more broad in the coming decades for this area. There is a need to merge and explore the potential of the combination of biofuels and nanotechnology area for viable and sustainable economic biofuels production.

Based on the current scenario, publication of the book *Nanomaterials in Biofuels Research* is much required effort in this series, and I am happy to write this positive message with great satisfaction. The book contains 11 essential chapters focusing on nanomaterial synthesis and characterization for biofuels application. Current global scenario of biofuels and role of nanotechnology in biofuels production, different immobilization patterns to improve biofuels production, and synthesis and mechanism of nanomaterial for economic biofuels production with the green approach are presented and discussed in details. The book suggests a new insight with advancement and sustainable solution to overcome existing loopholes in nanotechnology-based biofuels production process. The book predicated that nanotechnology will play a key role in the near future in the area of the biofuels production process, and hence, this book will serve as an asset for the researcher working in the relevant area including scientists, researchers, teachers, and students.

I am taking the opportunity to congratulate Dr. Manish Srivastava (DU, Delhi), Dr. Neha Srivastava (IIT-BHU, Varanasi), Prof. Dr. P.K. Mishra (IIT-BHU, Varanasi), and Dr. Vijai Kumar Gupta (TTU) for their effort to bring out the publication of *Nanomaterials in Biofuels Research* to cater the long-felt need of scientists, teachers, researchers, and students. My sincere thanks are for the editors for their untiring efforts, devotion, and dedication in this endeavor. All the authors and editors of this book deserve sincere appreciation for their commendable achievements.

Dr. Vijay Kumar Thakur

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21 March, 2019

# Acknowledgments

The editors are thankful to all the academicians and scientists whose contributions have enriched this volume. We also express our deep sense of gratitude to our parents whose blessings have always prompted us to pursue academic activities deeply. It is quite possible that in a work of this nature, some mistakes might have crept in text inadvertently, and for these, we owe undiluted responsibility. We are grateful to all the authors for their contribution to the present book. We are also thankful to Springer Nature for giving this opportunity to the editors and the Department of Chemical Engineering and Technology, IIT (BHU), Varanasi, UP, India, for all their technical support. We thank them from the core of our heart. Editor Manish Srivastava acknowledges the DST, Govt of India for awarding the DST-INSPIRE Faculty Award [IFA13-MS-02] 2014 and also Science and Engineering Research Board for SERB-Research Scientist Award-2019.

# Contents

# About the Editors

**Dr. Manish Srivastava** has worked as DST INSPIRE faculty in the Department of Physics and Astrophysics, University of Delhi, India during June 2014 to June 2019. Currently he is working as SERB-Research Scientist in the Department of Chemical Engineering and Technology IIT (BHU), Varanasi, India. He has published 45 research articles in peer-reviewed journals, authored several book chapters, and filed one patent. He worked as a postdoctorate fellow in the Department of BIN Fusion Technology, Chonbuk National University, from August 2012 to August 2013. He was an assistant professor in the Department of Physics, DIT School of Engineering, Greater Noida, from July 2011 to July 2012. He received his PhD in Physics from the Motilal Nehru National Institute of Technology, Allahabad, India, in 2011. Presently, he is working on the synthesis of graphene-based metal oxide hybrids and their applications as catalysts. His areas of interest are synthesis of nanostsructured materials and their applications as catalyst for the development of electrode materials in energy storage, biosensors, and biofuels production. His *fields of expertise* are synthesis of nanomaterials and their application as catalyst for the development of electrode materials in energy storage, biosensors, and biofuels productions.

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Linnean Society of London, UK, of Indian Mycological Association, and of Mycological Society of India. He has been honored with several awards in his career, including Indian Young Scientist Award, for his advanced research achievements in the field of fungal biology and biotechnology. He is the editor of few leading scientific journals of high repute, having many publications in his hands with h-index 21. He has edited many books for publishers of international renown, such as CRC Press, Taylor and Francis, USA; Springer, USA; Elsevier Press, the Netherlands; Nova Science Publisher, USA; Walter de Gruyter, Germany; and CABI, UK.

# Biofuel: Types and Process Overview

# 1

Ajay Kumar Chauhan

**Abstract**

Excessive use of conventional fossil fuels resulted in a hike in price, exhaustion, and change in climatic conditions. Therefore, a novel route to biofuel generation is another feasible option of sustainable process development. Continuous upgradation of technologies for biofuel generations from the first generation (1G) to the fourth generation (4G) gives new hopes to fulfill energy demands. Biofuel generation from multiple approaches such as physical, biological (includes microbial and enzymatic), chemical, and biochemical catalysis with nanotechnology from multiple feedstocks is the key to biofuel generation. Suitable conversion of cellulosic biorefinery and lignin biorefinery (via lignin valorization) is the key for complete utilization of lignocellulosic biomass. Utilization of the biological methods includes the use of microbial machinery from different domains which might open the door toward an environmentally benign process. Nanotechnology and its potential application with 1G to 4G have future promises for increasing yield and integration of technology. So, this book chapter covers a detailed process overview of biofuel generations and its challenges with the hope of overcoming it.

**Keywords**

Sustainable · Microbial machinery · Catalysis · 1G · 4G · Lignin valorization · Nanotechnology

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_1

## Abbreviations

| | |
|---|---|
| 1G | First generation |
| 2G | Second generation |
| 3G | Third generation |
| 4G | Fourth generation |
| BGA | Blue-green algae |
| C | Carbon |
| CBDH | Cellobiose dehydrogenase |
| Centimeter | cm |
| CH4 | Methane |
| CNG | Compressed natural gases |
| CO | Carbon monoxide |
| $CO_2$ | Carbon dioxide |
| DME | Dimethyl ether |
| DNA | Deoxyribonucleic acid |
| ED | Entner-Doudorof |
| EMP | Embden-Meyerhof-Parnas |
| ETBE | Ethyl butyl ether |
| FAME | Fatty acid methyl esters |
| FT | Fischer-Tropsch |
| GHG | Greenhouse gases |
| $H_2$ | Hydrogen |
| HC | Hydrocarbons |
| HDO | Hydrodeoxygenation |
| Lignin peroxidase | LiP |
| LPG | Liquefied petroleum gas |
| LPMO | Lytic polysaccharide monoxygenase |
| Manganese peroxidase | MnP |
| Mn | Manganese |
| MPa | Mega Pascal |
| $N_2$ | Nitrogen |
| $NO_x$ | Nitrogen oxide |
| NPs | Nanoparticles |
| PBR | Photobioreactor |
| PEG | Polyethylene glycol |
| PM | Particulate matter |
| SNG | Syngas |

## 1.1 Introduction

Increase in population and economic growth has increased the energy demand, and fossil fuels fulfill this energy demand as per the data revealed from the International Energy Agency (IEA) in 2011. As the consumption of fossil fuels is nonrenewable and emits greenhouse gases (GHG) which severely deplete environmental conditions (Buragohain et al. 2010), therefore, the notable increase in renewable energy sources such as solar, wind, and biomass gives new hope of a sustainable process. Biofuels are the renewable energy sources defined as the energy derived from biological carbon fixation, which plays a pivotal role in new energy sources. Therefore, the production of biofuel requires extensive research and development of technologies. These biomass require suitable conversion of biomass into biobutanol, bioethanol, biomethanol, bio-dimethyl ether, biomethane, Fischer-Tropsch (FT) fuel, biohydrogen, algae, and halophilic lipid-based biofuels.

Plant biomass is an abundant source of renewable energy which comprises of the various components such as carbon, hydrogen, oxygen, and traces of minerals. Availability of plant biomass is more reliable compared to another energy sources such as solar energy, wind energy, and hydropower, and it can easily grow in variable environmental conditions. Moreover, the low density of biomass over fossil fuel makes it more feasible for storing and transportation purposes. In developing countries, utilization of plant biomass for household purposes (burning of wood) underestimates its value as a potential biofuel. The burning of fossil fuels and plant biomass is the main contributor to the increase in $CO_2$ level and has a direct impact on global warming. GHG emissions and global warming open up the door for the novel processes of biofuel generation.

Lignocellulosic biomass is made up of highly complex biopolymer of cellulose (40–60%), hemicellulose (10–40%), and lignin (15–30%) as major component which gives support to plant, and it is resistant to various microbial degradations as well as biochemical conversions (Himmel et al. 2007; DeMartini et al. 2013; Schutyser et al. 2018). Cellulose and hemicellulose mainly consist monomeric sugar unit, and after pre-treatment, lignocellulosic biomass sugar component via further saccharification is converted into suitable biofuel (Chen 2014). However, the lignin component is made up of the phenylpropane unit, which is more recalcitrant than the cellulose and hemicellulose. During kraft pulping process, lignin is utilized for the co-power generation, which can be separated, and suitably valorized into useful precursors for biofuel generation. However, the hydrogenation process is being used as a suitable method for converting lignin into a valuable product and converting raw material into biofuel (Chen 2014; Abdullah et al. 2017). Lignin valorization strategy utilizes the complete conversion of lignocellulosic biomass into biofuel generation.

Different methodologies are adapted from time to time for treating the plant biomass for the biorefinery route. This methodology has its advantages and limitations. Here, pulp and paper industries are the best examples of lignocellulosic biomass biorefinery, and they are emerging as fermentative production of bioethanol (Ragnar et al. 2000; Hahn-Hägerdal et al. 2006; Limayem and Ricke 2012). Various existing conventional techniques result in low yield of primary products obtained from the lignocellulosic biomass, but in case of lignin, it can be suitably valorized into valuable products (Davis et al. 2013). Further, the optimization process is done by understanding the process. Even biofuels productions have a direct impact on carbon sink; therefore, biofuels produced from oil-based fuels always have a better choice compared to others GHG emissions into the environment critics of biofuels, which ultimately depends on the route of production. Another selection of biofuel has the debate over food versus fuel; land used for cultivation for feedstock always competes with food demand and land uses for productions. Therefore, the governments of the countries take so many initiatives for the continuous growth of the biofuel industry, which includes providing fund for research and development along with mandating laws of Environmental Protection Agency (EPA) for blending of biofuel with conventional fuel. Renewable Fuel Standard (RFS) provides harness power of biofuels and infrastructure, and high productions of biofuels must be produced and widely available for customers with the competitive price of conventional biofuel (Richards 2013).

## 1.2 Classification of Biofuels

On the basis of origin of raw material biofuels are classified into first-generation (1G), second-generation (2G), third-generation (3G), and fourth-generation (4G) (Demirbas 2011). The first-generation biofuel, sugar, starch, vegetable oils, and fats are converted into bio-alcohol from ($n$ = 1–4) fatty acid methyl esters (FAME). Second-generation biofuel depends on the carbon negative in terms of the carbon dioxide concentration in the environment, which majorly depends on the lignocellulosic biomass (plant material) (Gomez et al. 2008). The third and fourth generation of the biofuel utilize the algae and the blue-green algae (BGA) machinery for converting lipid into biofuel. In third generation, algae is directly used for the production of the biofuel, where in the fourth generation, metabolic engineered algae from the oxygenic photosynthetic microorganism create artificial carbon reservoir (Lü et al. 2011). Further, they are known as chemically synthesized biofuel and biologically synthesized biofuel. In chemical synthesis, biofuel is the generation with the help of a catalyst, and the action of various parameters such as pH, temperature, and pressure has played a significant role. Moreover, chemical methods, not benign environmental methods, in terms of the yield are preferable over biological methods. In the chemical process, the use of the catalyst is preferred over the various physical processes because of the more specific product formation and low cost of separation. Generally, physical processes yield more complex product formations, which are not easily separable and thus require a higher price for downstream processing. In biological methods, lignocellulosic biomass is microbially treated with bacteria, halophilic archaea, fungi, and algae. Moreover, other than these biological processes include various kinds of biocatalysts known as the enzymes used for the production of the biofuels' generation. These enzymes are cellulases, xylanases, and lignolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), laccase, lytic polysaccharide monooxygenase (LPMO), multicopper oxidases, cellobiose dehydrogenase (CBDH), and lipases. Moreover, enzymatic use of the various processes of biofuel generations is the most environmentally friendly process which overcomes the numerous barriers of the chemical processes. Benefits of the enzymatic process yield specific product formation at optimum pH, temperature, and pressure. Therefore, enzyme from extremophile enzymes have added extra-advantages of various product formations at broad pH range and different temperature ranges from 4 to 80 °C, and they even act on higher pressure making them suitable for industrial process. To date, all the biological processes require a higher cost of production than the chemical process, and the cost-effectiveness of the biological process can only be reduced by the processes understanding of the microbial pathways, enzymatic reduction, and its substrate utilization strategy. However, new biological modified strains have shown various promises to overcome all barriers. In the last decade, there had been four industrial plants that started for the commercial production of the biofuel listed as "Project Liberty" by the joint venture of POET-DSM, 2G ethanol at Dupont, Abengoa Bioenergy Hybrid Kansas, and Crescentino by Beta Renewables in Europe (Valdivia et al. 2016).

## Raw Materials

Availability of raw material is the key to success to achieve better economy and viability of the process. Biodiversity available on the earth is a good sink of energy as in the form of hydrocarbon and the production of hydrocarbon for biofuel generations are listed in the Table 1.1. As per requirement, feedstock can be classified on the basis of the source of origin. Feedstock can suitably be converted into biofuel and the abundance of this material in various forms are the source for different biofuel generations.

**Table 1.1** Classification of biomass feedstock (Speight 2011; Sikarwar et al. 2017)

| Biomass type | Examples |
|---|---|
| Forest product | Wood, logging, residue, trees, shrubs, and wood residues, sawdust, bark |
| Bio-renewable waste | Agricultural waste, crop residues, mill wood waste, urban wood wastes, urban organic waste |
| Energy crops | Short-rotation woody crops, sugar crops, forage crops, oilseed crops, switchgrass, *Miscanthus* |
| Aquatic plants | Algae, water weed, water hyacinth, reed, and rushes |
| Food crops | Grains, oil crops |
| Sugar crops | Sugar cane, sugar beets, molasses, *Sorghum* |
| Landfill | Hazardous waste, nonhazardous waste, inert waste, liquid waste |
| Organic wastes | Municipal solid waste (MSW), industrial organic |
| Algae | Prokaryotic algae, eukaryotic algae, kelps |
| Mosses | Bryophyta, Polytrichales |
| Lichen | Crustose lichens, foliose lichens, fruticose lichens |

## 1.3 First-Generation Biorefinery

Concept of the biorefinery comes in the limelight for the researcher's interest due to the limited resources of conventional energy sources and the rising price of the oil in the global market. 1G is produced from the food crops such as corn, sugar cane, rapeseed, cassava, etc. The first-generation biofuels are biodiesel, ethanol, and biogas, which are primarily producing so far. Biodiesel is the alternative biofuel to the diesel which is produced by the transesterification of the various vegetable oils and fats. However, small modification in the properties of biodiesel can make it a better substitute for the diesel. Whereas, bioethanol is the better substitute for the gasoline and is also known as the flexi fuel. Moreover, bioethanol is produced from various sources via fermentation of the sugar or starch material. Bioethanol is used as a feedstock for ethyl butyl ether (ETBE), which can easily blend with the gasoline. Another biofuel like biogas and biomethane is produced from the anaerobic digestion of the household and municipal waste. Biodiesel, bioethanol, and biogas can easily be produced from the food crop feedstock, but the increasing demand of the edible oils makes it difficult to use food crop for the biorefinery (Lee et al. 2014b). The big challenge of the production of the first-generation biofuel is the food to feedstock, high uses of the groundwater, fertilizer, and pesticide use on the crop.

### 1.3.1 Transesterification

The oil obtained from the various feedstock suitably transesterified into the fatty acid methyl esters (FAME) with the help of alcohol. The alcoholic group readily reacts with the triglyceride units in the presence of a suitable catalyst (homogenous or heterogeneous) with oil group and releases biodiesel and glycerol as high-value coproduct (Kulkarni et al. 2006; Meher et al. 2006). This mechanism can suitably be applied to all kinds of oil obtained from using algae, halophilic isolate, animal fat, and vegetable oil. The transesterification reaction is an equilibrium reaction in which continuous mixing is required for getting methyl esters (biodiesel). However, biodiesel obtained from transesterification reactions (given below) has similar characteristics as compared to diesel.

### 1.3.2 Ethanol Production

Ethanol is produced from sugar, starch, and cellulose-containing crops. Alcohol produced from the food crops and lignocellulosic biomass is known as the bioethanol, which can be easily produced by the biochemical process (Minteer 2016). Structure of starch consists of the polymeric unit of Dglucopyranose, which is first converted into glucose molecule with the action of enzymes "amylase." Amylase produces the maltodextrin by liquefaction process. The process of saccharification of dextrin and oligosaccharides is hydrolyzed by the enzymes pullulanase and glucoamylase. The action of these enzyme converts the substrate into the smaller unit of glucose, maltose, and isomaltose. Then further, fermentation is carried by the yeast at 30 °C for ethanol production (Lee et al. 2014b).

### 1.3.3 Fermentation

The fermentation process converts organic compounds into simpler products with the action of the microbes and enzymes. Ethanol production from glucose utilizes fermentative consumption of pyruvate as an intermediate product (Ingram et al. 1987). The process can be classified as the aerobic and anaerobic among microbes depending up on oxygen requirement. Many microorganisms such as yeast (*Saccharomyces cerevisiae*), bacteria (*Zymomonas mobilis*), and halophilic bacteria (*Halanaerobium saccharolyticum*) convert butyrate and ethanol from glycerol and hydrogen from *senegalensis* (Kivistö et al. 2010). Most eukaryotes and prokaryotes follow Embden-Meyerhof-Parnas (EMP) also known as glycolysis pathway, and *Zymomonas mobilis* follows Entner-Doudorof (ED) pathway. *Z. mobilis* produces up to 97% of the theoretical yield of ethanol and also produces a 2.5-fold higher yield compared to *S. cerevisiae* (Weber et al. 2010). Moreover, *Z. mobilis* is suitably engineered for utilizing various types of sugar of lignocellulosic biomass such as glucose, mannose, and xylose for fermentation (Yanase et al. 2012). Another microorganism such as *Clostridia* can secrete hydrolytic complex to utilize both types of sugars (Lütke-Eversloh and Bahl 2011). However, these microorganisms are capable enough to ferment into C6 and C5 sugar or a mixture of both.

### 1.3.4 Anaerobic Fermentation

This process produces biogas under the absence of oxygen. However, anaerobic condition facilitates the growth of microorganism on organic biomass and converts it into methane (60–70%) and carbon dioxide (Naik et al. 2010). When we consider from the economic point, anaerobic digestion is an economically feasible process and also produces the biofertilizers for agricultural uses. The conventional process generally produces the gaseous fuel for the household purposes and electricity generation, but newer technology now focuses on the liquid fuel to overcome the separation process cost (Lee et al. 2014b). Utilizing simultaneous saccharification and fermentation (SSF) and anaerobic digestion in the case of birch wood by *Saccharomyces cerevisiae* efficiently converts sugar into ethanol, and stillage was subsequently utilized for the biomethane production (Kalyani et al. 2017).

### 1.3.5 Whole-Crop Utilization

In this concept, the whole crop utilization is applied for conversion of the whole plant into a useful product. Like *Jatropha*, oilseed contains 30–40% of oil. In the whole-crop utilization, different parts of the crop are treated separately. For example, oil produces the biodiesel, which can further be used for the conversion into oleochemicals. Remaining de-oiled cake is used for the gasification purposes, which can further convert into the syngas. Syngas finally is utilized for the production of various fuels.

## 1.4 Second-Generation Biofuels

Second-generation biofuels are produced from the nonfood crops such as agricultural residues (wheat straw, rice straw, and sugar cane bagasse, etc.) and organic waste from municipal solid waste. 2G biofuels have a key advantage over the first generation in terms of no competition with the food resources, their product formation with less emission of GHG, and less land requirement for the raw material. However, the mixed feedstock concept involved heterogeneous feed. When compared with 1G, the technological risk of operation is higher and also requires higher input cost for the process development. Process understanding in terms of the route selection requires higher technical knowledge and process modification (Margesin and Schinner 2001). Plant biomass consists of the plant cell, which comprised of the 75% of polysaccharide as a potential source of carbon (Pauly and Keegstra 2008). Lignocellulosic biomass is converted into the advanced biofuels through hydrolysis and fermentation (bioethanol and biobutanol) or gasification (FT biodiesel, dio-DME, and SNG). Raw materials for the advanced biofuels are rotational crops (poplar and *Eucalyptus*), *Miscanthus*, switchgrasses, and agricultural waste residues (Naik et al. 2010). Advanced bioethanol can be a suitable alternative of gasoline, and the sugars obtained from the hydrolysis are converted into Fischer-Tropsch diesel or biomass to liquids for the full substitution of the conventional diesel. In this, the lignocellulosic biomass with the help of suitable gasification is converted into syngas. Further, these syngas is utilized as the source of liquid hydrocarbon (kerosene, diesel, or bio-SNG), or this syngas can have transformed into the methane or dimethyl ether (DME) (Naik et al. 2010). 2G biofuels involve two main route strategies. First one is the thermochemical conversion route in which biomass conversion utilizes the thermal decay and chemical reformation in the presence of different concentrations of the oxygen. In the second one, biochemical conversion utilizes the conversion with the help of microbes using catalysts/biocatalysts. The major advantage of the thermochemical conversion over the biochemical is the conversion of the whole organic component into biofuel, but in the case of the biochemical conversion, it is only the polysaccharide that is converted into biofuel (Gomez et al. 2008).

### 1.4.1 Physical Process

#### 1.4.1.1 Mechanical Extraction

In the mechanical extraction processes, crude oil from the oilseeds is recovered from the action of an external mechanical pressure generated by screw press. However, a small amount of the de-oiled cake (around 15–20% of oil) from the oilseed also comes out with the recovered oil. In some mechanical extraction processes, pre-pressing is followed by the solvent extraction for the high oil recovery around 30–40% (Stevens and Verhé 2004). On the other hand, in the full pressing system, high pressure of 9.5 MPa is applied to take out as possible oil content.

#### 1.4.1.2 Briquetting

Various biomass resources obtained from agricultural, forest residues and other waste biomass come under second-generation fuel and are difficult to use as a direct biofuel due to uneven bulk. Therefore, this problem is overcome by the method of densification of the material to give near uniform compact shape. These densification methods are employed as pressing and maceration or combinations of both methods. In press, the density of the material increases by applying mechanical pressure in the early stage of compression. Further, an increase in the pressure has a negative impact on the density of material until it reaches the density of water, whereas in maceration, no such correlation of pressure exists, but chopping, grinding, and pulverization of the large material (like branches of the tree) are more effective methods. These methods are more effective compared to ultrafine grind material (Stevens and Verhé 2004; Osamu and Carl 1989).

#### 1.4.1.3 Distillation

In this process, raw materials are crushed followed by steam distillation. Further, collective vapors are condensed separately which then turn into liquid form. Advanced distillation process known as molecular distillation is applied where conventional methods cannot be applied for the productions of the fragrances (Stevens and Verhé 2004).

### 1.4.2 Thermochemical Conversion

Thermochemical conversion involves heating of biomass followed by chemical conversion into biofuel. This process includes combustion, gasification, liquefaction, and pyrolysis under oxygen-deficient or oxygen-controlled conditions and results in the generation of syngas majorly comprising of $H_2$ and CO. Further, this syngas is processed into gaseous or liquid products (Lee et al. 2014b).

#### 1.4.2.1 Combustion

In the combustion process, the chemical reactions usually take place in the air where fuel and air react with each other. Therefore, the products formed during this process are carbon dioxide and water, and it liberates energy in the form of heat (Lee et al. 2014b). Combustion is the simplest way to use biomass into energy with the low yield of products.

#### 1.4.2.2 Gasification

Gasification is a type of pyrolysis carried out at a very high temperature and produces a mixture of gases known as producer gas (CO, $H_2$, $CH_4$, $CO_2$, and $N_2$). In the case of biomass, gasification producers are methanol, ammonia, and ethylene. Gasification utilizes conversion of the lignocellulosic biomass into the biofuel since the past 30 years. Gasification has many processes which simultaneously go on, such as drying, pyrolysis, and partial oxidation (Bridgwater and Boocock 2013). The syngas produced from the gasification process can be through catalytic and non-catalytic processes. In the non-catalytic process, higher temperature ranges 1000–1300 °C for the syngas production; in the catalytic process, it is operated at around 900 °C due to more advances in catalysis (Lee et al. 2014b). The key in gasification is controlling $H_2$/CO ratio for getting the different product in higher concentration (Melin et al. 2015).

#### 1.4.2.2.1 Biomethanol

The reaction of the biomass requires oxygen to produce producer gas, which is the mixture of various gases such as CO, $CO_2$, $H_2$, $CH_4$, and $N_2$ (known as syngas). These producer gases have the potential to produce stationary power generation. Gases are first cooled down to 300 °C. Further, gases are fed to shift reactor with the controlled condition of $H_2$/CO ratio provided to 2.1 for the methanol synthesis. Remaining acid gases are then removed by scrubber or dissolved in solvent (Vamvuka et al. 2004). Further, high pressure of 6.1 MPa is used to compress purified syngas to increase methanol synthesis:

$$\mathrm{CO} + 2\mathrm{H}_2 \leftrightarrow \mathrm{CH}_3\mathrm{OH}$$
$$\mathrm{CO}_2 + 3\mathrm{H}_2 \leftrightarrow \mathrm{CH}_3\mathrm{OH} + \mathrm{H}_2\mathrm{O}$$

#### 1.4.2.2.2 Methane

All conditions for the preparation of the syngas follow the same procedure. Here, the use of catalyst which catalyzes tar with methane decomposition requires higher $H_2$/CO ratio of 3.1, which forms methane at 31 bar of pressure and temperature between 300–600°C, whereas the chemical reactions are:

$$\mathrm{CO} + 3\mathrm{H}_2 \leftrightarrow \mathrm{CH}_4 + \mathrm{H}_2\mathrm{O}$$
$$\mathrm{CO}_2 + 4\mathrm{H}_2 \leftrightarrow \mathrm{CH}_4 + 2\mathrm{H}_2\mathrm{O}$$

#### 1.4.2.2.3 Bioethanol Production

Biomass is majorly used for the power steam generation. Wherein, $SO_2$ feed with the steam explosion around 205 °C for a short time of 5 min. Then, steam is recovered by forming the product. This product flash and vapor formed during flash is condensed to recover furfural. After this steam recovery, the liquid is recovering with the help of additional water use for the simultaneous fermentation. Saccharification to form the simplest sugar is done at low temperature up to 40 °C. Therefore, saccharification forms the simplest sugar of hexoses and pentoses, which further are converted into bioethanol via the fermentation process:

$$\mathrm{C}_6\mathrm{H}_{10}\mathrm{O}_5 + \mathrm{H}_2\mathrm{O} \rightarrow \mathrm{C}_6\mathrm{H}_2\mathrm{O}_6$$

$$C_5H_8O_4 + H_2O \rightarrow C_5H_{10}O$$

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

$$2C_5H_{10}O_5 \rightarrow 3CO_2 + 3C_2H_5OH$$

Combine production of the methanol and ethanol is achieved by using the following strategy. First, hydrolyzed biomass of cellulose and hemicellulose is used for ethanol production. The remaining part such as lignin with the remaining residue of the hydrolyzed biomass is used for the methanol production (Melin et al. 2015). In other strategies, biomass is hydrolyzed and coupled with hydrogenation with the use of a catalyst such as lignin with the remaining residue of the hydrolyzed biomass used for the methanol production (Melin et al. 2015). In other strategies, biomass is hydrolyzed and coupled with hydrogenation with the use of a catalyst such as Ru at 160 °C and 50 bar pressure. The major advantage of this method is the stability of sugar which produces alcohols:

$$C_6H_{12}O_6 + H_2 \rightarrow C_6H_{14}O_6$$

### 1.4.3 Liquefaction

Biomass is subjected to the presence of alkali, glycerine, propanol, butanol, or direct liquefaction (Demirbas 2004). This technique product is water insoluble, with high viscosity, which requires solvents, carbon monoxide (reducing gases) or hydrogen, or catalyst to be present in biomass.

In thermochemical conversion, lignocellulosic biomass is directly converted into the liquid as heavy liquid oils.

### 1.4.4 Pyrolysis of Biomass

The basic thermochemical process converts biomass into hydrocarbon-rich gas mixture in the absence/controlled oxygen conditions (Demirbaş 1998). In this process, biomass is converted into the vital product known as bio-oil, and other liquid products like acetic acid, acetone, and methanol. Solid products like charcoal, and gaseous products like non-condensable gases are also formed on heating 477 °C in the presence of air. However, around 70% of bio-oil are produced on a mass basis, whereas this process economically viable does not require pre-treatment of the biomass (Alonso et al. 2010). Therefore, the corrosive nature of the bio-oil towards equipment, and poor thermal stability has to be overcome by upgrading oxygen content, whereas, hydrogenation leads alkali removal and oil require catalytic cracking (Demirbaş 1998). Pyrolysis of lignocellulosic biomass such as wood is a zonal process, in which thermal degradations of hemicellulose, cellulose, and lignin occur (Chum 1991). Moreover, pyrolysis comprises of five stages (Demirbaş 2000):

First stage: Removal of moisture with some volatile loss
Second stage: Degradation of hemicellulose and emissions of CO and $CO_2$
Third stage: Exothermic reaction and emission of $CH_4$, $H_2$, $C_2H_6$
Fourth stage: External energy supply
Fifth stage: Completion of the process

#### 1.4.4.1 Fast Pyrolysis

This process is performed in the higher temperature range of 577–977 °C with fast heating rate achieved in short residence time of 0.5–10 s. In fast pyrolysis, biomass is converted into vapors, aerosols, and char. With further cooling and condensation of vapor, the aerosol's dark color liquid is formed, whereas in terms of yield, 60–75% bio-oil, 15–25% char, and 10–15% non-condensed gases are formed (Shafizadeh 1982).

#### 1.4.4.2 Flash Pyrolysis

Flash pyrolysis occurs in higher temperature than fast pyrolysis of 777–1027 °C with the fast heating rate of more than 723 °C/s (<0.5 s short retention time) and very fine particle size of <0.2 mm. Bio-oil is formed from flash pyrolysis, is mixed with char, and makes a mixture of bioslurry. Further, this bioslurry is fed to the gasifier at 26 bar pressure with 654–954 °C to convert into syngas with the conversion efficiency of 70%. This bio-oil formed from flash pyrolysis is used for the engines and turbines for the power generation (Demirbas 2004).

Biomass-derived syngas is used for different biofuel productions contaminated with various particulates, tars, nitrogen, sulfur contaminants, and halides with trace elements. These contaminants are removed before entering into the catalytic reactor system (Sikarwar et al. 2017).

Production of the 2G biofuel not an issue for the feed to food compared with 1G biofuel, but fiber crops used as raw material, compete with the food crops. Moreover, the pesticides and fertilizer use also the primary concern for the production of the second-generation biofuel.

## 1.5 Third-Generation Biofuels

In the early stage of development, algae are used for the generation of the 3G biofuel. Algae include the microalgae, macroalgae from seaweed, and cyanobacteria, also known as blue-green algae (BGA). More than 800,000 algal species are exist on the earth, and algae can rapidly grow and harvested in a month, and minimal nutrient is required for their growth; even economic production in wastewater is an added advantage (Bowyer et al. 2018). The potential of oil production from algae is 100 times greater than in area basis than the soya bean and canola oils (Hu et al. 2008). For the better growth of the algae, the optimal temperature range is between 20 and 30 °C and slow growth observed is lower than 16 °C, whereas above 35 °C is deleterious for the algae growth (Menetrez 2012). Microalgae produce lipid

**Table 1.2** Various lipid extraction methods with their advantages and disadvantages Halim et al. (2011), Harun et al. (2010), Wang et al. (2015), Kanda et al. (2013) and Mubarak et al. (2015)

| Method | Adv Wang antages | Disadvantages |
|---|---|---|
| Oil expeller | Easy to operate<br>No solvent required | Large biomass size required<br>Slow operation<br>Higher power utilization and scale up problem |
| Ultrasonication | Reduced extraction time<br>Low solvent required<br>Release of cell content higher in solvent | Filtration and centrifugation add extra cost for removal of solid residue<br>Efficiency poor, target compound, volatile in nature |
| Microwave | Economical<br>Environmental friendly<br>Reduced solvent use, time<br>Improved extraction yield | Extraction time long<br>Large volume solvent required, which is toxic and highly flammable<br>Solvent recovery is highly energy intensive<br>High operational and infrastructure cost |
| Solvent extraction | Simple and economical viable<br>High efficiency, better for small scale | |
| Supercritical $CO_2$ | Reduced time<br>Low toxicity of solvents<br>Flexibility to change pressure and temperature and improves selectivity<br>Favorable for mass transfer<br>Solvent-free extract | |
| Wet extraction | Energy saving for drying biomass<br>Reduced solvent uses | Low-quality lipid extracted |

through photosynthesis, and lipid content reaches around 20–80% of dry weight depending on the species. However, changes in the nutritional requirement can change the product yield of the lipid, protein, and carbohydrate (Shen et al. 2009). After that, lipid extracted by different extraction processes is used for the production of the biodiesel, and the leftovers, carbohydrate and starches (residual), are further processed and can be used for the production of ethanol (Shen et al. 2009; Coppola et al. 2009). Lipid extraction process includes mechanical and chemical extractions (Halim et al. 2012). Table 1.2 summarizes the various method of extraction.

### 1.5.1 Open Pond

A natural water reservoirs like lake and lagoon and artificial reservoirs system like ponds, shallow ponds, and tanks are the open pond system. Advantage of the open system is that it is easy to construct and operate, but poor utilization of the light, evaporation loss, loss of the $CO_2$ to the environment, and chances of contamination are the significant key challenges of the open pond system. This system can be suitably installed with the wastewater treatment plant (Beal et al. 2012). But the major drawbacks of this system lacks control of various parameters.

### 1.5.2 Photobioreactor (PBRs)

The system is closed, therefore it provides a better internally maintained control system for the growth of pure algal culture as they maintain various parameters of inadequate amount such as $CO_2$, water, temperature, light intensity, mixing density, and pH of the system (Lee 1999; Mussgnug et al. 2007). In PBRs, algae are cultured in glass or plastic tube, where they are grown in light of the particular wavelength which supports the optimal growth of the algae. Approximately, 7.6–10 cm light penetrates inside the PBRs due to the turbidity of the algal growth into PBRs (Lee 1999; Mussgnug et al. 2007; Müller et al. 1993; Rapala and Sivonen 1998). For the easy operations of the PBRs, various conditions optimized like volume size to the area for the optimum light penetration, spatial distribution of light, $CO_2$ transfer rate, and monitoring of the growth inside the reactor (Menetrez 2012). Their installation and operational costs are higher, but product yield in terms of oil is obtained around 40–55% (Hu et al. 2008; Chisti 2007). Due to the less expensive operation of the open system, it can be suitably hybrid with the closed type of PBRs for optimizing cost and product yield (Menetrez 2012).

Algae are an excellent source to produce various biofuels such as biodiesel, ethanol, and petroleum. From various reports, productivity from the algae is 602 times higher than various crops like corn and switchgrasses (Menetrez 2012). The significant advantage of algae is higher productivity, and small land for the cultivation is required. Transesterification of oil recovered from algae produces biodiesel chemically and biologically. Moreover, biological production utilizes a lipase enzyme which converts lipids into biodiesel at room temperature. This operation at room temperature cuts the energy requirement for biodiesel production (Fig. 1.1).

## 1.6 Fourth-Generation Biofuel

The use of the new synthetic biological tools has emerged as the fourth-generation biofuel. In this generation, the breakthrough has made promises to photobiological biofuels and electro biofuels (Aro 2016). Synthetic biology of the algae and cyanobacteria involves various designs of the new promising strains by using recombinant

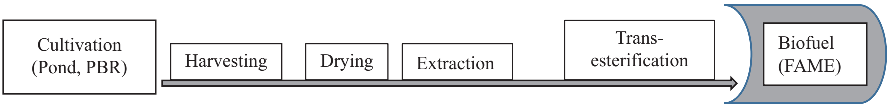

**Fig. 1.1** Biofuel from algae

**Table 1.3** Comparisons of different biofuel generations and its challenges

| Generation | Source | Current conditions | Problems |
|---|---|---|---|
| First | Food crop sources<br>Starch and sugar<br>Oil crops | Commercially available. Technically developed | Competition with food supply and land uses<br>Benefit of greenhouse gases is dependent on the feedstock |
| Second | Lignocellulosic biomass<br>Agro-waste and energy crop | Advanced status of development and demonstration step | Higher cost, selection cheap, and sustainable raw material<br>Additional cost of pre-treatment<br>Enzyme cost is higher |
| Third | Algae | Fast-growing, cultivation land not required, Various potential algae strains are available, “under research and development” | High cost of downstream processing |
| Fourth (extension of third generation) | Algae and cyanobacteria | Primary stage of genetic engineering and new recombinant strains development, more promising than 3 G | Under research. |

deoxyribonucleic acid (DNA) technologies. This technology uses the synthesis of the new strain by using new devices and the integration of the new biological parts or redesigning of the biological system for the biofuel generation.

The fourth-generation biofuels are inexhaustible, cheap, and readily available resources compared in (Table 1.3) with other generation of biofuel. During photosynthesis process, water molecule splits into hydrogen and oxygen by the action of solar energy with the interaction of photosystems I and II which is the key for the biofuel production at a large scale (Inganäs and Sundström 2016). Moreover, artificial photolysis is emerging as the new door for the biofuel generation (Inganäs and Sundström 2016). Even $CO_2$ water can also use as an alternative strategy for biofuel production, which utilizes carbon-based solar fuel.

### 1.6.1 Direct Process for Solar Fuel

This technology utilizes the combination of sensitizers and catalyst for performing individual reactions and generating approximately 30% of the solar fuel from water, but no complete device to date is made for direct fuel productions (Inganäs and Sundström 2016). In this process, oxidation of the water and reduction of the substrate are principle mechanisms. Further, proton from substrate utilized for the hydrogen fuel generation. The catalyst used is generally made up of abundant metals like cobalt, iron, and manganese, etc. In another way, an artificial photosynthesis follows the molecular mechanism; it involves the enzymes which are catalytically active used as biocatalyst like hydrogenase enzyme, which is used for hydrogen production by dark fermentation (Tamagnini et al. 2007). In direct process, with the help of the nanotechnologies, hydrogen production follows the non-molecular mechanism light-driven catalysis on the surface. This surface area can be metal, semiconductors, or fabrication of nanostructured carbon-based material. Limitations of the non-molecular mechanism are expensive, and rare metals are used as the catalyst. Involvement of molecular and non-molecular mechanism involved makes the process complex thus less understood. Limitations of the non-molecular mechanism are the use of rare metals as the catalyst which makes it expensive.

## 1.7 Microbial Conversion

Production of the biofuel is still a challenge because the production cost is more than the raw material. In bioprocessing, microbial conversion step converts lignocellulosic biomass into biofuel by microbial action (no action of enzyme). This method has gained more interest from researchers over the first-generation biofuel production in terms of the cost (Lynd et al. 2005). The new trend of bioprocess states that the potential of ethanol production through microbial route or its combinations mainly utilizes the cellulose machinery with other components (hemicellulose and lignin) of the pre-treated biomass with the high yield of the ethanol. Various aerobic fungi can degrade pre-treated feedstocks directly into the $CO_2$, and only a minor amount of ethanol is produced. However, the electrons yielded are transferred to the respiratory chain for oxidative phosphorylation, and the metabolic pathway does not utilize the substrate level phosphorylation for ethanol production. The rule of thumb of alcohol production from sugar is that 2 moles of alcohol is produced from each mole of hexose sugar. Yeast like *Saccharomyces cerevisiae* uses the glycolytic pathway to form alcohol. All the industrial processes are operated at higher pH and temperature range, which suitably use consortia of potent fungal strain like *Sarocladium strictum* with thermophiles like *Halomonas* for the improved degradation of wheat straw (Cortes-Tolalpa et al. 2018) (Table 1.4).

## 1.8 Enzymatic Conversion to Biofuel

Biofuel production from lignocellulosic biomass has given the new option to fulfill global energy demand but has a greater challenge of global warming. Production of biological-derived energy known as "bioenergy" is a major concern with the biocatalysis (utilizes the various enzymes to catalyze biomass conversion) (Rubin 2008). The complex structure of the lignocellulosic biomass first gets hydrolyzed

**Table 1.4** Microorganism for producing biofuels (Kumar and Kumar 2017)

| Microorganism | Biofuel | Biofuel yield (g/L) | References |
|---|---|---|---|
| *Clostridium acetobutylicum* | Butanol | 3 | Lütke-Eversloh and Bahl (2011) |
| *Clostridium thermocellum* | Isobutanol | 5.4 | Lin et al. (2015) |
| *Escherichia coli* | Butanol | 30 | Shen et al. (2011) |
| *Escherichia coli* | Ethanol | 25 | Romero-García et al. (2016) |
| *Saccharomyces cerevisiae* | Fatty acids | 0.38 | Yu et al. (2016) |
| *Saccharomyces cerevisiae* | Isoprenoid based biofuel | 40 | Westfall et al. (2012) |
| *Pseudomonas putida* | Butanol | 0.05 | Nielsen et al. (2009) |
| *Cryptococcus* | Lipids | 7.8 | Deeba et al. (2016) |
| *Zymomonas mobilis* | 2,3 butandiol | 10 | Yang et al. (2016) |
| *Zymomonas mobilis* | Ethanol | – | Kremer et al. (2015) |
| *Caldicellulosiruptor bescii* | Ethanol | 0.7 | Chung et al. (2014) |
| *Trichoderma reesei* | Ethanol | 10 | Huang et al. (2014) |
| *Yarrowia lipolytica* | Fatty acids | 55 | Beopoulos et al. (2009) |
| *Synechococcus* sp. | Limonene | 0.04 | Davies et al. (2014) |
| *Synechococcus elongatus* | 1,3 propanediol | 0.28 | Hirokawa et al. (2016) |

into its simplest fractions with various hydrolyzing enzyme cocktails. However, the use of enzyme cocktail proved the maximum conversion into industrial product formation from lignocellulosic biomass conversion. Enzyme consortium is another way used to deconstruct recalcitrant substrate into a useful product (Lopes et al. 2018). This enzyme consortium conversion requires detailed studies on various enzymes and their action to work synergistically. To date, Sprizyme® from Novozyme, a commercial enzymatic cocktail is available in three versions (in the market) for the complete conversion of sugar. Moreover, Cellic®CTec3 (2017) from Novozyme is using cellulose conversion into ethanol. Enzyme cocktails enable the enzyme to hydrolyze cell wall structure due to their specific action. Moreover, the product of one enzyme acts as the substrate of another enzyme and also gives an option to the individual enzyme to act on an available substrate. During enzyme-substrate reaction, enzyme inhibitors are also produced, which can inhibit the hydrolysis process. Therefore, a complete enzyme mechanism can be studied to increase lignocellulosic biomass conversion. Wherein, each substrate in cellulosic biomass has its characteristic and feature which require the distinct condition to convert it into its simplest form.

### 1.8.1 Cellulases

There are a group of three enzymes which act synergistically on cellulose and completely convert it into glucose. These enzymes are endoglucanase, exoglucanase, and β-glucosidase.

Endoglucanase: This enzyme randomly hydrolyzes β 1-4 glucosidase linkages at the amorphous region and forms 2 Carbon units of cellobiose from cellulose chain.

Exoglucanase: Other names are cellobiohydrolase, avicelase, and exocellulases, which cleaves at exterior ends of the crystalline part of cellulose and produces cellobiose as a product.

β-glucosidase: This enzyme cleaves 2C chain molecule (cellobiose) into glucose.

These all three enzymes are collectively known as cellulytic enzyme, majorly produced from *Trichoderma reesei*. Various thermophilic potent strains such as *Thermophilic clostridia* and *Thermoanerobacterium* are also reported in producing cellulases (Lamed and Zeikus 1980).

### 1.8.2 Xylanases

Xylan sugar is an important component of hemicellulose which is made up of 5C chain unit (pentosans). Xylanases are the enzymes, which are used to degrade xylan unit linked with the lignin and cellulose component. Removing the xylan unit from lignocellulosic biomass gives easy accessibility to other hydrolyzing enzymes or chemicals for the increasing saccharification of cellulose. Various microbial species like *Trichoderma reesei* and *Humicola insolens* are the potent strain which degrades xylan at higher temperatures of 40–60 °C (Binod et al. 2018).

### 1.8.3 Lignolytic Enzymes

These are lignin-degrading enzymes which utilize lignin phenolic and non-phenolic group as substrates comprising of lignin peroxidase (LiP), manganese peroxidase (MnP), laccases, versatile peroxidase (VP) and dye-oxidizing enzymes (DyP A and DyP B). The action mechanism of LiP acts on the surface of the phenolic and non-phenolic aromatic component, and MnP acts on phenolic substrates by the action of $Mn^{3+}$, and VP has characterstics of both LiP and MnP (Schoemaker and Piontek 1996). Lignin-degrading enzymes remove lignin in the pre-treatment step of lignocellulosic biomass and expose site for easy degradation of cellulose.

### 1.8.4 Cellobiose Dehydrogenase (CBDH)

This enzyme is the oxidative enzyme used to oxidize various sugars such as lactose, glucose, and cellobiose. Wherein, the substrate is oxidized by the action of CBDH and reduction of flavin into flavin adenine dinucleotide (Knöös et al. 2014). In another way, CBDH activates cellulases by product inhibition of cellobiose. Moreover, another enzyme of this class is lytic polysaccharide monooxygenase copper (LPMO) enzyme, and it needs an external electron donor for its activity to cleave C1, C4, or both glycosidic bonds of cellulose. LPMO acts on first C-H bond of cellulose followed by the oxygen-dependent chain for the degradation into the product (Eibinger et al. 2014).

## 1.9 Effect of Surfactant on Enzymatic Hydrolysis

Surfactant positively enhances enzymatic hydrolysis and reduces hydrolysis time and dosage of enzymes (Helle et al. 1993). Surfactant hydrophobically interacts with the lignin part of lignocellulosic biomass and doubles the yield by increasing surface modifications or disruption; even surfactant acts as enzyme stabilizer (Kim et al. 1982). However, nonionic surfactant used in enzymatic hydrolysis such as Tween (80, 20) and polyethylene glycol (PEG) has more effect than the ionic surfactant as it increases higher surface for hydrolysis (Binod et al. 2018) (Table 1.5).

## 1.10 Biofuel from Nanotechnology

Nanotechnological advancement for improving bioprocess requires nontoxic NPs for microbial growth, which is economically produced and environment friendly. However, nanomaterial provides high surface area, high catalytic activity, stability, storage, durability, and higher potentials of 3R principle (recovery, reusability, and

**Table 1.5** Advantages and disadvantages from biofuels in terms of GHG emissions (Nylund et al. 2008)

| Biofuel | Advantage | Disadvantage |
|---|---|---|
| Ethanol | Reduction CO, HC, particulate matter (PM), NOx, 1,3 butadiene, aromatics | Increase acetaldehyde emissions |
| FAME | Reduction in PM | Increases in NOx and aldehyde emissions |
| Synthetic fuel | PM, NOx and aldehyde, 1,3 butadiene, benzene, polycyclic aromatic hydrocarbons (PAHs), and mutagenicity | |
| Natural gases | Low PM, NOx<br>Methane performance improved by adding hydrogen | Formaldehyde, PAHs |
| DME | Clean burning | Challenging as a vehicle fuel |
| Hydrogen | Zero emissions | |
| CNG, LPG, biogas, and hybrids | GHG reductions with hybrids like CNG and LPG | |

recycling). Previous reports showed the successful implementation of nanotechnology with all types of biofuel-producing processes such as transesterification, anaerobic digestion, gasification, FAME, and renewable hydrocarbons shows efficient and economic feasible only at lab scale (Zhang et al. 2010). Various nanocatalysts such as titanium dioxide, magnesium oxide calcium oxide, and strontium oxide are developed with high catalytic performance. Nanotechnology is also applied with the enzyme (biocatalyst) immobilization in nanoencapsulation, entrapment with silaffin, and adsorption for providing higher surface area, higher stability, higher enzyme loading and also gives the chance of reusability. Nanoparticles are used for the extraction of lipid from algae without harming cells. Nanotechnology can suitably bring breakthrough in advanced fermentation, pyrolysis, jet fuels, catalytic conversion gasification, biofuel cell, carbon capture storage, and nano-based precision forming technology (Nizami and Rehan 2018). Nanoparticles recently gained lots of interest due to their feasibility to enhance effect metabolic engineering by improving performance or product yield. Biohydrogen produced by the microbes shows enhanced production in anaerobic conditions using NPs. Whereas, these NPs improve reaction kinetics with increased transfer of electrons. Though several NPs have been reported to enhance dark fermentation for hydrogen production with incorporation of gold NPs, which shows stimulatory effect on substrate utilization by 56% and increases yield by 46%, this enhanced surface area to volume ratio provides better accessibility of binding site with bacteria as well as enzyme (Zhang and Shen 2007; Sekoai et al. 2019). Various mesoporous NPs have been developed to overcome the barrier of higher dosing of NPs for improving the production of biofuel. When NPs supplemented (zero-valent metals, metal oxide, and carbon-based NPs) with the anaerobic fermenting bacteria which further increase the hydrolysis process of organic material by increasing substrate utilization, lipase catalyzing biodiesel production by transesterification reaction produces biodiesel; this enzyme suitably nano-immobilized, produces biodiesel, and increases the reusability of the enzyme. Therefore, the use of nanotechnology also improves bioethanol production by immobilizing cellulases using manganese oxide dependent (NPs), it increases hydrolysis and catalytic efficiency of cellulase. Immobilization of enzyme by NPs acts as enzyme protector from the intermediate inhibitor. Nevertheless, NPs modified enzyme can sustain in extreme environmental conditions and supports higher product formation with reusability. Since nanotechnological implementation is still under laboratory scale, it requires higher knowledge and techno-economic assessments for process modification before its commercialization.

## 1.11 Lignin Strategy to Biofuel

### 1.11.1 Lignin Structure

Lignin matrix consists of heterogeneous structure of aromatic alcohol such as p-coumaryl, coniferyl, and sinapyl alcohols. The aromatic monolignols from lignin form guaiacol (G) from coniferyl alcohol, p-hydroxy phenyl (H) from coumaryl alcohol, and syringyl from sinapyl alcohol (S) gives lignin with distinctive characteristics from each other. Variation in monolignol compositions forms distinctive properties of wood. Where G/S-Units ratio are highly present in softwood lignin, and a variable composition ratio of G/S/H is present in hardwood lignin (Laskar et al. 2013; Lee et al. 2014a). Aromatic structure of lignin transferred to hydrocarbon (HC) fuels via chemical treatment produces products having similar properties like gasoline and diesel (Ragauskas et al. 2006).

### 1.11.2 Lignin Valorization

In the cellulosic biorefinery, the cellulosic part, which is consist of 45–60%, is utilized for the ethanol production; the rest of the ~20% part of lignin leftover is the key motivation for the lignin valorization into biofuel and generation of value-added by-product. This strategy involves depolymerization of lignin by using hydrogen as the reducing agent to form aromatic product with low oxygen content with higher stability. Due to the presence of different lignin monomers, degree of interaction varies to form useful product (Ragauskas et al. 2014). This barrier of interaction overcome by different pre-treatment strategies of lignocellulosic biomass. In case of hydrogenation operated at mild conditions with the help of a suitable catalyst, which ensures the desired product formation in complex reaction. In an in situ catalytic characterization, hydrogenation process yields guaiacol conversion with cyclohexanol selectivity of 99% and ~94% in 7 h, 220 °C, and initial pressure of 2 MPa with the suitable ration of water/methanol/lignin as feedstock (20:5:0.5) (Yu et al. 2013). Presently, in highly selective hydrogenation at mild conditions, step-by-step precipitation is done with the help of catalyst (Ni/$SiO_2$). This highly selective hydrogenation process is operated in 7h, 120 °C, and 2 MPa for complete conversion into guaiacol (Shu et al. 2016). Moreover, in wet impregnation technique of hydrogenation palladium is suitably doped with alumina as a catalyst, where 3 weight % of catalyst converts 4-ethylphenyl(cyclohexanol) in aqueous phase reaction operated for 6h, 60 °C (Yi et al. 2016). In lignin valorization other than the chemical catalyst, biological catalyst like lignolytic enzyme is also utilized for future trend of biofuel generation. The value added HC fuels produced from lignin are able to converted into C7-C18 hydrocarbon, which have suitable applications in jet fuel (Wang et al. 2015). This lignin to biofuel strategy involves degradation of lignin in such a way that $O_2$/C and H/C ratios match to conventional fuel. In case of hydrodeoxygenation (HDO) technique, lignin monomers increase the ratio of H/C while decreasing their O/C ratio. This process includes cleavage of CO bond in monomeric lignin unit and oxygen removal achieved by high-pressure hydrogen, which forms water vapor with the decrease in O/C ratio. Further more, double bond of aromatic carbon is saturated with the help of hydrogen using catalyst (palladium and active carbon) (Ge et al. 2017).

## 1.12 Sustainability Criteria

### 1.12.1 Food and Feedstock

Crop food used for the biofuel generation in 1G affects the food requirement in developing countries; even increasing the price of agricultural product, cereal, and sugar is the key public agenda. Therefore, biofuel expansion requires both increased agricultural productivity for food and feedstock for animal and biomass material for biofuel. This would lead conversion of forest land, and further, this would affect the increase in GHG and a potential risk to biodiversity. Moreover, 3G biofuel gives hope for the high product yield, and land requirement, but the downstream cost is higher to be reduced.

### 1.12.2 Water Requirement

Even 2G biofuel requires nonfood crop, but it competes with land for cultivation of lignocellulosic biomass such as *Jatropha*, which also requires other resources like water uses (400–700 times higher than other energy sources) and 90% more than the production of feedstocks (Mandil and Shihab-Eldin 2010).

### 1.12.3 Emissions

As expected biofuel reduces GHG emissions by 60–94% compared to the fossil fuels, but when we compare the scenario with climatic change and lifecycle assessment, biofuel is worse than gasoline (Highina et al. 2014; Holma et al. 2013).

### 1.12.4 Biodiversity

Biofuel production and conversion of land affect the environment, sustainability, and biodiversity. Conversion of land and forest may affect a wide range of flora and fauna of the ecosystem. No methods are available, which can tell about the direct impact on biodiversity and its damage (Araújo et al. 2017). Therefore, biodiversity is considered foremost, integrated with the biofuel generation planning and agricultural farming (Morgera et al. 2009).

### 1.12.5 Policies

Policies and its implementation for biofuel generation play a pivotal role in the development of sustainable development, reducing barriers, greater economics, better implementation of technologies, and funding agencies. However, many

**Table 1.6** Showing the biofuel blend strategy of countries (Araújo et al. 2017)

| Country | Ethanol blend aim | Biodiesel blend aim | Year of implementation |
|---|---|---|---|
| Brazil | 27% | 10% | 2019 |
| China | | 15% (10% mandate) | 2020 |
| EU | 10% | 20% | 2020 |
| India | | 20% petroleum | 2017 |
| USA | | 0.1 | Implemented |

countries like Brazil, the USA, China, and India have their own regulation policies for the development of biofuel and blending with the flexi fuel. These policies suitably help for reducing subsidies to conventional fuel and mandate blending of biofuel to curb out $CO_2$ emissions (Table 1.6).

## 1.13 Conclusions

In this book chapter, biofuel production disscussed on different process methodologies, uses of different feedstocks, its key challenges. The production of biofuel plays a pivotal role in the current scenario. In case, 1G technology impacts on food prices, and competition with the food crop is the key challenge. Wherein, the concept of 2G comes into the biofuel production, which is economically better than 1G, but again its competition with the land uses and other resources makes it difficult. Further, concept of 3G uses of algae and BGA strains, which require low area and no competition with food feedstock and high product yield opens a new door, but its downstream processing cost is still a challenge. This 3G technology is still in the developmental phase for increasing higher product yield. The genetic manipulation and engineered strain reduces its downstream cost and yield known as 4G of biofuel. Apart from 1G to 4G new advancement in nanotechnology, has potential with technological advancement increases the effectiveness of processes. Generation of biofuel from the chemical process is commercially viable but still have challenge to environment. Therefore, biologically and biochemically preccesses are promising and environmentally friendly. Furthermore, several attempts are made for making the enzymatic process economically viable, and still industrial important enzymes are required. Biofuel production requires several issues for careful analysis such as; feedstock selection, process design, reduction of cost, cultivation practices, land uses and its practices, GHG, cocktail preparation, effect on soil, and mineral, etc. Shortly, the processes compacted and designed by removing pre-treatment strategy. Lignin valorization strategy is also gaining interest to convert lignin as biofuel in recent trends. The suitable blending of biofuel with existing fuel is the alternate best option. However, large-scale production is the ultimate requirement shortly to fulfill our energy need. Nevertheless, there is still a need for technological understanding with advancement and integration of the concept of chemistry, bioprocess, and chemical engineering in biofuel generation.

## 1.14 Summary

This book chapter gives the idea about how biofuels are formed from various feedstocks using multiple technology strategies. Biofuel production requires various chemical, biological, biochemical processes, and nanotechnologies. Moreover, biofuel formed from these technology showed similar characteristic properties as fuel from fossil fuel would be blended suitably to open the doors of sustainable process development. In the future complete development of biofuel technology will reduce dependence on fossil fuels.

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# Applications of Plant-Based Natural Products to Synthesize Nanomaterial

# 2

Muhammad Irfan, Mamoona Saeed, Bushra Iqbal, and Misbah Ghazanfar

**Abstract**

Plants are the important means of various kinds of phytochemicals with several applications in nanotechnology. Nanotechnology refers to the building and use of materials whose components exist at the nanoscale up to 100 nm in size. These biotechnological tools have been synthesized by the use of different kinds of plants. Plants have numerous natural products like tannins, saponins, flavonoids, steroids, alkaloids, and other nutritional products that can be obtained from several plant parts like seed, barks, leaves, roots, shoots, flowers, and stems. It has been reported that the extracts from plants act as a powerful pioneer for a nanomaterial production in safe procedures. As the plant extracts have numerous secondary metabolites, it plays part as stabilizing and reducing factors for the bioreduction reaction to form new metallic nanoparticles. These plant-based nanoparticles have various applications in different fields especially in biofuel production.

**Keywords**

Nanoparticles · Plants · Natural · Nanomaterials · Applications · Substances

## 2.1 Introduction

In the present days, nanoparticles and its various items have become principal components of our items in routine, so metallic nanoparticles like silver in antiperspirants and particles with improved discharge properties into meds are known as nanoparticles and the whole distance to "nano impregnations" of spray lodges,

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_2

baths, and cleaning bowls (Moss and Siccardi 2014; Vincent and Loeve 2014; Kettler et al. 2016). As these nanomaterials, which include particles having diameters in the range of hundred nanometers, have now significant role in our routine life, there are also concerns about a possible toxic effect of these nanomaterials on humans and environment as well (Roy et al. 2017). However, such evaluations usually disregard the truth that nature is a proficient nanotechnologist itself, with various precedents of general nanomaterials emerging from sources that are natural, like mineral springs, volcanoes but specifically from living beings also. Actually, life rotates around the cell which itself is microscopic in size and metabolize molecules which are picoscopic (Kajander et al. 2001; Ciftcioglu et al. 2006; Urbano and Urbano 2007).

Presently, we sometimes fortuitously judge the emerging field of nanomaterials. Here, we will shortly discuss the rising area of natural nanoparticles. Actually, there is a demand for a difference among natural nanomaterials, such as already found or synthesized in the environment without man involvement, and substances which are "bio" and "nano" as well. In another case, the predicate "natural" corresponds to "biological," as in "natural products," which mentions mainly biological substances (Mukherjee et al. 2001; Patel et al. 2015a, b; Pasula and Lim 2017). Through the earliest starting point, all of us ought to complement to grounds of nanoparticles (NPs) as is broad and incidental. Figure 2.1 illustrates the synthesis of nanomaterials from plants and their applications in various fields.

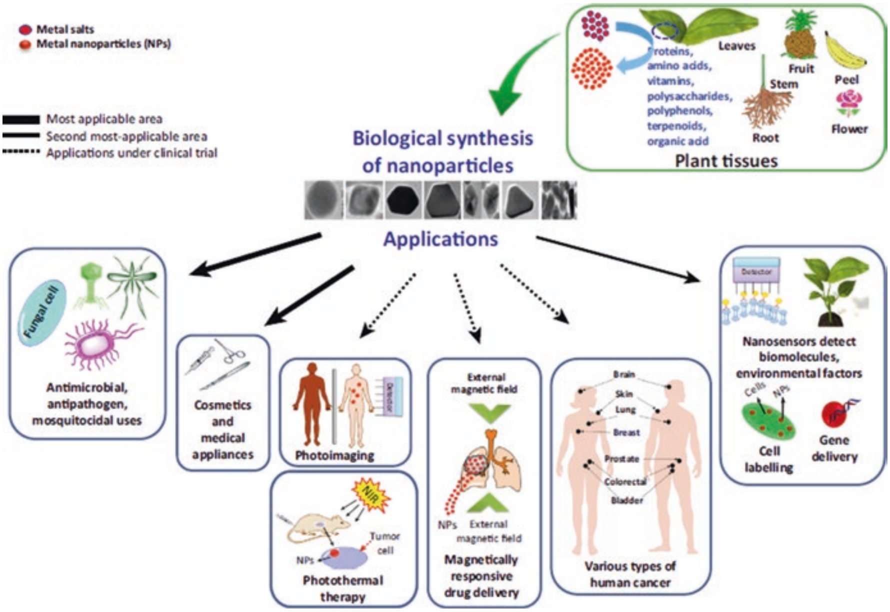

**Fig. 2.1** Biological synthesis and applications of metallic nanoparticles in environmental and biomedical areas. (Modified from Singh et al. 2016a, b)

## 2.2 Inorganic Nanoparticles Derived from Natural Sources

In the earth, so many inorganic nanoparticles are present which are derived from natural sources. So such particles are common, so far more often than nonnatural. Volcanic powder mists contain a comprehensive assorted diversity in polydispersity miniaturized scale plus nanomaterials. Such particles extend in size somehow from 100 to 200 nm and are synthetically basically made out of composites of iron and silicates. These particles are deliberately floating in air and if only once heaved can prompt attentive pulmonary disorders. “Carbon nanotube” sediment gathered from the cremation of Texas pine, for example, in point, involves many layers (15–70 nanometers of size) of carbon nanotubes. Such carbon-based particles deliberately turned out to be airborne and present serious well-being perils to creatures and the human populace (Murrand Guerrero 2006).

Miraculously, drinking water, when seen under microscope, which could be loaded with variously sized nanoparticles just like minute strong materials of every shape. Generally, these particles are not of good quality and cannot easily be molded and are very much characterized. There are inexhaustible instances of characteristic nanomaterials structured thusly, for example, the $CaSO_4$ and silicate particles in spring water (Wu et al. 2016). In reality, these inorganic substances meanwhile urged counterparts to produce a scope of similar particles dependent on normally happening particles, for example, refined nanoparticles of $Fe_3O_4$ and $MnO_2$ (Song et al. 2010; Cho et al. 2017).

Likewise, in such “straightforward” physical occasions, there are comparable however progressively controlled molecule-inducing forms, frequently made on regular oxidation. One constantly noticed precedent of hydrogen sulfide gas oxidation (HS) condensed in volcanic eruptions or/and in wells which are common in numerous locality of our planet (Ezoe et al. 2002; Berlo et al. 2014). All things considered must be well characterized—at any rate with respect to synthetic course of action—and could get significant consideration.

## 2.3 Biological Synthesis of Nanomaterials

To be sure, the living cell is habitually managing “nanotechnology,” in other words with objects of a nanoscopic rate. Just to wind up an inclination for sizes, basic strand of DNA in width is almost equal to 2.5 nm (Shors 2011). Henceforth some cells that lie in microbes are specific and are included in the field of nanotechnology. Some procedures are somewhat very much perceived and contemplated with respect to some metals like selenium and sulfur just as metallic NPs (Kuppusamy et al. 2016). The particles created by such biogenic production lines are generally of decent quality, for example, little circular states of a practically uniform size. Not astoundingly, such natural procedures might be inappropriate ultimately to create materials of good quality and then to deliver.

To be sure, the subtraction of ecological contaminants (e.g., overwhelming metals, natural and inorganic toxins), from defiled destinations utilizing nanomaterials or nanoparticles shaped in or by plants, parasites, and microbes with the aid of nanotechnology, frequently referenced to as nanobioremediation (NBR), is a growing, earth-responsive, and practical substitute to customary compound strategies (Singh and Walker 2006; Yadav et al. 2017). At this point, the three fundamental methodologies of present-day bioremediation grasp the utilization of organisms, plants, and sequestered chemicals, for event, nitrate reductase, or laccase (Sanghi et al. 2011). All things being equal, there is likewise an extra plus point of that strategy. Presently, the nanoparticles made by those life forms are not all encompassing spotted as contaminants however basically as esteemed nanoparticles of a pretty much common birthplace. Inside this specific situation, a portion of these bioreductively framed "normal" nanomaterials have been found currently with focus on likely restorative and horticultural entries (Salata 2004; Li et al. 2011; Duhan et al. 2017; Iavicoli et al. 2017). It is conceivable, for example, to tenderfoot innocuous microorganisms, for example, *Staphylococcus carnosus* and *Saccharomyces cerevisiae*, to create genuinely homogeneous selenium nanoparticles from selenite, an ion with normal measurements of 60 and 80 nanometers, correspondingly (Zhang et al. 2012; Estevam et al. 2017). Such particles are assembled from the microbes and yeasts after rupture of the cell. The investigators have wandered about conceivable implementations as nourishment increments and most likely as antimicrobial operators as a portion of such particles uncover an unambiguous antimicrobial movement (Zhang et al. 2012; Estevam et al. 2017; Skalickova et al. 2017).

In the area of farming, likely implementations have considerably much surfaces and a conceivable "cap trap" of immediately enhancing dirt with selenium for braced sustenance items, of giving plants components for their characteristic obstruction frameworks, and of dispensing with plant pathogens appears to be doable (Estevam et al. 2017). Inside this unique situation, one needs to push such normally delivered particles are not proportionate to modernly created materials. They are not "artificially unadulterated" and every now and again likewise contain a "characteristic" covering of proteins whose design is an impression of the yeasts or microscopic organisms they have been made in. Later the organic action of such characteristic particles may come from the mass material of the molecule itself, for example, selenium, from different mixes kept or restricted to the molecule and furthermore from the covering, which is as often as possible wealthy in proteins (Prakash et al. 2009; Wang et al. 2010; Yazdi et al. 2012; Estevam et al. 2017). In cases like these, a broad "intracellular diagnostics" is needed to explain the correct target(s) and point-by-point method(s) of activity (Mániková et al. 2014). Eventually, one may foresee a complex procedure by which microorganisms are created or tainted and by remediating that dirt delivers unmistakable nanoparticles which might be reaped and push off in prescription, horticulture, or several more appropriate applications. The significant merits of these techniques might be impressive and are not unrealistic additionally, as germane contaminants, for example, extensive metals, regularly likewise describe the establishment of mostly intriguing particles. In any case, there might be some further advantages, particularly with regard to pathogenic organisms and microorganisms. Various examinations have built up that the arrangement of nanomaterials by or inside pathogenic microscopic organisms is a compelling instrument to cancel those living beings. It has been seen, for example, that pathogenic strains of *Staphylococcus aureus*, for example, HEMSA and HEMSA 5 M, reduce $SeO_3^{2-}$ to basic selenium when tested with incredibly extraordinary convergences of this anion (around 2 mM) in a shallow endeavor to manage this experience (Estevam et al. 2015). At last, this defensive plan comes up short, and the stores of selenium framed inside the microscopic organisms kill these cells. This sort of "self-destructive normal nanotechnology" is found between numerous microorganisms and organisms, just as pathogenic ones. It somewhat clarifies to some degree the antimicrobial activity frequently related with reactive selenium species (RSeS), such as $SeO_3^{2-}$, $SeO_4^{2-}$, $TeO_3^{2-}$, and $TeO_4^{2-}$. These activities might be explicit for specific life forms, giving these operators and chaperon forms with specific "sensor/effector" properties. Later on, this sort of common nanotechnology thusly can give an intriguing chance to trade off, debilitate, harm, or potentially even execute such pathogenic living beings (Estevam et al. 2017).

## 2.4 Processing Natural Materials

Various methodologies had great impact on processing of natural materials. The subsequent particles of such regular items are of an elite nature, as they are common items, yet being twisted to an uncommon, unnatural shape and size. It is along these lines barely amazing that numerous normal items have been nanosized in the most recent few years. Cancer prevention agents, for example, rutin, have been transformed into alleged "nanocrystals" utilizing an expressive procedure which incorporates wet globule processing (WBM) and high-weight homogenization (HPH) (Müller and Keck 2008; Mauludin et al. 2009). Here, nanotechnology can be utilized to create nanoparticles with a drastically enhanced solvency, phenomenal discharge energy, and later a decent bioavailability and organic action. This technique is especially alluring in the area of—frequently sparingly dissolvable—cell reinforcements and plant items wealthy in such cancer prevention agents, i.e., materials which initially have deficient discharge energy on the lipid/fluid skin surface; however on account of the new innovation, it can nowadays be utilized effectively, for example, in beauty care products. To be sure, the guideline of nanosizing coarse materials to advance their organic movement is extremely straightforward and is for the most part dependent on the Noyes-Whitney condition, one of the real conditions in biopharmacy.

For this situation, the immersion dissolvability increments because of a higher disintegration weight, which is elucidated by a greater shape of the particles (Kelvin condition) and the diffusional separate likewise diminished (Prantl condition). Nanosizing prompts an imperative increment in the general speed of disintegration, which is particularly intriguing if dynamic constituents break up gradually or not properly soluble in water. Besides, nanosizing enhances the bioactivity of inadequately solvent dynamic fixings. Because of the expansion in dissolvability, the fixation slope, when contrasted with bigger estimated materials, is expanded (Keck and Müller 2006). Because of these more prominent structures and the simplicity of generation, nanosizing, i.e., the creation of nanocrystals, has turned into a noteworthy readiness guideline in pharmaceutics to propel the bioactivity of dynamic fixings (Müller and Keck 2012; Scholz and Keck 2015). A portion of these processed plant materials have been assessed as likely nourishment supplementation and even as characteristic medications and antimicrobial specialists (Griffin et al. 2016). NPs of normal items must be utilized in the grounds of sustenance, medication, and beautifying agents or on account of substantial scale getting together, in "green" horticulture. The exercises trial for those nanosized materials are regularly encouraging, yet there is a well parity of contentions which should be estimated. Therapeutic plant is equivalently clear and altogether simpler than withdrawal, refinement, and detailing of the dynamic component(s) kept in that. It additionally delivers no or minimal waste. Also, NPs that are basically "normal," in any event to the extent their concoction organization is unstable, that contain every one of the elements in plant will not face any broad alterations and would not be treated with natural solvents. In a perfect world, they even portray a characteristic moderate discharge assembly of biologically available and naturally dynamic fixings. On account of high-pressure homogenization (HPH), such materials are additionally sanitized as progressively current investments.

## 2.5 Plant-Based Synthesis of Metallic NPs and Their Applications

NPs have exceptionally fascinating applications covering many areas (Chandran et al. 2006). Biological ways of making natural elements capable of reusing are shown in Fig. 2.2.

### 2.5.1 Traditional Strategies of Metals

Anciently metals were considered symbol of strength, and even nowadays some metals are considered as influential like gold and silver. In the eighteenth era, gold was used for mental and spiritual purification. Egyptians utilize gold

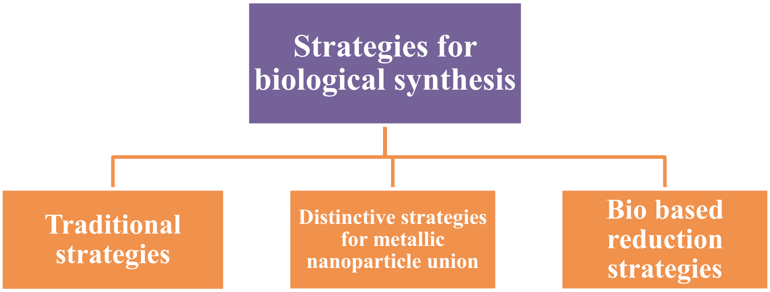

**Fig. 2.2** Biological ways for synthesis of metallic nanoparticles

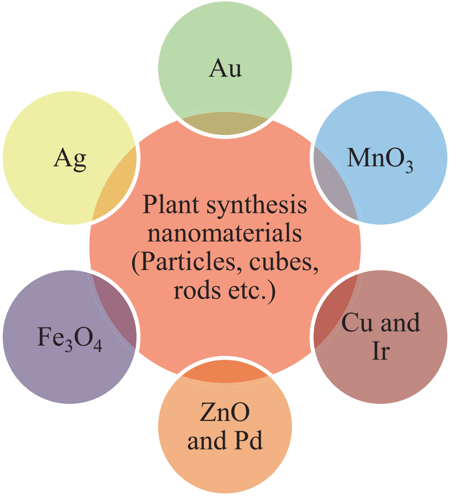

**Fig. 2.3** Different plant-derived metallic nanoparticles

metal-solubilized water. The significance of gold is as yet respected in countryside areas, as the employees there prepare their rice dishes with a gold pellet to supersede the mineral lack in the body by nourishment admission. Silver is used for the healing of wounds and ulcer removal (Singh et al. 2013). Truth be told, the silver nanoparticles (AgNPs) which are colloidal in nature were utilized against antimicrobes, as wound dressing material, as tooth bond, and as a water purifier (Narayanan and Sakthivel 2011).

## 2.5.2 Distinctive Strategies for Union Metallic Nanoparticle

Some techniques are employed for the union of nanoparticles (nanoparticles, such as natural, compound, enzymatic, and physical). Physical strategies include ball processing, warm dissipate, beat laser desorption, atomic shaft, and flame amalgamation dispersion of NPs (Joerger et al. 2000). Synthetic techniques are utilizing radiation of high frequency and settling operators which are hurtful to human well-being. Figure 2.3 exhibits various kinds of metallic nanoparticles formed from plant means.

## 2.5.3 Bio-based Reduction Strategies

Silver is the biochemical feedback of silver nitrate that basically prompts the arrangement of AgNPs with the help of plant stock (Tripathy et al. 2010). The plants possess many vital biomolecules, like proteins, amino acids, and chemicals; moreover, they have some metals too. Then all these biomolecules must engage with bioreduction mechanism. So the conclusion in this metal like gold was the conversion and reduction of $Au^+$ into metallic $Au_0$ nanoparticles in the redox catalysts (Thakkar et al. 2010).

## 2.6 Parts of Plants Used to Synthesize Nanomaterials

Different parts of plants are being employed in the formation of eco-friendly nanoparticles.

### 2.6.1 Flowers

Noruzi et al. (2011) considered an environment-friendly disposed strategy amalgamation of gold nanoparticles (GNPs) by utilizing flower petals. The concentrated media contains sufficient proteins and sugars. A portion of these utilitarian mixes are the critical wellsprings of tetrachloroaurate salt, which declines into the main part of GNPs. Additionally, flowers of *Clitoria ternatea* and *Catharanthus roseus* are utilized for the metal nanoparticle combination with required shape and sizes. The plant material-incorporated nanoparticles are viably handling disease-causing microbes, and correspondingly the therapeutic utilizable *Nyctanthes arbor-tristis* flowers for GNPs extracts are obtained through green science technique (Das et al. 2011). Fluid concentrate of flowers of *Mirabilis jalapa* goes about as a reducing agent and delivered GNPs with eco-friendly technique (Vankar and Bajpai 2010). Table 2.1 delineates the plant metabolites which speak to in the bioreduction response to union of metal NPs and their applications in pharmacology.

**Table 2.1** Different types of plant-based metallic nanoparticles (Kuppusamy et al. 2016)

| Plant used | Part of plants | Nanoparticles | Shape | Size (nm) | Plants involved in bioremediations |
|---|---|---|---|---|---|
| *Acalypha indica* | Leaf | Ag, Au | Spherical | 20–30 | Quercetin, plant pigment |
| *Alternanthera sessilis* | Whole | Ag | Spherical | 40 | Amine, carboxyl group |
| *Aloe vera* | Leaf | $In_2O_3$ | Spherical | 50 | Biomolecules |
| *A. paniculata* | Leaves | Ag | Spherical | 67–88 | Alkaloid, flavonoids |
| *A. mexicana* | Leaves | Ag | Spherical | 20–50 | Proteins |
| *Boswellia serrata* | Gum | Ag | Spherical | 70–90 | Secondary metabolites |
| *Caria papaya* | Fruit | Ag | Spherical | 15 | Catechins |
| *Cassia fistula* | Stem | Au | Spherical | 55–98 | Hydroxyl group |
| *Cinnamon zeylanicum* | Leaves | Ag | Spherical | 45 | Water-soluble organics |
| *Citrullus colocynthis* | Calli | Ag | Triangle | 5–70 | Polyphenols |
| *Citrus sinensis* | Peel | Ag | Spherical | 35 | Water-soluble compound |
| *Dillenia indica* | Fruit | Ag | Spherical | 11–24 | Biomolecules |
| *Dioscorea bulbifera* | Tuber | Ag | Rod-triangular | 8–24 | Ascorbic acid |
| *Euphorbia prostrata* | Leaves | Ag | Rod, spherical | 52 | Proteins, phenols |
| *Mirabilis jalapa* | Leaves | Au | Spherical | 90–150 | Polysaccharides |

### 2.6.2 Stem

Shameli et al. in 2012 investigated the stem of *Callicarpa maingayi* with removed methanolic group utilized for amalgamation of AgNPs and shaped [Ag (Callicarpamaingayi)] + complex. The plant extracts have aldehyde collections, and it's for the most part associated with the decrease of silver particles into metallic Ag nanoparticles. The distinctive useful gathering demonstrates amide and polypeptides that are dependable mixes with topping of ionic substances into metal NPs. The atomic examinations on biosynthesis of silver precious stones are intricate and not yet completely comprehended. Be that as it may, some past investigations are proposed demonstrating components of nanoparticle cooperation with pathogenic living beings. The biologically produced silver nanoparticles have protein external cell mass of microscopic organisms, growths, or entities of viruses that degrade the lipoproteins of microbe cell divider. At last the division of cell was ceased and cell prompts passing. At room temperature silver nanoparticle photosynthesis utilized extracts of *Cissus quadrangularis* (Vanaja et al. 2013). The removed stem of some portion of plants demonstrates the distinctive utilitarian gatherings, especially the amine, phenolic, carboxyl, and aggravates that are associated with the decrease of silver particles. Thus, incorporated silver nanoparticles indicate greater action against *Bacillus subtilis* and *Klebsiella planticola* pathogenic microscopic organisms. Along these lines, the biosynthesized metal nanoparticles went about as great antibacterial specialists. Various plant parts involved in formation of nanoparticles are shown in Fig. 2.4.

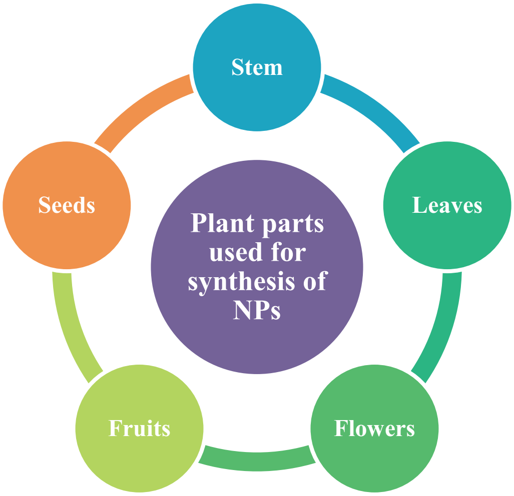

**Fig. 2.4** Different types of parts of plants involved in synthesis of nanomaterials

### 2.6.3 Seeds

A few seeds that extricate like that of fenugreek contain high substance of secondary metabolites like flavonoids and other bioactive items, for example, lignin and nutrients. *Chloroauric corrosive* could be utilized for breakdown of solid parts of the fenugreek seeds. The COO gathering (carboxylic) and C=N and C=C useful gatherings are separately lying in the seed. The useful gathering of metabolites goes about as a surfactant of GNPs, and the flavonoids can settle the electrostatic adjustment of GNPs (Mittal et al. 2013). The fluid drawouts of *Macrotyloma uniflorum* impact the decrease of silver particles. Caffeic corrosive might be expected in the concentrate. Subsequently, decreased caffeic corrosive response could happen in one moment.

### 2.6.4 Fruits

Gopinath et al. (2012) utilized plant organic product of *Tribulus terrestris* extricate with expansion of various molar convergences of silver nitrate arrangement so as to incorporate eco-accommodating silver NPs with certain morphological highlights. The concentrate contains dynamic phytochemical that are subject for the single step decrease response. The round states of AgNPs were delivered by the *T. terrestris* extricates and proved commendable antimicrobial action against multidrug safe human pathogens. There is comparable cover utilizing polyphenol from grapes to combine palladium NPs and act viably against bacterial maladies (Amarnath et al. 2012). Not withstanding this, *Rumex hymenosepalus* serves as a balancing out operator for AgNPs. The utilization of ideal physic-concoction strategies to create nanomaterials is exceptionally valuable in pharmacological proposition to treat numerous endemic illnesses.

### 2.6.5 Leaves

Leaves that extricate are utilized as mediators to shape nanoparticles. Leaves of *Murraya koenigii*, *Centella asiatica*, and *Alternanthera sessilis*, including various leaves separate, are studied. Most recently leaves of *Piper nigrum* contained a critical bioactive material which is taking an interest in the nanoparticle arrangement by eco-philic strategy. The naturally orchestrated silver nanoparticles of 100 lg/ml concentration were dynamic medication, which focus on HEp-2 and HeLa cell line to control the ordinary biological work in disease cells. The AgNPs are powerful medication in malignant growth treatment to fix oncology in addition to terrifying disorders. *P. nigrum* removes longumine, it goes about as a topping operator for the arrangement of AgNPs and may upgrade the cytotoxic impacts of the tumor cells (Jacob et al. 2012). A green blend of AgNPs utilizing plant *Artemisia nilagirica* leave extracts has been depicted by Vijayakumar et al. (2013). It is by all accounts a crucial device for antimicrobial operators for now and close fates like that of silver nanoparticle amalgamation from the plants that control diverse pathogens in human.

## 2.7 Plant-Derived Formation of Silver Nanoparticles

Both physical and chemical methods had been used for the synthesis of silver nanoparticles for an extended period, but now for this purpose, developments have found the principal role of biological systems (Fig. 2.5).

Chemical and physical procedures are energy-exhaustive operations which indicate high production cost. Synthesis of AgNPs by chemical methods such as ethylene glycol, hydrazine hydrate, sodium borohydride, and dimethyleformamide can cause absorption of dangerous chemicals on the nanoparticle surfaces leading to toxicity problems (Iravani 2011). Furthermore, aqua chemical paths form nanocrystalline silver colloids which show accumulation with time, which means accommodation with the size factor upon storage. Plant derivatives have been explored as much better nominees over other biological systems, e.g., fungi and microorganisms, among the bio-based formation of silver nanoparticles, as they do not demand

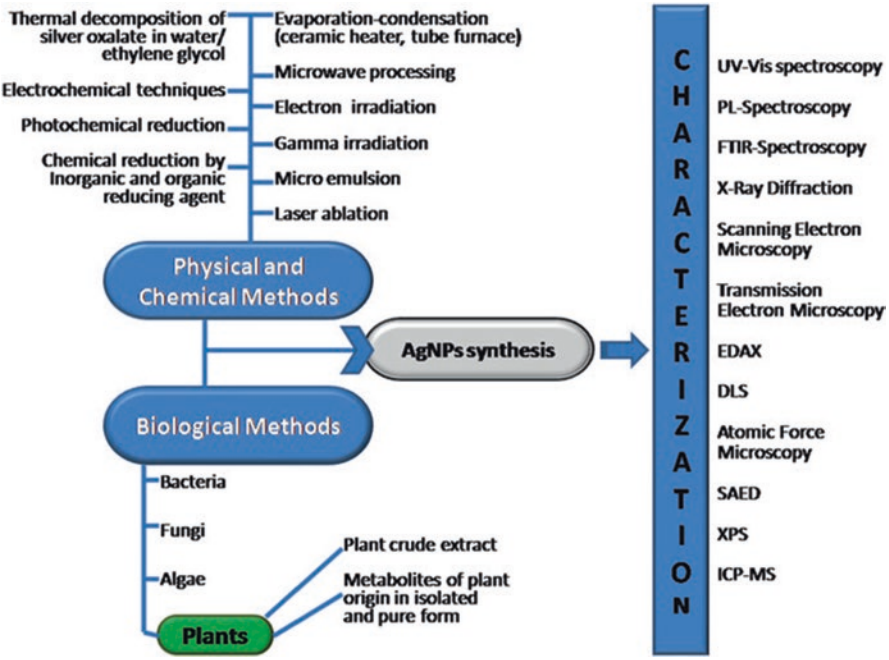

**Fig. 2.5** Chemical, physical, and biological methods for the synthesis of AgNPs and the techniques employed for the characterization of produced nanoparticles (Patel et al. 2015a, b)

**Table 2.2** Investigations by several researchers on plant-based AgNP synthesis and characteristics of formed nanoparticles

| Plants | AgNP specifications | Plant parts | References |
|---|---|---|---|
| *Citrus sinensis* | 10–35 nm | Peel | Kaviya et al. (2011) |
| *Allium sativum* | Spherical, 7.3 nm | Garlic cloves | Rastogi and Arunachalam (2011) |
| *Allium cepa* | Spherical, 33.6 nm | Leaves | Saxena et al. (2010) |
| *Capsicum annuum* | Spherical and crystalline, 10–70 nm | Fruit | Li et al. (2007) |
| *Daucus carota* | 31–52 nm | Taproot spherical | Mukunthan and Balaji (2012) |
| *Eucalyptus globulus* | 5–50 nm | Bark | Astalakshmi et al. (2013) |
| *Mangifera indica* | Triangular, hexagonal, and nearly spherical, 20 nm | Leaves | Philip (2010) |
| *Ocimum sanctum* | 5–10 nm | Root and stem | Ahmad et al. (2010) |
| *Piper betle* | 17–120 nm | Leaves | Rani and Rajasekharreddy (2011) |
| *Zingiber officinale* | 6–20 nm | Rhizome | Kumar et al. (2012) |
| *Trachyspermum ammi* and *Papaver somniferum* | 60–80 nm | Seeds | Vijayaraghavan et al. (2012) |
| *Rosa rugose* | 12 nm | Leaves spherical | Dubey et al. (2010) |
| *Svensoniahyderobadensis* | 24 nm | Roots | Rao and Savithramma (2013) |
| *Parthenium hysterophorus* | 20–70 nm | Leaves | Anwar et al. (2015) |

harmful capping and reducing factors, high temperature, radiation, fungal/microbial strains and expensive media for growth of microbes/fungi as well as for the production of nanoparticles. During formation and implementation zones, they also minimize the possibility of contamination or infection (Borase et al. 2014).

Many other researchers have also investigated the participation of several plant-based metabolites like amines (Prasad et al. 2011), flavonoids, polyphenols, terpenoids (Marimuthu et al. 2011), aldehydes, ketones (Chandran et al. 2006), starch (Vigneshwaran et al. 2006), arabinose and galactose (Kora et al. 2010), and saponins (Elavazhagan and Arunachalam 2011) in AgNP formation. Table 2.2 shows utilization of various plant parts in the formation of silver nanomaterials.

## 2.8 Plant-Based Gold Nanoparticle

Gold-derived nanoparticles have several applications in various fields. Au(3+) ion is reduced to the Au atom by binding of the atom to the cell surface, whereas further reduced Au also combines and clumps to produce GNPs. Au ions have good chances

**Table 2.3** Plant-based synthesis of gold nanoparticles

| Plants | AuNP size | Plant parts | References |
|---|---|---|---|
| *Citrus maxima* | 25.7710 nm | Fruit | Yu et al. (2016) |
| *Ixora coccinea* | 5–10 nm | Flower | Nagaraj et al. (2011) |
| *Coleus forskohlii* | 5–18 nm | Root | Naraginti et al. (2016) |
| *Cassia fistula* | 55.2–98.4 nm | Stem bark | Daisy and Saipriya (2012) |
| *Punica granatum* | 70 nm | Fruit peel | Ganeshkumar et al. (2013) |
| *Argemone mexicana* | 22–26 nm | Leaf | Varun et al. (2015) |
| *Hygrophila spinosa* | 50–80 nm | Leaf | Koperuncholan (2015) |
| *Abelmoschus esculentus* | 45–75 nm | Seed | Jayaseelan et al. (2013) |
| *Morinda citrifolia* | 12.17–38.26 nm | Root | Suman et al. (2014) |
| *Hibiscus sabdariffa* | 10–60 nm | Leaf and stem | Mishra et al. (2014) |

at the starting phases to attach with the atom and produce clumps (Cai et al. 2011). GNPs have applications in the field of medicine (Honary et al. 2012). The bio-based synthesis of nanoparticles has specific morphology and determined shapes such as hierarchical tubes, triangle, hexagonrods, decahedrons, icosahedrons, and nanotriangles as well as nodous ribbons. Different nanoparticles have different efficient applications in various fields; thus researchers are now focusing on their shapes (Du et al. 2007). GNPs are being produced by utilizing several plant parts, e.g., stem, leaf, root, and fruit as showed in Table 2.3.

## 2.9 Plant-Based Zinc Oxide Nanoparticles

Zinc oxide (ZnO) nanoparticles are currently of greater interest due to their certain characteristics, e.g., their production is cheap, safe to use, and can be synthesized easily (Elumalai et al. 2015). The reason of the attention of researchers toward zinc oxide NPs is their vast applications in the sector of optics, electronics, and biomedical systems (Azizi et al. 2014). ZnO nanoparticles show enormous applications as anti-inflammatory (Ramesh et al. 2015) and wound-healing characteristics (Nagajyothi et al. 2013). They have ultraviolet filtering characteristics thus being used in cosmetics, e.g., sunscreen creams and lotions. They have also vast applications in medicines such as anticancer, drug delivery, antifungal, antibacterial, antidiabetic, and agricultural characteristics (Ramesh et al. 2015). They have also been used in manufacturing rubber and paint, in applications in dentistry, and in eliminating arsenic and sulfur from water (Ali et al. 2016). Anbuvannan et al. (2015) reported ZnO nanoparticles have several morphologies like nanorods, nanowires, nanoflowers, nanoflakes, and nanobelts. Table 2.4 shows plant-based synthesis of ZnO NPs derived from different plant parts like flowers, stem, leaves, and fruit peels.

**Table 2.4** Plant-based synthesis of ZnO nanoparticles

| Plants | Shape | Plant parts | References |
|---|---|---|---|
| *Rosa canina* | Spherical | Fruit extract | Jafarirad et al. (2016) |
| *Solanum nigrum* | Hexagonal and quasispherical | Leaf extract | Ramesh et al. (2015) |
| *Cocos nucifera* | Spherical and hexagonal | Coconut water | Krupa and Vimala (2016) |
| *Gossypium* | Spherical and nanorod | Cellulosic fiber | Aladpoosh and Montazer (2015) |
| *Calotropis gigantea* | Spherical | Fresh leaves | Vidya et al. (2013) |
| *Nephelium lappaceum* | Spherical and hexagonal | Fruit peels | Yuvakkumar et al. (2015) |
| *Coptidis rhizoma* | Spherical and rod shaped | Dried rhizome | Nagajyothi et al. (2014) |
| *Vitex negundo* | Hexagonal | Flowers | Ambika and Sundrarajan (2015) |

## 2.10 Biofuel Applications of Nanoparticles

Commercialization process of biofuels is limited due to hinders in their production processes. Productions of high-energy yield and cost-effective biofuels are the main challenges. Nanoparticles in this regard may play a significant role in forming this process economically viable.

### 2.10.1 Role in Pretreatment

Most frequently found biopolymer is the lignocellulosic biomass rich in carbohydrate content. Two-thirds of the lignocellulosic biomass consists of cellulose and hemicellulose and are among the cheapest sources for production of biofuel (Srivastava et al. 2015a). Lignin barrier is removed prior to saccharification by pretreatments to expose cellulose and hemicellulose (Alvira et al. 2010). Biological and chemical pretreatment methods are common among various pretreatment procedures for breakdown of lignocellulosic matter (Srivastava et al. 2015a). Although pretreatment is considered as the expensive phase in the transformation of cellulose, it has the ability to enhance the efficiency remarkably and lower the net cost of biofuel production (Alvira et al. 2010; Srivastava et al. 2015a). Degrading enzymes, e.g., cellulases and hemicellulases, are employed after the pretreatment to liberate the fermentable sugars (Rawat et al. 2014). Thus many investigators are working on pretreatment process to improve the bioconversion efficiency of lignocellulosic biomass. Wei et al. (2015) observed that production of sugar from corn stover was improved in the existence of iron oxide nanoparticle by employing the acid pretreatment. Furthermore, nanoparticle-acid pretreated substrate exhibited nearly 13–19% more xylose and glucose relative to control (acid-treated substrate without nanoparticles). This study found that iron oxide nanoparticle can aid the pretreatment process, as results exhibited improved sugar formation over the metallic iron nanoparticles. The experiment was conducted at 100 °C which showed its economic viability, and the reported data exhibited a positive link among the concentrations of released sugar and iron during the biomass pretreatment. In another investigation done by Yang et al. (2015), breakdown of cellulosic biomass was improved in the existence of decreased graphene oxide functionalized with iron oxide nanoparticles. The iron oxide reduced graphene oxide-$SO_3H$ ($Fe_3O_4$-RGO-$SO_3H$); nanocomposite was successfully produced employing the reduced graphene oxide (RGO), containing iron oxide nanoparticles and benzene sulfonic acid which were directly harbored on the surface of RGO through C-C covalent bonds. The distinctive structure of $Fe_3O_4$-RGO-$SO_3H$ nanocomplex along with increased dispersion in water sustains the reachability of cellulose to the active sites and enhances the production of sugar which can further be employed for the biofuel production. Although these investigations have explored a new zone for the production of biofuels employing the nanoparticles, currently this avenue is at the very initial phase. So, many more attempts to improve the economic sensibility are needed.

### 2.10.2 Role in Cellulase Production and Stability

After the pretreatment process, enzymatic hydrolysis is done by cellulases to degrade lignocellulosic biomasses. For enhanced enzymatic hydrolysis, greatly efficient cellulases are needed, which are capable of working in unrelenting conditions. Furthermore uses of several cofactors, e.g., metal ions, are reported in the many investigations other than various substitute methods to make enhanced cellulase efficiency and its production in industries (Srivastava et al. 2014). To increase the stability of enzyme application of nanoparticles is a new avenue in the area of bioenergy production (Srivastava et al. 2014; Singh et al. 2016a, b). Few but encouraging and potential investigations have been outlined in this field currently. The study by Dutta et al. (2014) noticed an enhanced production of cellulase in the existence of hydroxyapatite nanoparticle employing the bacterial strain. Highly thermostable enzyme was used in this research which kept its half-life at 80 °C. Besides enhanced thermal stability, these authors also observed an enhancement in reducing sugars when substrates, e.g., rice husk and rice straw, were used. Similarly Srivastava et al. (2015a) reported an improved cellulase production, its thermal strength, and sugar production in the existence of iron oxide/alginate nanocomplex. A higher sugar yield was observed in this study by employing thermotolerant fungal sp. *Aspergillus fumigatus* AA001 in the solid-state fermentation using the same nanocomplex. Furthermore, thermal stability of cellulase enzyme was also improved besides its production in the existence of uncovered iron oxide nanoparticle as relative to control. The production of cellulase was enhanced in the existence of pure iron oxide nanoparticle and iron oxide/alginate nanocomposite by 35% and 40%, respectively, when compared to control. In addition, iron oxide/alginate nanocomplex treated cellulase exhibited its thermal stability at 70 °C for 8 h by maintaining its 56% of relative activity, whereas crude cellulase could keep only 19%. These investigations distinctly evince that the nanoparticles may play a significant part to change the whole process of bioconversion. Srivastava et al. (2014) suggested that the major cause of enhancement in the thermal stability of cellulase was cellulase immobilization on to the nanoparticles. Besides, thermal stability and an improved cellulase production have been noticed in the existence of nickel cobaltite nanoparticle by thermotolerant *Aspergillus fumigatus* NS (class: Eurotiomycetes) through solid-state fermentation. Furthermore, untreated cellulase showed thermal stability at 80 °C for 7 h in the presence of $NiCo_2O_4$ nanoparticles, whereas at the same temperature, untreated cellulase was stable up to 4 h. Moreover it was also observed that production time was lessening in the existences of nanoparticles in above studies. Therefore, in the near future, nanoparticles may prove their capability in biofuel production process. Verma et al. (2013) reported improvement in the above stated investigations; there are also many researches that have concluded improvements in the hydrolysis efficiency of cellulases besides its better production and thermostability in the presence of various kinds of nanomaterials, e.g., iron oxide, zinc oxide nanoparticles, etc. Ansari and Husain (2012) immobilize cellulase on iron oxide magnetic nanoparticles. They reported that the nanoparticles may act as a carrier and provide resistance against the unfavorable pH and inhibitor besides thermal stability. Verma et al. (2013) observed that the thermostability of the enzyme β-glucosidase was enhanced in the occurrence of iron oxide magnetic nanoparticles and showed the half-life of that enzyme at 70 °C. As nanoparticles have explored a new path to enhance the production of cellulase and its thermal stability, their exact mechanism is not well known. So, further studies are focusing on this specific area for its industrial and economic viability.

### 2.10.3 Role in Saccharification

Saccharification or hydrolysis of lignocellulosic biomass is the next phase after pretreatment to liberate sugars using cellulase enzyme. Temperature 45–50 °C needed for enzymatic hydrolysis makes the whole process slow, more vulnerable to contamination by microbes, and usually incomplete, ending in decreased production of fermentable sugars and demand greater enzyme quantity. Hence, it is suggested that these bottlenecks can be overcome by employing the thermostable cellulase enzymes and thermophilic microbes (Yeoman et al. 2010). Cellulase that can tolerate increased temperatures can also work at higher hydrolysis temperatures. The presence of nanoparticles enhances the thermal stability of cellulase, and these thermostable cellulases may perform significantly at higher degradation temperatures. Some current investigations concluded the enhanced thermostability and degrading efficacy at increased temperatures (Dutta et al. 2014; Srivastava et al. 2015a, b).

Dutta et al. (2014) employed rice husk/rice straw as substrates, and improved thermal stability of cellulase enzyme was observed resulting in enhanced production of sugar in saccharification process. In this study calcium hydroxyapatite nanoparticles were used, and substrates were treated at 80 °C to get reducing sugars. Srivastava et al. (2015b) used iron oxide/alginate nanocomposite and found betterment in degradation efficacy of cellulase enzyme and sugar yield at 70 °C. Under the solid-state fermentation, greater sugar yield was reported at higher temperature employing *Aspergillus fumigatus* AA001 in the occurrence of iron oxide/alginate nanocomplex. Though nanoparticles have greater capability to enhance the degrading efficacy of cellulase enzyme, their mechanism is not very clear; so attention must be paid in this aspect for industrial scale production.

## 2.11 Optional Metabolite Impact on Bio-decrease Response

A considerable portion of the optional metabolites and a few proteins have reasonably advanced the combination of NPs which are metallic in nature from the other ionic mixes. The decreased response principally included plant biomolecules (optional metabolites, e.g., sugars (polysaccharides), proteins, natural mixes, shades, and plant tars. Plant normal items are engaged with the decrease response to incorporate green nanoparticles. Plants are especially taking an interest in guard components to create different substance mixes, for example, polyphenols, saponins, and cell reinforcement chemicals, like alkaloids. The proposed decrease response illustrated the primary components for the biological synthesis of metallic NPs. The plant removes various useful collections, for example, alkenyl, phenolic and liquor, amine, and carboxylic group. It is for the most part indicated as plant auxiliary metabolites and may be miniaturized scale and large-scale biomolecules (Jha et al. 2009). These substances are completely taking an interest for the NP generation. For example, *R. hymenosepalus* plant removes the advances of NP unions with quick response energy. Consequently, the dissolvable concentrate of *R. hymenosepalus* is rich in polyphenols, for example, stilbenes and catechins, atoms that go about as diminishing and balancing out specialists for silver NPs creation (Awwad et al. 2013).

## 2.12 Business Uses of Biosynthesized Nanoparticles

### 2.12.1 NPs in Waste Treatment NPs

Nanoproducts have various applications in everyday life. There are different ecologically accommodating products accessible in business showcase which are highly efficient, for example, bone and teeth concrete and handcrafted items (Kouvaris et al. 2012). For example, Au, Ag, and platinum NPs have wide applications in beauty products and they are utilized as fixings in different items, like, to protect against the UV rays sun blockers, toothpastes, mouthwash, and fragrances and shampoos (Kumar and Yadav 2009). NPs of silica act in fixing business items. The altered silica NPs are utilized as pesticide, and it is utilized in an assortment of non-farming implementations.

### 2.12.2 Beautifiers

Metallic NPs are utilized as additive specialists in restorative enterprises. New element of metallic nanoparticles is utilized for various business applications, for the most part beauty care products, medical materials, and sustenance additives (Songand and Kim 2009; Kokura et al. 2010). The metallic NPs like the platinum, gold, and Ag are connected for some business items, for example, soap, detergent, shampoo, and shoes.

### 2.12.3 NPs in Food Industry

Some metals like silver are quick warmth directing; therefore, nano-Ag is utilized in different mechanical frameworks. It is predominantly utilized in warmth-inclined instrumentation like in PCR cover (Weiss et al. 2006). In food businesses, sustenance items have very high microbial sullying because of different procedures, for example, assembling and handling of crude materials. Consequently, requirement is there to build up a savvy biological sensor to decide the nature of the items.

## 2.13 Component Blend of Metallic NPs

Difference highlighted in hydrogen particles is basically a reaction by variation in size and states of NP arrangement. Shankar et al. (2003) revealed that the extracts of *Aloe vera* provided Au-Ag center NPs in different sizes by changing the pH of the medium which is dissolvable. So, biologically synthesis of NPs by hay plant concentrate have variable size.

## 2.14 Conclusion

Environmental hazards caused by chemical synthesis of nanoparticles have urged synthesis of nanoparticles using plant means, as it includes eco-friendly methods for the synthesizing nanoparticles. This chapter focused on the plant-based nanoparticles and methods of their synthesis. Various plant parts, e.g., leaves, flowers, roots, stems, fruit and its peels, etc., are being used for the green synthesis of nanoparticles especially metallic nanoparticles. This chapter also overviews the applications of these plant-based nanoparticles in different fields, e.g., medical, agriculture, food industry, and cosmetic industry.

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# Application of Plant-Based Natural Product to Synthesize Nanomaterial

## 3

Mohammad Khajeh Mehrizi and Zahra Shahi

**Abstract**

Nanotechnology is rapidly advancing and is used for a wide range of applications. The chemical and physical methods used to synthesize nanoparticles are expensive and often raise questions of environmental risk because of the use of toxic and hazardous chemicals. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plant extracts have been suggested as alternatives to chemical and physical methods. The methods have drawn the attention of researchers due to their eco-friendly nature and low-cost, energy-efficient, non-toxic, and easier steps for synthesis. Among these methods, plants (*Thuja occidentalis, Ocimum sanctum, Curcuma longa, Ficus benghalensis* leaf extract, etc.) are the best candidate and suitable for large-scale biosynthesis of nanoparticles. Many biomolecules in plants such as proteins, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins could be involved in bioreduction, formation, and stabilization of nanoparticles. So, using plants for biosynthesis of nanoparticles has become a major focus of researchers.

**Keywords**

Nanotechnology · Chemical synthesis · Biosynthesis · Plant

## 3.1 Definition of Nanoparticles

“Nano” is a Greek word synonymous to dwarf meaning extremely small. The prefix is used to indicate one billionth of a meter or $10^{-9}$ m. The nanosized world includes systems whose size is above molecular dimensions and below macroscopic

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The original version of this chapter was revised. A correction to this chapter can be found at https://doi.org/10.1007/978-981-13-9333-4_12.

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_3

(generally >1 nm and <100 nm). Nanoparticles play a basic role as building blocks of nanotechnology. In other words, nanotechnology is a branch of science (often referred to as the "tiny science") which deals with matter having at least one dimension size from 1 to 100 nm (Pal et al. 2011, 2018).

## 3.2 Physicochemical Properties and Application of Nanoparticles

Physicochemical properties of nanoparticles are large surface area, mechanically strong, optically active, high thermal conductivity, and chemically reactive. Their reactivity, toughness, and other properties are dependent on their unique size, shape, and structure. Due to these characteristics, they are suitable candidates for various commercial and domestic applications (Khan et al. 2017).

Nowadays, nanotechnology is widely applied in different fields mainly in water purification, electronics (capacitors), coatings, packaging, antibacterial, cosmetic, drug delivery, imaging, chemotherapy, and catalysis (Pal et al. 2018; Sujitha and Kannan 2013; Thakkar et al. 2010).

### 3.2.1 Silver Nanoparticles (Ag NPs)

Silver nanoparticles have many important applications. It is used as an antimicrobial agent in textiles, cosmetics, and household appliances (Khalil et al. 2014). Nanosilver-sprayed bandages can be used to avoid bacterial infections in wounds (Aondona 2018). Also, these nanoparticles can be exploited in medicine for burn treatment (Rai et al. 2009). Silver nanoparticles are suitable for biological sensing and imaging. Due to their high conductivity, silver nanoparticles are applied in conductive inks and pastes for a range of electronic devices. Also, these nanoparticles are used as catalysts for accelerating some chemical reactions (Khalil et al. 2014).

### 3.2.2 Zinc Oxide Nanoparticles (ZnO NPs)

Zinc oxide nanoparticles have been widely used in many industries such as solar cells, photocatalysts, and pharmaceutical. It is used as a UV blocker in sunscreens (Sangeetha et al. 2011). Also, these nanoparticles, with a wide band gap (3.37 eV), are semiconductors for nano-electronic applications (Qu et al. 2011).

### 3.2.3 Titanium Nanoparticles ($TiO_2$ NPs)

Titanium nanoparticles possess optical, dielectric, antimicrobial, antibacterial, chemical stability, and catalytic properties which lead to industrial applications such as pigment, fillers, and photocatalyst (Sundrarajan and Gowri 2011).

The photocatalytic activity of titanium nanoparticles is ideal in antimicrobial applications including air and water purification and also antimicrobial coatings on biomedical devices. Also, the US Food and Drug Administration (FDA) has approved the use of these nanoparticles in human food, drugs, and cosmetics (Fernando et al. 2018).

### 3.2.4 Copper Nanoparticles (Cu NPs)

Copper nanoparticles, due to their unique physical and chemical properties and low cost of preparation, have been of great interest recently. These nanoparticles have industrial use such as catalytic processes, high-temperature superconductors, and solar cells (Honary et al. 2012). Furthermore, copper nanoparticles, due to their antimicrobial properties, have been used for coating of medical devices (Fernando et al. 2018).

### 3.2.5 Gold Nanoparticles (Au NPs)

Gold nanoparticles have been used at atomic force microscopy and surface plasmon resonance imaging (Iravani 2011). Also, gold nanoparticles have been studied as potential drug and gene delivery systems in cancer therapy (Fernando et al. 2018).

Based on all of the above, the synthesis of metallic nanoparticles is an active area of academic and, more importantly, “application research” in nanotechnology, and there is a great interest in synthesized nanoparticles due to their extraordinary properties.

## 3.3 Synthesis of Nanoparticles

Various methods can be employed for the synthesis of nanoparticles. These methods are divided into two main classes (bottom-up and top-down approach). In top-down method, larger molecules are decomposed into smaller units, which are then converted into suitable nanoparticles (destructive). Also, bottom-up method synthesizes the material from atomic or molecular species via chemical reactions; therefore this approach is also called building up approach. Some important manufacturing methods used in nanoparticle synthesis are showed in Fig. 3.1 (Khan et al. 2017).

Physical methods (arc discharge, physical vapor condensation, energy ball milling method, etc.) generally employ high temperatures, and the disadvantages of this method include the required space, time, and high energy. The absence of solvent contamination and the uniformity of the distribution of nanoparticles are the advantages of the physical methods in comparison with chemical processes. Chemical method of synthesis can be subdivided into chemical reduction, electrochemical, and pyrolysis methods. Chemical methods have the advantage of higher yield compared to physical methods (Fernando et al. 2018; Siddiqi et al. 2018).

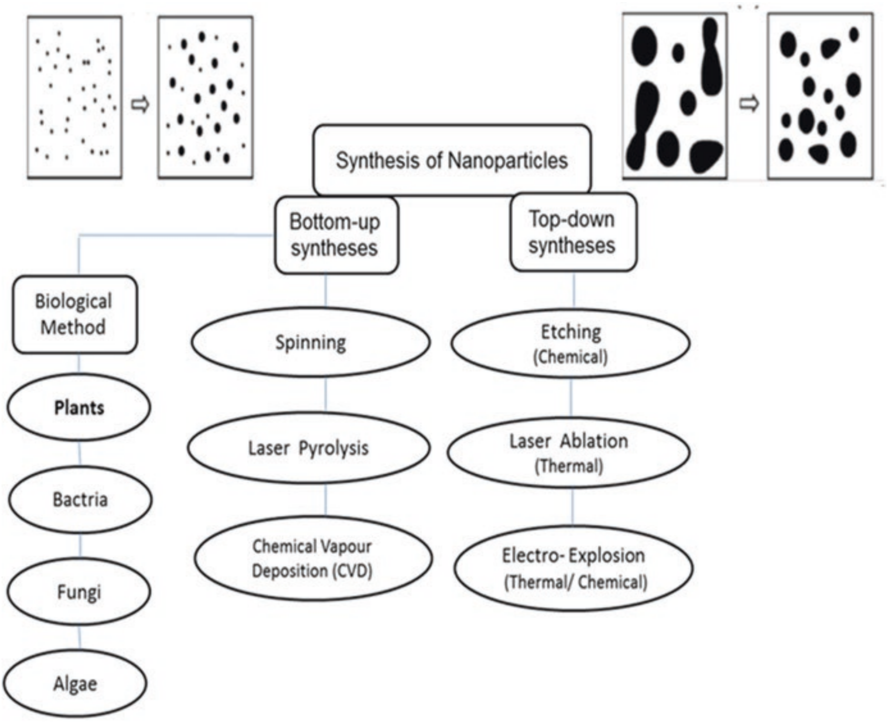

**Fig. 3.1** Manufacturing methods used in nanoparticle synthesis (Khan et al. 2017)

Generally, although physical and chemical methods are more popular in the synthesis of nanoparticles, they have limitations such as increase cost of production, release of hazardous by-products, long time, high pressure, difficulty in purification, and not suitable for biological applications. Therefore, development of nontoxic and eco-friendly methods for synthesis of nanoparticles is required (Nasir et al. 2016).

## 3.4 Biosynthesis of Nanoparticles Using Plants

Bio-nanotechnology has emerged in developing biosynthetic and environment-friendly technology for the synthesis of nanomaterials (Rai et al. 2009). Biosynthesis of nanoparticles using biological agents such as plant extract, bacteria, fungi, yeast, and algae has gained popularity in nanotechnology (Siddiqi et al. 2018).

Developing of biosynthetic has added much importance because of its eco-friendly products, biocompatibility, and economic potential and also to avoid adverse effects during their application especially in the medical field (anticancer activity, drug delivery, etc.) compared with physiochemical nanoparticles (Sundrarajan and Gowri 2011). Additionally, plants and microorganism constituents act as stabilizing and capping agents and do not require stabilizing agents (Singh et al. 2016). Among the various biosynthetic methods, the use of plant extracts has advantages such as readily available, safety in handling, and possessing a broad variety of metabolites (Sundrarajan and Gowri 2011). Also, because of their cost-effective and environment-friendly nature, they are easily used for large-scale synthesis and in this method there is no need to use high pressure, energy, and toxic chemicals (Prasad and Elumalai 2011). Plant systems are suitable and reliable for high demand in nanoparticle synthesis with applications in the biomedical and environmental areas (Singh et al. 2016).

Various parts of plants, such as leaves, stems, roots, shoots flowers, barks, and seeds, have been successfully used for the biosynthesis of nanoparticles (Siddiqi et al. 2018). The dry form of plants compared to fresh is preferable due to different content of water in various plant parts (Mohamad et al. 2014).

Recently, nanoparticle synthesis was achieved using plant extracts of *Azadirachta indica*, *Camellia sinensis, Nyctanthes arbor-tristis, Coriandrum, Nelumbo nucifera, Ocimum sanctum, Alfalfa, Lemon grass, Emblica officinalis, Aloe vera,* and several others which are compatible with the green chemistry principles (Sangeetha et al. 2011; Patidar and Jain 2017).

However, there are certain limitations in using plants. The heterogeneity of the size and shape of nanoparticle synthesis of plants hinder their use in specific applications. Also, efficient extraction and purification of nanoparticles from plants is a difficult method with a low recovery (Makarov et al. 2014).

### 3.4.1 The Role of Plant Metabolites in the Reduction of Metal Ions

The mechanism of nanoparticle synthesis by plants includes (a) the activation phase by the reduction of metal ions, (b) the growth phase that the small nanoparticles spontaneously coalesce into larger particles, and (c) the termination phase determining the final shape (Makarov et al. 2014).

The synthesis of nanoparticles can be done by reducing metal ion using some chemical molecules of plants containing amino acids, organic acid, fats, proteins, nucleic acids, reducing sugar, and types of secondary metabolites, such as flavonoids, alkaloids, polyphenols, heterocyclic compounds, and polysaccharides, which have significant roles in metal salt reduction and, furthermore, act as capping and stabilizing agents for the synthesis of nanoparticles without producing any toxic by-product (Siddiqi et al. 2018; Makarov et al. 2014). In other words, the hydroxyl and carboxylic groups in plants may act as stabilizing agents in the synthesis of nanoparticles (Mohamad et al. 2014). Figure 3.2 shows the flowchart of the biosynthesis of nanoparticles using plants.

Examples of the main types of compounds capable of reducing metal ions are shown in Fig. 3.3.

**Terpenoids** are a class of organic polymers synthesized in plants. Eugenol is the main terpenoid of *Cinnamomum zeylanisum* (cinnamon) extracts that plays an essential role in the bioreduction of tetrachloroauric acid ($HAuCl_4$) and silver nitrate ($AgNO_{3)}$ solution to nanoparticles.

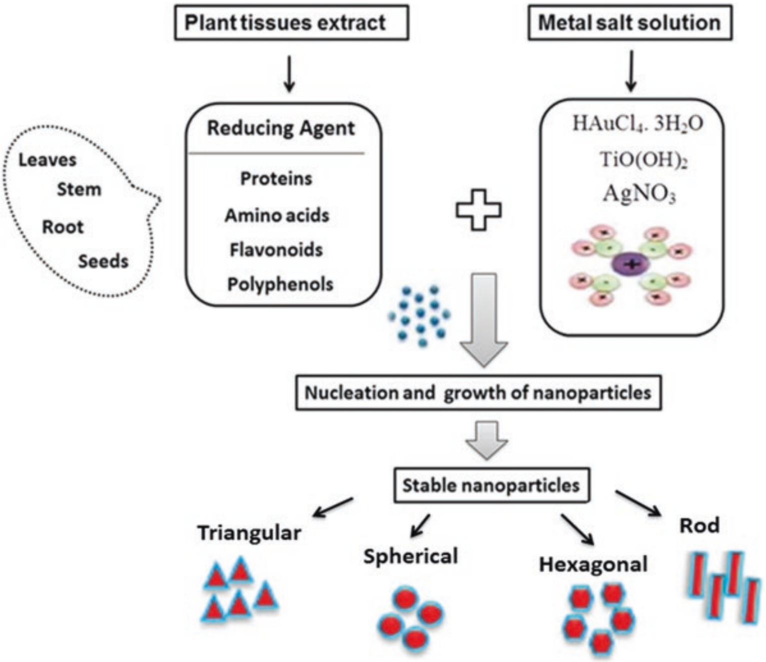

**Fig. 3.2** Flowchart representing the biosynthesis of nanoparticles using plants

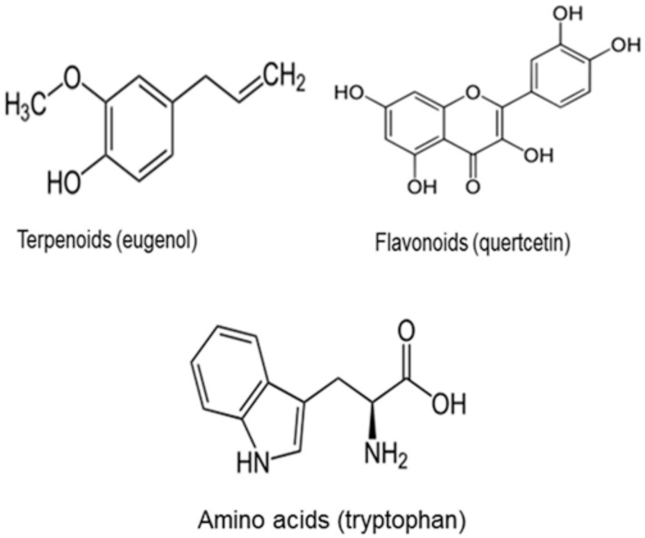

**Fig. 3.3** The main types of plant metabolites involved in the synthesis of nanoparticles

**Flavonoids** are a large group of polyphenolic compounds that comprise several classes, such as anthocyanins isoflavonoids, flavonols, and chalcones which can reduce metal ions into nanoparticles. The tautomeric transformations of flavonoids from the enol to the keto form release a hydrogen atom that can reduce metal ions.

**Amino acids** can bind to metal ions through the amino and carbonyl groups of the chain, such as the carboxyl groups of glutamic acid, a nitrogen atom of the imidazole ring of histidine or thiol groups of cysteine; studies have shown amino acids such as lysine cysteine, arginine, and methionine are capable of binding silver ions and aspartate can reduce $HAuCl_4$ solution to form gold nanoparticles.

Also the sugars present in plant extracts can induce the formation of metal nanoparticles, for example, monosaccharides containing a keto group (e.g., fructose) have tautomeric transformations from a ketone to an aldehyde (Singh et al. 2016).

### 3.4.2 Factors Affecting the Biological Synthesis of Nanoparticles Using Plants

The reduction process of metal ions besides the nature of a plant extract containing reducing agents with different concentrations, dependent on the pH, incubation temperature, reaction time, salt concentration, mixing ratio, and electrochemical potential of a metal ion plays a key role in nanoparticle synthesis. Each of these factors is briefly discussed as follows (Singh et al. 2016; Makarov et al. 2014):

**Influence of pH**

The pH of a plant extract is one of the influential factors and different nanoparticles can be synthesized at different pH values. A change in pH results in a charge change in an extract, which affects their ability to bind and reduce metal ions, and also it causes change in shape, size, and yield of nanoparticles synthesized (Makarov et al. 2014).

Larger nanoparticles can be produced at a lower pH values compared to high pH values. For example, synthesized gold nanoparticles using *Avena sativa* plant were in larger size (25–85 nm) at pH 2 and smaller size (5–20 nm) at pH 3 and 4. In other words, more accessible functional groups at pH = 3–4 within the plants extract were available for nanoparticle nucleation (Shah et al. 2015).

**Influence of Reactant Concentration**

The studies showed that by varying the amount of leaf extract in the reaction medium could significantly influence the shape and size of the nanoparticles. In the study, by changing the *Aloe vera* leaf extract concentration, hexagonal, triangular, and spherical gold nanoparticles in different sizes (50–350 nm) have been produced (Shah et al. 2015).

**Influence of Reaction Time**

The reaction time is an important factor in synthesizing nanoparticles. The study revealed that increasing the reaction time (30 min and 4 h) in the biosynthesis of Ag nanoparticles using *Azadirachta indica* leaf extract tended to produce particles with increasing size (10–35 nm) (Shah et al. 2015).

#### 3.4.2.1 Influence of Reaction Temperature

Temperature is an important physical parameter in the formation of nanoparticles in plant.

In general, temperature increases the reaction rate and efficiency of nanoparticle synthesis.

In other words, higher rate of reduction at higher temperature is due to the consumption of metal ions in the formation of nuclei. Temperature is an important factor in determining the size and shape of nanoparticles synthesized via plant. In the research, by raising reaction temperature (25–60 °C) in the synthesis of silver nanoparticles using *Citrus sinensis* peel extract, the average particle size decreased (35–10 nm) (Makarov et al. 2014; Shah et al. 2015; Punjabi et al. 2015).

## 3.5 Major Nanoparticles Synthesized by Plant Extracts

### 3.5.1 Biosynthesis of Silver Nanoparticles

**Olive Leaf Extract**

Khalil et al. synthesized the silver nanoparticles (20–25 nm) with the bioreduction method using aqueous *olive leaf* extract and stock solution of silver nitrate. The reduction process $Ag^+$ to $Ag^0$ nanoparticles was followed by the color change of the solution from yellow to brownish-yellow to deep brown by changing the contact time, concentration, temperature, and pH. Figure 3.4 shows UV-visible spectra of

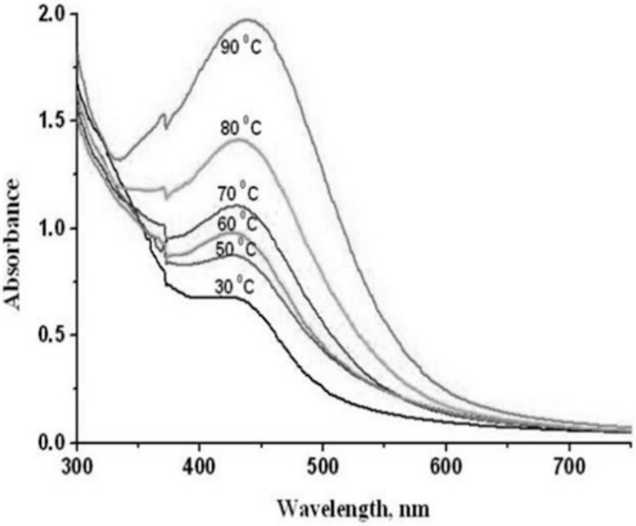

**Fig. 3.4** UV-vis spectra of Ag NPs as a function of temperature

the Ag NPs prepared at different temperatures. It can be seen that the absorbance increases with raising temperature. In other words, increasing the reaction temperature led to a rapid reduction of the $Ag^+$ ions.

Also, results indicated that the reduction of silver ions was increased at elevated pH due to increased activity of olive leaf extract constituent. As a result, the number of nucleus and size of the silver nanoparticles decreased with increased pH of the reactions (Khalil et al. 2014).

### *Telfairia occidentalis* Leaf Extract

Aondona et al. synthesized the silver nanoparticles using dry fluted pumpkin (*Telfairia occidentalis*) leaf extract as reducing agent and 0.01 M silver nitrate solution. The produced nanoparticles had an average size of 30 nm and angular and cubicle shape (Aondona 2018). The green synthesis of nanoparticle includes three steps: (i) selection of solvent, (ii) the reducing agent, and (iii) the nontoxic capping agent (Nasir et al. 2016). The work has shown that dried fluted pumpkin leave extract can be used as a reducing agent without any capping to produce nanoparticle. Due to the eco-friendly method of nanoparticle biosynthesis, these can be used on wound bandage (Aondona 2018).

### *Thuja occidentalis* Leaf Extract

Barua et al. synthesized polyethylene glycol-stabilized colloidal silver nanoparticles (7–14 nm) using the reductive potency of *Thuja occidentalis* leaf extract under ambient conditions. The silver ions in the polyethylene glycol matrix are stabilized by electrostatic interaction. The leaves contain considerable amounts of tannic acid, fenchone, and isothujone as the main components. Also, it includes carvotanacetone, myrcen, and camphen that may be directly related to the excellent reducing potential of the leaf extract. The silver ion is reduced by −OH group of the active components of the leaves, which is oxidized to carbonyl group.

The peak at around 420 nm (pH = 8) in the UV-vis spectrum was observed which indicates the formation of silver nanoparticles (Fig. 3.5) (Barua et al. 2013).

Kurian et al. synthesized the silver nanoparticles (20–50 nm) using *Curcuma longa* (turmeric) and *Zingiber officinale* rhizome extracts and silver sulfate solution. The reaction was completed at 3 h using turmeric rhizome as reducing agent and it reported one of the fastest biosynthesis route. Silver nanoparticles showed greatest antibacterial activity against *Staphylococcus aureus* pathogen (Kurian et al. 2016).

### *Ocimum sanctum* Leaf Extract

Singhal et al. synthesized the stable silver nanoparticles using *Tulsi (Ocimum sanctum)* leaf extract. It was observed that *Tulsi* leaf extract can reduce silver ions into silver nanoparticles within 8 min of reaction time. Thus, this method can be used for rapid and eco-friendly biosynthesis of stable silver nanoparticles in the medical industry. The TEM images of the prepared silver nanoparticles are shown in Fig. 3.6. It was observed that silver nanoparticles were circular in shape with maximum particles in range size within 4–30 nm (Singhal et al. 2011).

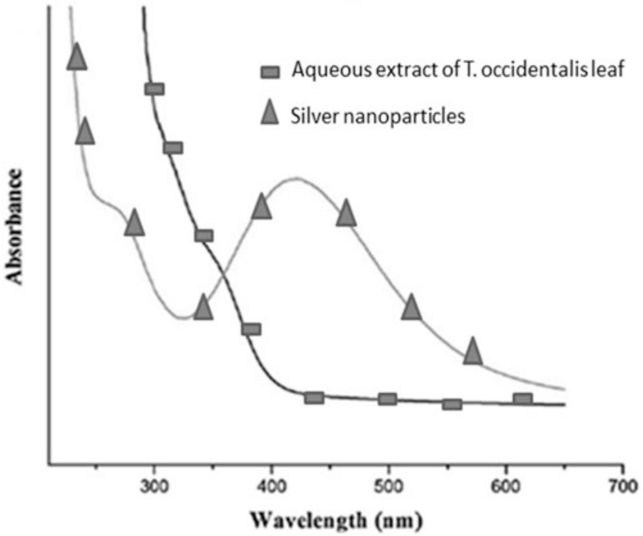

**Fig. 3.5** UV-vis spectra showing the formation of the PEG/Ag nanoparticle *Curcuma longa*/*Zingiber officinale* extracts

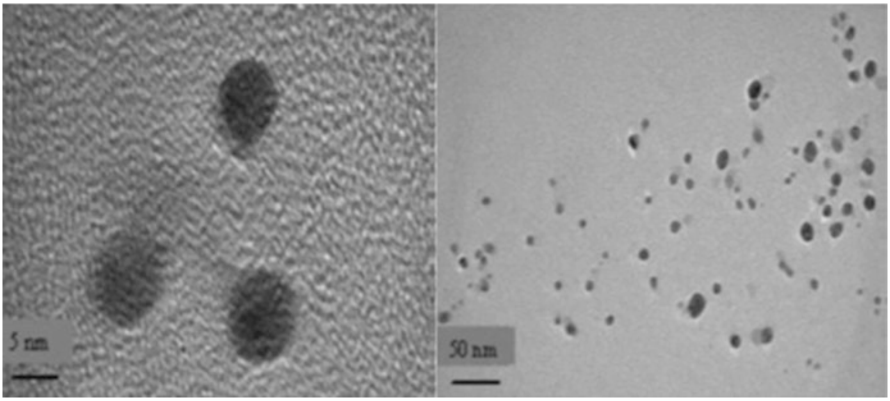

**Fig. 3.6** TEM images of biosynthesized silver nanoparticles

Ahmad et al. synthesized silver nanoparticles (~10 nm) from the dried stem and root of basil plant. The size of the nanoparticles derived from the stem (~62%) is more uniform than those derived from the root (~30%). Rosmarinic acid and flavonoids like luteolin, apigenin, and isoeugenol were the most abundant components identified in basil. The formation of enol form of the luteolin liberates reactive hydrogen which is responsible for the conversion of $Ag^+$ to $Ag^0$ (Fig. 3.7a and b). Also, there is liberation of reactive hydrogen in the keto form of the rosmarinic acid

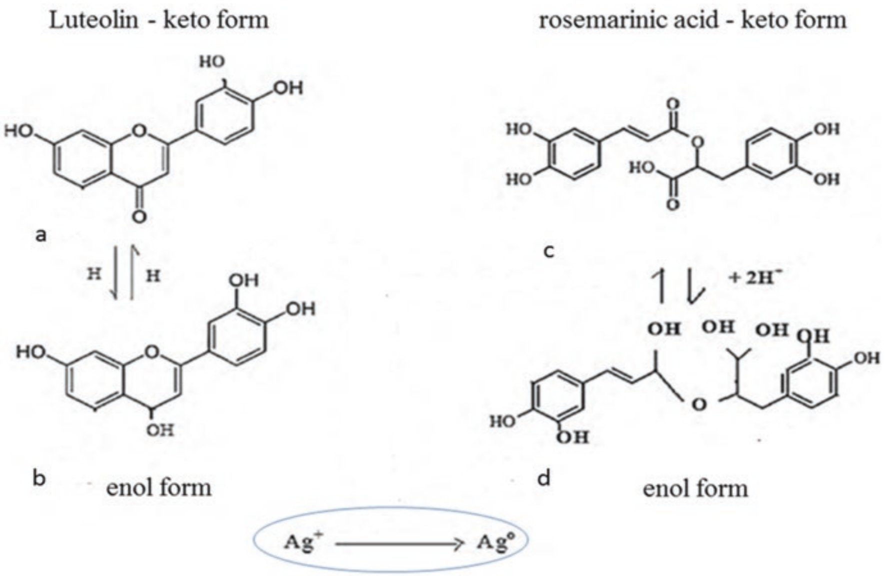

**Fig. 3.7** Mechanism of biosynthesis of Ag nanoparticles

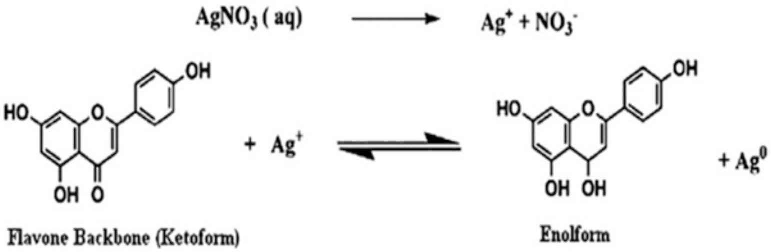

**Fig. 3.8** Mechanism of biosynthesis of silver nanoparticles

to unstable enol form (Fig. 3.7c and d). Thus, in both of them the reactive hydrogen is liberated which participates in the synthesis of silver nanoparticles (Ahmad et al. 2010).

*Ficus benghalensis* **Leaf Extract**

Saxena et al. developed a green synthetic method for silver nanoparticles using *Ficus benghalensis* leaf extract which acts as a reducing and capping agent. *F. benghalensis* plant leaf contains high levels of flavonoids. Phenolics and other chemicals within the plant leaf extract reduce silver salts (Fig. 3.8). Also proteins and enzymes present in the extract facilitate the formation of pure nanoparticles by reduction of the metal ions.

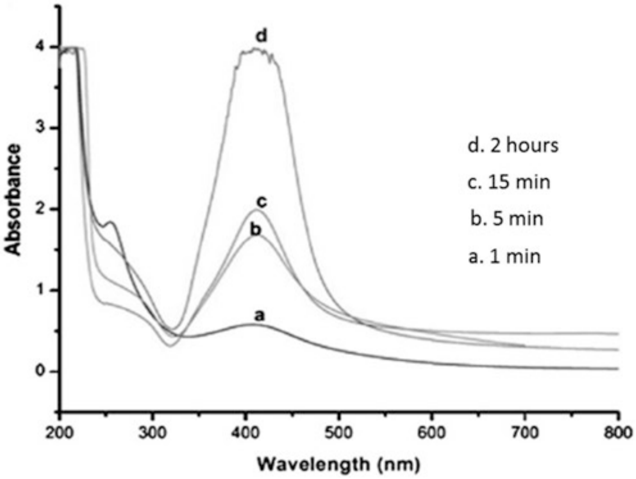

**Fig. 3.9** UV-vis spectra showing absorption recorded as a function of time

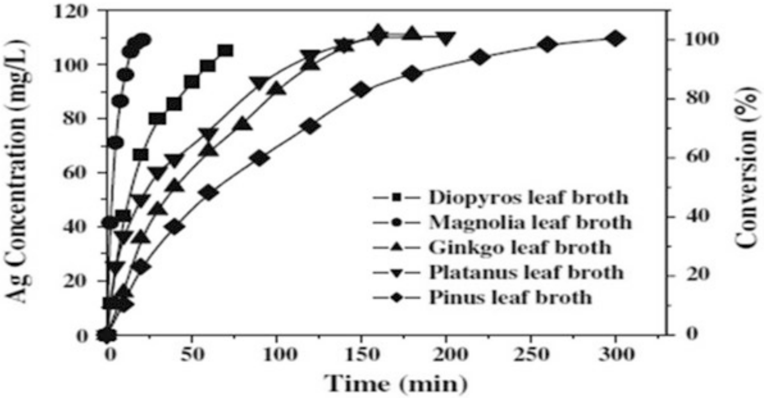

**Fig. 3.10** Time courses of silver nanoparticle formation obtained from various plant leaf extracts

UV-vis spectra were recorded as function of reaction time (Fig. 3.9). The results showed that after 5 min the surface plasmon resonance of silver occurs at 410 nm. So, the use of *Ficus benghalensis* extract is a fast method for the synthesis of silver nanoparticles and reduces silver ions without using any harsh conditions (Saxena et al. 2012).

### Pine, Persimmon, Ginkgo, Magnolia, and Platanus Leaf Extract

Song et al. synthesized silver nanoparticles (average particle size ranged from 15 to 500 nm) from the five plant leaf extracts (pine, persimmon, *Ginkgo, Magnolia*, and *Platanus*). *Magnolia* leaf extracts are the best reducing agent in the synthesis of silver nanoparticles (11 min, 95 °C) (Fig. 3.10).

This method of silver nanoparticle production can potentially be used in various human contacting areas such as cosmetics, foods, and medical applications (Song and Kim 2009).

### 3.5.2 Biosynthesis of Gold Nanoparticles

**Olive Leaf Extract**

Khalil et al. synthesized gold nanoparticles using olive leaf extracts as reducing agent and $HAuCl_4$. $3H_2O$ solution. The high phenolic content of the hot water extract of olive leaves helped in the reduction of $Au^{3+}$ to Au nanoparticles. The TEM images showed that a mixture of shapes (triangular, hexagonal, and spherical) was formed at lower leaf concentration and high pH, while smaller spherical shapes were obtained at higher concentration and low pH (Fig. 3.11) (Khalil et al. 2012).

***Pelargonium graveolens*** **Leaf Extract**

Shankar et al. reported the use of *geranium* leaves (*Pelargonium graveolens*) in the synthesis of gold nanoparticles. The reducing and capping agents in gold nanoparticles synthesized using geranium leaves appear to be terpenoids. The nanoparticles grown showed a variety of shapes that included rods, flat sheets, and triangles (Fig. 3.12).

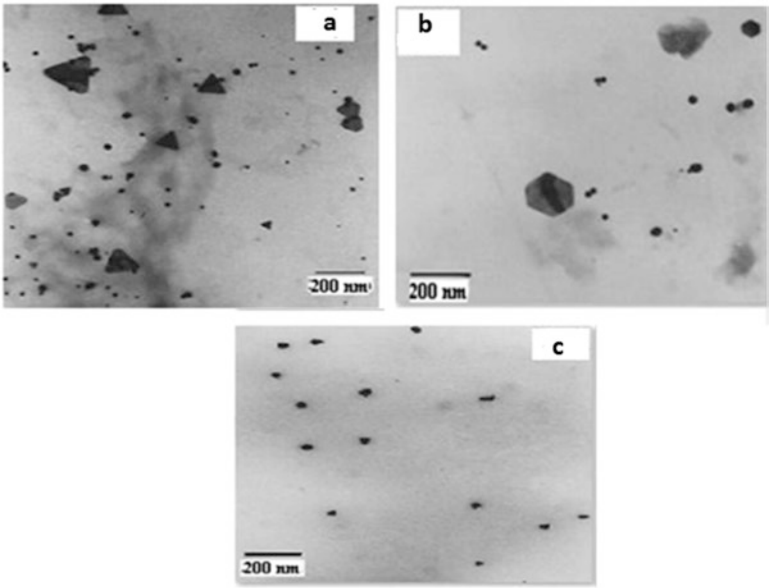

**Fig. 3.11** Effect of the leaf extract quantity on the size and shape of the gold nanoparticles. (**a**) TEM image measured at 0.5 ml (triangle shapes), (**b**) 0.5 ml (hexagonal shapes), (**c**) 5 ml of extract

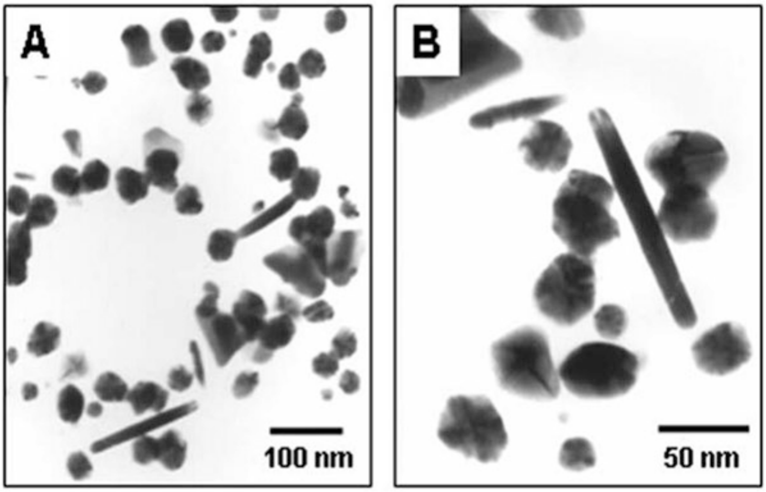

**Fig. 3.12** TEM images of gold nanoparticles

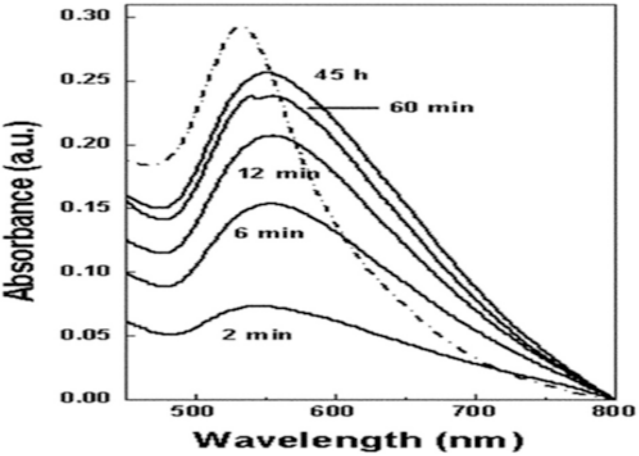

**Fig. 3.13** UV-vis spectra recorded as a function of time of reaction

Also, the results showed that the gold surface plasmon resonance occurs at 547 nm after 2 min (Fig. 3.13) (Shankar et al. 2003).

### Honey

Philip synthesized gold nanoparticles (~15 nm) using honey as reducing and capping agent. The reducing agent is fructose, and the capping material responsible for stabilization is proteins present in honey (Philip 2009).

**Citrus Fruit Extract**

Sujitha et al. reported a novel method for the biosynthesis of gold nanoparticles through the reduction of aqueous $AuCl_4$ ions using citrus fruit extract (*C. limon, C. reticulata,* and *C sinensis*). The rich source of citric acid and ascorbic acid in the citrus fruit extract may be responsible for the reduction of metal ions and stabilization of synthesized nanoparticles.

TEM studies showed the particles to be of various shapes and sizes (15–80 nm) that could be changed by altering the concentration of citrus extract. At lower concentrations of the extract, different shapes of nanoparticles, while at higher concentrations spherical particles are observed (Sujitha and Kannan 2013).

### 3.5.3 Biosynthesis of Palladium Nanoparticles

***Cinnamomum camphora*** **Extract**

Yang et al. synthesized palladium nanoparticles (3.2–6.0 nm controlled by initial concentration of the palladium ions) using *Cinnamomum camphora* leaf without capping agent. The polyol components and the heterocyclic components were responsible for the reduction and stabilization of palladium nanoparticles, respectively. Reaction between the hydroxyl group of the polyols in the *C. camphora* leaf broth and the palladium ions is shown below (Yang et al. 2010).

$$\mathrm{nPd}(\mathrm{II}) + 2\mathrm{R} - (\mathrm{OH})_n \rightarrow \mathrm{nPd}(0) + 2\mathrm{nR} = \mathrm{O} + 2\mathrm{nH}^+ \tag{3.1}$$

**Coffee and Tea Extract**

Mallikarjuna et al. synthesized the palladium nanoparticles (in the size range of 20–60 nm) using coffee and tea extract at room temperature. Caffeine and polyphenols in the extracts can form complexes with metal ions and reduce them to the corresponding metals (Nadagouda and Varma 2008).

***Cinnamomum zeylanicum*** **Bark Extract**

Sathishkumar et al. synthesized the palladium nanoparticles (15–20 nm) using an extract of *Cinnamomum zeylanicum* bark and palladium chloride ($PdCl_2$) solution. This palladium nanoparticle biosynthesis is performed under a moderate pH (=5) and room temperature (30 °C). Figure 3.14 shows a representative TEM picture of the palladium nanoparticles that were synthesized for 72 h (Sathishkumar et al. 2009).

### 3.5.4 Biosynthesis of Titanium Dioxide Nanoparticles

***Eclipta prostrata*** **Leaf Extract**

Rajakumar et al. synthesized titanium dioxide nanoparticles using *Eclipta prostrata* leaf extract and $TiO(OH)_2$ solution. The synthesized nanoparticles are quite polydisperse and range in size from 36 to 68 nm (Fig. 3.15).

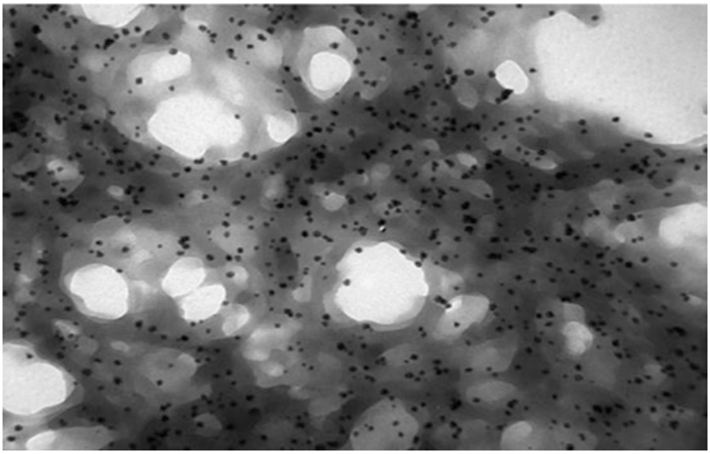

**Fig. 3.14** TEM image of palladium nanoparticles

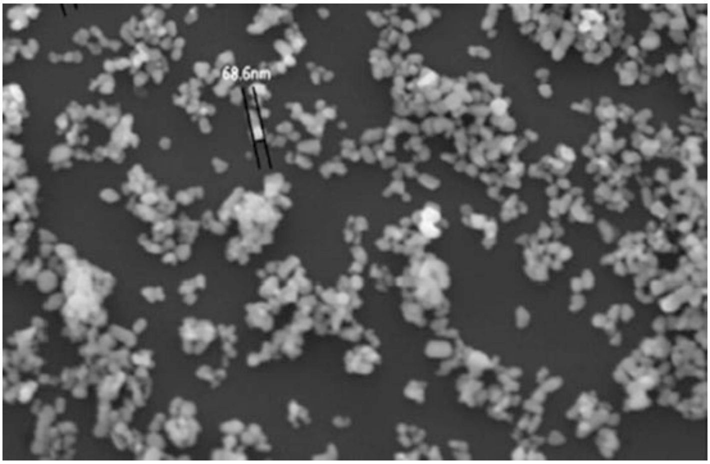

**Fig. 3.15** FESEM image of the $TiO_2$ nanoparticles

Alcohols, phenols, alkanes, and aliphatic amines in *Eclipta* may participate in the process of nanoparticle synthesis. Also, heterocyclic compounds such as flavones are the reducing and capping ligands of the nanoparticles (bioreduction of $TiO(OH)_2$ to $TiO_2$ (Rajakumar et al. 2012).

### *Moringa oleifera* Leaf Extract

Patidar et al. synthesized titanium dioxide nanoparticles using *Moringa oleifera* leaf extract and titanium tetraisopropoxide solution. Anatase phase $TiO_2$ nanoparticles with mean crystalline size of 12.22 nm and band gap of 3.9 eV at wavelength 318 nm was obtained by UV-visible spectroscopy (Sundrarajan and Gowri 2011).

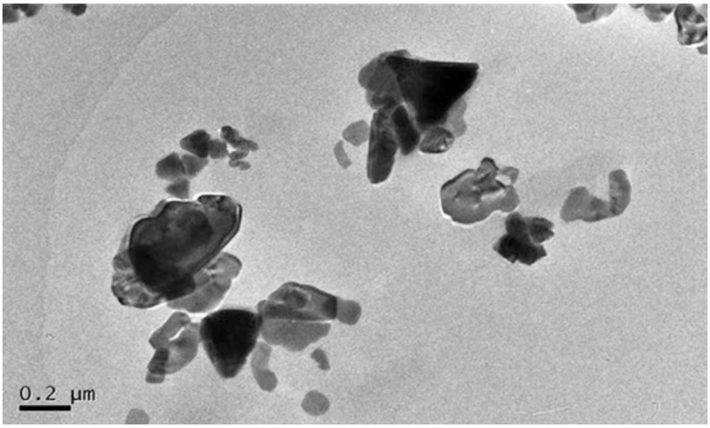

**Fig. 3.16** TEM image of synthesized zinc oxide nanoparticles

*Vigna radiata* **Seed Extract**

Chatterjee et al. synthesized oval titanium dioxide nanoparticles using *Vigna radiata* (green gram) legume extract. *Vigna radiata* has been confirmed to be a good source for the biosynthesis of titanium nanoparticles. *Vigna radiata* seeds were chosen for the experiment because seeds have strong reducing capacity. The particles were found to be effective against pathogens and can be further used in antibiotic drugs (Singh et al. 2016).

### 3.5.5 Biosynthesis of Zinc Oxide Nanoparticles

*Physalis alkekengi* **Extract**

Qu et al. synthesized zinc oxide nanoparticles using *Physalis alkekengi* plant. The TEM images showed that nanoparticles are not uniformly distributed and the morphology of most nanoparticles is triangular and elongated with a mean size of 72.5 nm (Fig. 3.16) (Qu et al. 2011).

*Aloe vera* **Leaf Extract**

Sangeetha et al. synthesized zinc oxide nanoparticles (25–40 nm) using *aloe vera* leaf extract and zinc nitrate. The nanoparticles obtained have been found to be predominantly spherical and the particle size could be controlled by varying the concentrations of leaf solution. Figure 3.17 shows the effect of leaf concentration on the nanoparticle synthesis. The rate of synthesis increased with increase in concentrations. Increasing the leaf concentration to more than 25% resulted to 100% conversion to nanoparticle after 6–7 h.

The zinc oxide nanoparticles prepared from *Aloe vera* leaf are expected to have extensive applications in biomedical and cosmetic industries (Sangeetha et al. 2011).

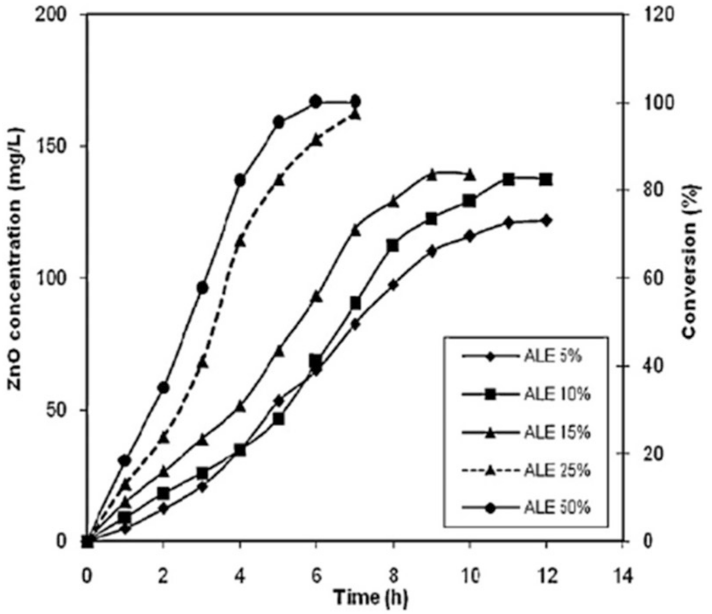

**Fig. 3.17** Effect of various concentrations of *Aloe* leaf extract (ALE) on the time course of nanoparticle synthesis

### 3.5.6 Biosynthesis of Iron Nanoparticles

*Camellia sinensis* **Extract**

Hoag et al. synthesized zerovalent iron (ZVI) nanoparticles using tea (*Camellia sinensis*) polyphenols and $Fe(NO_3)_3$ solutions. The tea extracts (polyphenols) used function as both a reducing and capping agent for iron. The reaction between polyphenols and ferric nitrate occurs within a few minutes at room temperature and is indicated by color changes from yellow to dark brown in the formation of iron nanoparticles (Hoag et al. 2009).

*Dodonaea viscosa* **Leaf Extract**

Daniel et al. reported the biosynthesis of zerovalent iron nanoparticles (27 nm) using the leaf extract of *Dodonaea viscosa*. The *Dodonaea viscosa* leaf contains flavonoids such as santin, saponins, alizarin, and tannins as active substances. The flavonoids help in the reduction of metal salts, and the tannins and saponins may act as the capping agents which prevents the further oxidation of nanoparticles. Further oxidation of nanoparticles (Fig. 3.18) showed reduction of metal into nanoparticles (Daniel et al. 2013).

*Aloe vera* **Extract**

Phumying et al. synthesized magnetite ($Fe_3O_4$) nanoparticles using ferric acetylacetonate ($Fe(C_5H_8O_2)_3$) solution and *Aloe vera* extract. The synthesized nanoparticles are crystalline and have particle sizes of ~6–30 nm. The XRD patterns of the

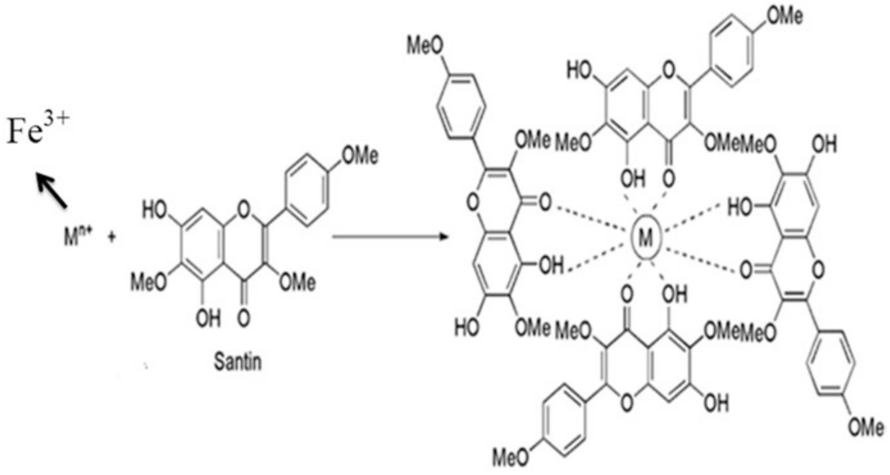

**Fig. 3.18** The reduction mechanism of metal into nanoparticles

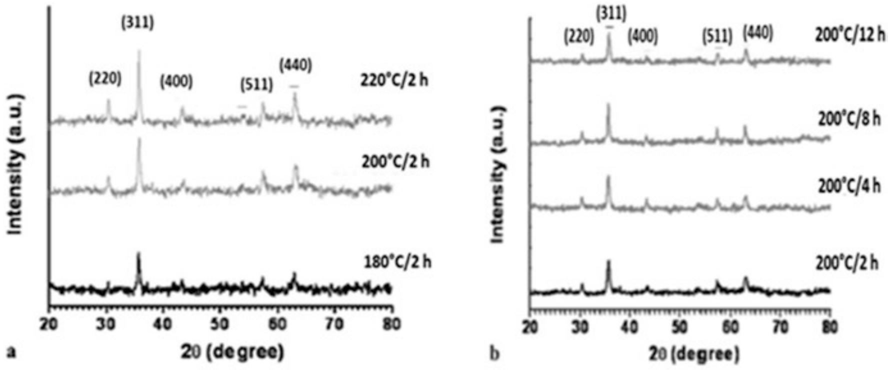

**Fig. 3.19** XRD patterns of $Fe_3O_4$ nanoparticles: (**a**) different hydrothermal temperatures and (**b**) different hydrothermal times

nanoparticles are shown in Fig. 3.19. No diffraction peaks of impurities were observed, which indicates that a high purity of final products was successfully under the experimental conditions. It was seen that the peaks become sharper and narrower along with the increased reaction temperatures and times indicating the enhancement of crystallinity (Phumying et al. 2013).

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# Green Synthesis Approach to Fabricate Nanomaterials

## 4

Ramchander Merugu

**Abstract**

Nanotechnology can overcome many technical hurdles due to their unique properties and small size compared to macrosize particles. Carbon and graphene are the major nanomaterial sources that are widely explored. They are widely produced by chemical, physical, and biological methods. Compared to the chemical and physical methods which are energy intensive, green approaches are economical and eco-friendly. Chemical synthesis includes the use of metal precursors and reducing agents which are generally toxic and non-eco-friendly. To prevent agglomeration of the synthesized nanoparticles, capping agents are also required. Physical methods are energy intensive and have many disadvantages. Biological methods do not require capping agents as the biomolecules present in it can act as capping agents. Different methods of synthesis using plants, microorganisms like bacteria and fungi, actinomycetes, algae, viral particles, and biomolecules have been reviewed in this chapter. Green fabrications have its own advantages and cover a lot of scope for potential applications in the future. The toxicity of the nanomaterial when it is released into the environment is the only cause of concern which needs to be researched further. In this chapter, various methods of fabrication of nanomaterials using biological methods are discussed in the light of existing literature.

**Keywords**

Synthesis · Characterization · Biological methods · Plant based · Microbial based · Green synthesis · Bacterial · Fungal · Biomolecules

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_4

## 4.1 Introduction

Feynman (1959) introduced the term "nanotechnology," which refers to the study of materials at nanoscale. Nanotechnology is that branch of interdisciplinary areas which deals with the study of nanomaterial and analysis of matter on an atomic or molecular scale. They show unique and extraordinary properties. Many different nanomaterials form a component such as sensing devices, therapeutic systems, and consumer-friendly goods such as wrinkle-resistant clothes and tubeless tires (Singh and Singh 2012). Hassan and Abul Kalam (2013) claimed that the biofuels are ten times easily biodegraded than renewable sources. Nanomaterials exhibit unique and improved properties as they have larger surface area to volume ratio. Nanotechnology aims at miniaturization of matter to achieve enhanced performance by enhancement of their functionalities. Nanotechnology offers solutions for overcoming potential drawbacks associated with these problems. Engelmann et al. (2013) proposed that the nanomaterials exhibit new and unique properties with applications in all areas of energy. Nanotechnology can also be used for generation, storage, and distribution of energy. Verma et al. (2013a, b) have envisaged the applications of nanomaterials in bioenergy and biosensors. Nanotechnology has a hidden potential which can bring a drastic change to meet the ever-increasing energy requirements. Easily available raw materials and inputs deliver no toxicity or chemical risks to the environment. Green nanoscience involves application of green chemistry principles to the design, development, and applications of nanomaterials. Nanoparticles possess different chemical, physical, and optical characteristics when compared to other materials of different sizes. At the nanoscale, properties of the particles change unpredictably, making them behave differently with the same substance at the macro scale. Nanoscale materials can be used efficiently for the oxidation of carbon-based biomass into carbon dioxide. Nanoparticles also convert small-chain fatty acids along with minute quantities of n-heptane and n-octane which are very useful as sources of energy. Both can be used for assessing the quality of petroleum and petroleum-based products.

## 4.2 Synthesis and Characteristics of Nanomaterials

Generally, two methods of synthesis are widely used, namely, the top-down and bottom-up synthesis approaches.

### 4.2.1 Top-Down Approach

In the top-down (physical) approach, nanoparticles are obtained from their bulk materials using irradiation, laser ablation, thermal decomposition, arc discharge, etc.

### 4.2.2 Bottom-Up Approach

In this technique chemical and biological approaches are used where nanoparticles are obtained from their basic building blocks which generate nanoparticles of the desired shape and size.

### 4.2.3 Chemical Approach

Generally, chemical synthesis includes the use of metal precursors, reducing agents, and stabilizing/capping agents. These methods are generally toxic and non-eco-friendly. Capping agents are used to prevent agglomeration of the synthesized nanoparticles. Carbon at nanoscales shows increased surface area, tenacity, elasticity, strength, and electricity. Nowadays, the focus is on conversion of lignocellulosic biomass into ethanol which reduces greenhouse gas emissions by 86% over fossils fuels. Fukuda et al. (2001) reported that biofuels can be obtained commercially from feedstocks such as animal fats, vegetable oils, and starch using transesterification and fermentation. Ongoing efforts are to produce biofuels from nonfood feedstocks which have many process-related problems. To overcome these potential barriers nanotechnology and nanomaterials stand as a possible solution.

## 4.3 Green Synthesis Approaches

Green-based methods for fabricating nanoparticles are being investigated for increasing the production rate of biofuels. Green fabrication of nanoparticles involves the use biomolecules like proteins, amino acids, carbohydrates, enzymes, and cells of microorganisms and plants. Green synthesis has received good attention as it is an inexpensive and eco-friendly way to fabricate nanoparticles. During the green synthesis process, modulation of the particle parameters such as size, chemical composition, and surface area is possible. To reduce metal toxicity, the microorganisms reduce the metallic compounds to nanoparticles. Prokaryotic as well as eukaryotic organisms can be used as nanofactories for different kinds of nanomaterials. Factors that influence the biosynthesis approach is pH, temperature, and incubation time. Sathishkumar and Narendhirakannan (2011) opined that the method of synthesis and incubation temperature determines the nature of the nanoparticle formed. Higher temperatures speed up the reaction rates, giving a better yield when compared to lower reaction temperatures (Sathishkumar et al. 2010). Rai et al. (2006) observed that there is less agglomeration at higher temperatures when compared to lower temperatures. In biogas generation the construction of the anaerobic digester is crucial for productivity. If the digester is not properly maintained, corrosion, wear, and tear decrease the output of biogas. These limitations can be overcome using nanomaterials at the construction stage. Application of nanomaterials incorporated in the form of photovoltaics can achieve excellent absorption of sunlight in the biogas plants which will make the process quicker, efficient, and more

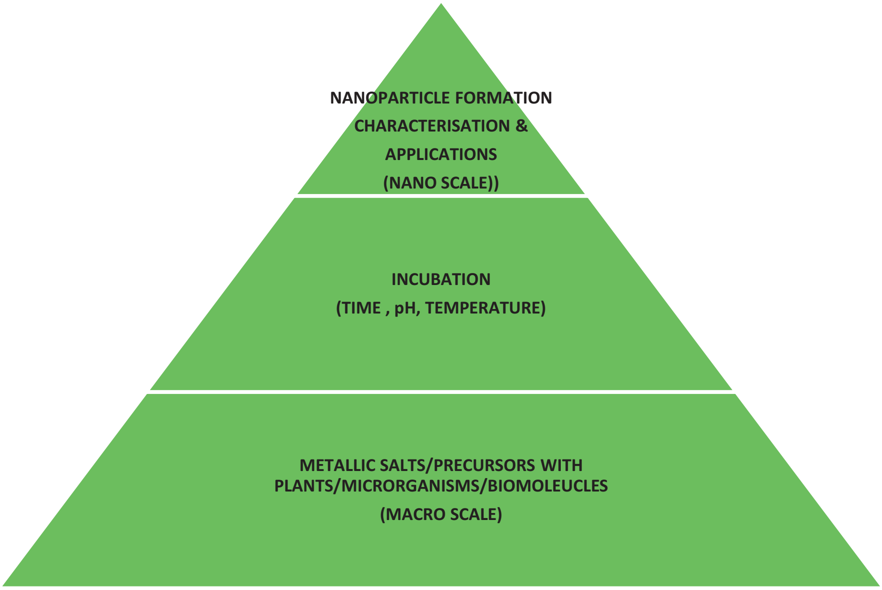

**Fig. 4.1** Green fabrication of nanoparticles

reliable. Lo et al. (2012) have reported improved production of biogas from micro-nano municipal solid waste incinerator fly ash and bottom ash. Green fabrication approaches are shown in Fig. 4.1, while the methods for characterization of synthesized nanoparticles are shown in Fig. 4.2.

## 4.4 Plant-Based Synthesis

Phytomining is a process where plants which hyperaccumulate metals are grown on metal-rich soils. These metal ions are converted into nanoparticles due to plant metabolites present in them (Rai and Yadav 2008). But it is a time-consuming process. Imran and Rani (2015) suggested that to overcome this, whole plant biomass or plant extracts are used for the synthesis. Alkaline pH was found to favor the formation of metal oxide nanoparticles (Singh et al. 2016). Zinc oxide is one of the major biofabricated nanoparticles due to its antimicrobial activity and photocatalytic activity, etc. Iron oxide metal nanoparticles also have been synthesized (Ahmed et al. 2017). Plant-based synthesis has an advantage of lack of pathogenicity when compared to bacteria or fungi (Pantidos and Horsfall 2014).

Datta et al. (2017) investigated the *Parthenium hysterophorus* for synthesis of zinc oxide nanoparticles with a particle size ranging between 16 and 45 nm. Using the aqueous extract of tomatoes, Sutradhar and Saha (2016) prepared zinc oxide nanoparticles. Sindhura et al. (2013) reported zinc nanoparticle fabrication using *Parthenium hysterophorus*. Sangeetha et al. (2011) investigated the production of nanostructure zinc oxide particles using *Aloe vera* leaf extract. Jain and Pradeep

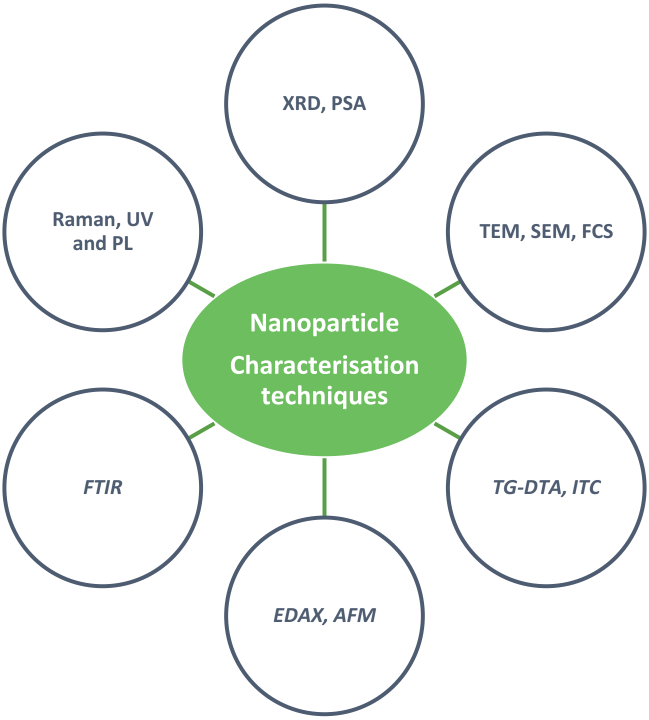

**Fig. 4.2** Methods for characterization of synthesized nanoparticles

(2004) stated that green fabrication method can also be used for large-scale synthesis. Green synthesis of nanoparticles is simple where biomaterials act as reducing agent. *Brassica juncea* and *Medicago sativa* were reported to accumulate 50-nm silver nanoparticles when grown with silver nitrate (Harris and Bali 2008). In *M. sativa* 4-nm-sized gold nanoparticles were formed (Gardea-Torresdey et al. 2002). Similarly in *Iris pseudacorus* copper particles sized 2 nm were formed (Manceau et al. 2008). The main mechanism for the green synthesis of silver nanoparticles is plant-assisted reduction due to alkaloids, flavonoids, terpenoids, amino acids, saponins, and phenols carbohydrates which act as reducing, capping, and stabilizing agents. Plant-mediated synthesis of nanoparticles is advantageous over other methods compared to microbial synthesis by eliminating the elaborative and complicated processes of maintaining microorganism cultures and scale-up problems. Sadhasivam et al. (2010) suggested the use of experimental procedures for the fabrication of green nanomaterials. Nadeem et al. (2017), Asha et al. (2016), Marimuthu et al. (2013), Jalill et al. (2016), and Singh (2016) have used biosynthetic approach for nanoparticle production. Biosynthesis of $TiO_2$ nanoparticles were synthesized using an eco-friendly approach by Pantidos and Horsfall (2014). Bao et al. (2012) have synthesized nanoparticles with controlled size and shape. Mittal et al. (2014a, b) considered plants to be better suited for synthesis of nanoparticles. Plant-based compounds such as phenolic acids, alkaloids, proteins, and carbohydrates can be used for the regulation, reduction, and stabilization of nanoparticles (Dobrucka 2017). Roopan et al. ( 2012) synthesized spherical $TiO_2$ nanoparticles using *Annona squamosa* at room temperature. Velayutham et al. (2012) investigated *Calotropis gigantea* and *Catharanthus roseus* leaf extracts for nanoparticle fabrication. Sundrarajan et al. (2017) reported bio-mediated $TiO_2$ nanoparticle formation from *Morinda citrifolia* leaf extracts with a size of 15 nm. Sivaranjani and Philominathan (2016) reported the synthesis of *Moringa oleifera* Lam. leaf extract mediated $TiO_2$ nanoparticles. Nickel nanoparticles have been used (Kidwai et al. 2006) to reduce aldehydes. Cobalt nanomaterials also play a role in changing the fatty acid chemistry. Rajiv et al. (2013) fabricated zinc oxide nanoparticles using *Parthenium hysterophorus* leaf extracts, whereas Ghidan et al. (2016) produced nanoplates. pH changes have a direct influence on the size of nanoparticles. Soni and Prakash (2011) stated that pH alteration causes a change in the secondary metabolites which help in reduction of the metal ions compared to acidic environments. The nanofabrication of nanoparticles using plants is presented in Table 4.1.

## 4.5 Bacterial Synthesis

Bacteria are prokaryotic organisms with faster growth rate. They can also be used as precursors for nanoparticle synthesis. The growth conditions can be easily controlled. Bacteria are also utilized in bioremediation and bioleaching. Magnetotactic bacteria and S-layer bacteria are also reported to be used for the synthesis of these nanoparticles (Pum and Sleytr 1999; Spring and Schleifer 1995). In order to overcome metal toxicity, bacteria produce proteins which bind with the metal ions and reduce them to nanoparticles. Saifuddin et al. (2009) established a synthesis approach for silver nanoparticle synthesis using *Bacillus subtilis* and microwave irradiation in water. Extracellular silver nanoparticle synthesis (~40 nm) with supernatant of *Bacillus licheniformis* was showed by Kalishwaral et al. (2008). Wei et al. (2012) used solar irradiation of *Bacillus amyloliquefaciens* extracts to obtain nanoparticles. Satyanarayana et al. (2010) predicted that proteins with a molecular weight between 25 and 66 kDa and 66 and 116 kDa induced the formation of gold nanoparticles and silver nanoparticles. Konishi et al. (2007a, b) investigated the bacterium *S. algae* which grew anaerobically in lactate medium at pH 2.0 for the production of gold nanoparticles within the bacterial cells and outside the bacterial cells. Liangwei et al. (2015) designed a Hb-coli-nAu-glassy carbon electrode for electrochemical deposition of gold nanoparticles. The effect of pH on gold nanoparticle formation using the bacteria *E.coli* and *D. desulfuricans* was also observed by Deplanche et al. (2010) where they concluded that in acidic pH, spherical gold nanoparticles and at neutral and basic pH, triangles, hexagons, and rod-shaped nanoparticles were formed. *Plectonema boryanum* UTEX 485 was shown to produce gold nanoparticles of desired morphology at temperatures between 25 and 200 °C as reported by Lengke et al. (2006). Klaus-Joerger (2001) opined that cobalt, chromium, and nickel metals could be introduced along with magnetite crystal nanoparticles. Jahn et al. (2005) discussed the mechanism for electron transfer and

**Table 4.1** Plant-mediated synthesis of nanoparticles

| Name of the plant | Nanoparticle synthesized | Size | Shape | References |
|---|---|---|---|---|
| *Aloe vera* | Au and Ag | 50–350 | Spherical, triangular | Chandran et al. (2006) |
| *Aloe vera* | $In_2O_3$ | 5–50 | Spherical | Maensiri et al. (2008) |
| *Camellia sinensis* | Ag, Au | 30–40 | Spherical, triangular, irregular | Nestor et al. (2008) |
| *Curcuma longa* | Pd | 10–15 | Spherical | Sathishkumar et al. (2009) |
| *Eucalyptus macrocarpa* | Ag | 10–100 | Spherical, cubes | Poinern et al. (2013a, b) |
| *Eucalyptus macrocarpa* | Au | 20–100 | Spherical, triangular, hexagonal | Poinern et al. (2013a, b) |
| *Diospyros kaki* | Pt | 15–19 | Crystalline | Song et al. (2010) |
| *Citrullus colocynthis* | Ag | 31 | Spherical | Satyavani et al. (2011) |
| *Avena sativa* | Gold | 5–20 | Rod-shaped | Armendariz et al. (2004) |
| *Brassica juncea* | Silver | 2–35 | Spherical | Haverkamp and Marshall (2009) |
| *Cinnamomum camphora* | Gold and silver | 55–80 | Spherical | Huang et al. (2007) |
| *Carica papaya* | Silver | 60–80 | Spherical | Mude et al. (2009) |
| *Citrus limon* | Silver | < 50 | Spherical, spheroidal | Prathna et al. (2011) |
| *Cycas* sp. | Silver | 2–6 | Spherical | Jha and Prasad (2010) |
| *Eucalyptus hybrid* | Silver | 50–150 | Crystalline, spherical | Dubey et al. (2009) |
| *Jatropha curcas* | Silver | 15–50 | Spherical | Bar et al. (2009) |
| *Ludwigia adscendens* | Silver | 100–400 | Spherical | Mochochoko et al. (2013) |
| *Mangifera indica* | Silver | 20 | Spherical, triangular, hexagonal | Philip (2011) |
| *Pelargonium roseum* | Gold | 2.5–27.5 | Crystalline | Shankar et al. (2003) |
| *Psidium guajava* | Gold | 25–30 | Spherical | Raghunandan et al. (2009) |
| *Pyrus* sp. *(pear fruit extract)* | Gold | 200–500 | Triangular, hexagonal | Ghodake et al. (2010) |
| *Psidium guajava* | Gold | 25–30 | Spherical | Raghunandan et al. (2009) |
| *Rhododendron dauricum* | Silver | 25–40 | Spherical | Mittal et al. (2012) |
| *Terminalia catappa* | Gold | 10–35 | Spherical | Ankamwar (2010) |

insoluble iron formation from iron-reducing bacteria. The marine bacterium *Desulfuromonas acetoxidans* was found to reduce Fe (III) and Mn (IV) by Roden and Lovley (1993). Kashefi and Lovley (2000) stated that *P. islandicum* reduced various metal salts at 100°C with an electron donor and electron acceptor. It was observed by these workers that this bacterium was not capable of reducing As (V) and Se (VII). Platinum nanoparticle formation of size 5 nm within 10 minutes at pH 7 and temperature 25 °C using *S. algae* was illustrated by Konishi et al. (2007a, b). The process could take place only in the presence of lactate. Yong et al. (2002) found that the platinum ions were adsorbed to the sulfate-reducing bacterium *D. desulfuricans*. A 90-kDa protein of *Tetrathiobacter kashmirensis* bioreduced selenite to elemental selenium (Hunter and Manter 2008). Both intracellular and extracellular formation of nanostructured selenium using *P. aeruginosa* SNT1 was reported by Yadav et al. (2008). *Bacillus selenitireducens* formed nanorods of elemental tellurium with 10-nm size (Baesman et al. 2007). *Bacillus* sp. BZ could produce elemental rod-shaped tellurium nanoparticles (Zare et al. 2012).

Holmes et al. (1995) have investigated the exposure of cadmium ions to *Klebsiella aerogenes* which produced intracellular CdS nanoparticle formation with size range between 20 and 200 nm. Similarly, Cunningham and Lundie (1993) observed that *Clostridium thermoaceticum* was able to form cadmium extracellularly in the presence of cysteine after 12-h exposure. Labrenz et al. (2000) found zinc sulfide particle formation of 2–5-nm diameter by sulfate-reducing bacteria. Periplasmic hydrogenases could be responsible for Pd (II) reduction resulting in the formation of palladium nanoparticles which were found to be inhibited by Cu (II) (Lioyd et al. 1998). Microbial Au (III) reduction was also investigated in *Thermotoga maritima*, *Geothermobacterium ferrireducens*, *Shewanella alga*, *Desulfovibrio vulgaris*, *Desulfovibrio desulfuricans*, and *Escherichia coli*. The natural product piperitone inhibited the formation of silver nanoparticles in *Klebsiella pneumoniae* which confirmed that nitroreductase enzymes play a major role in the formation of silver nanoparticles. Spherical platinum nanoparticles using cyanobacterium, *Plectonema boryanum*, were biosynthesized by Lengke et al. (2006). Riddin et al. (2009) reported the formation of platinum nanoparticles in which sulfate-reducing bacteria were used to understand the mechanism involved in platinum nanoparticle production. Prozorov et al. (2007) have used recombinant Mms6 protein for the formation of $Fe_2O_4$ nanocrystals. Microorganisms have also been suggested as nanofactories, for nanoparticle production (Mandal et al. 2006). Duran et al. *(*2005*)* highlighted the role of NADH and NADH-dependent nitrate reductase enzymes in metal nanoparticle fabrication. Several enzymes and their role in nanoparticles by microorganisms have been studied. Nangia et al. (2009) synthesized gold nanoparticles using a NADPH-dependent reductase enzyme. UV-vis absorption is used to understand the specific absorption of the prepared nanoparticle. Energy-dispersive X-ray (EDAX) analysis is used for studying the elemental composition of the synthesized nanoparticles. Electron microscopy techniques such as transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are used to study the size and shape of the nanoparticles. Fourier-transform infrared (FTIR) analysis reveals the specific chemical bonds which are involved in binding to the nanoparticles. X-ray diffraction (XRD) analysis is used for understanding the crystal nature of the nanoparticles. The nature and specific composition of nanoparticles can be understood by photon correlation spectroscopy (PCS) and atomic force microscopy (AFM). *Thermomonospora* sp. produced gold nanoparticles extracellularly (Ahmad et al. 2003). Gericke and Pinches (2006) highlighted the role of pH, temperature, substrate concentration, and exposure time in synthesized nanoparticle size. pH was known to influence gold nanoparticle fabrication by *Rhodopseudomonas capsulata*. Baia et al. (2009a, b) used TEM analysis to know the size of nanoparticles synthesized by *Rhodopseudomonas palustris.* Gold nanoparticle synthesis using *Yarrowia lipolytica* occurred in a pH-dependent manner (Agnihotri et al. 2009). UV-vis spectroscopy, XRD, and SEM were used for characterization of gold nanocubes synthesized by *Bacillus licheniformis* (Kalishwaralal et al. 2008). The nanofabrication of nanoparticles using bacteria is presented in Table 4.2.

**Table 4.2** Bacteria-mediated green fabrication of nanoparticles

| Bacteria | Nanoparticle | References |
|---|---|---|
| *Aeromonas* sp. *SH10* | Silver | Rai et al. (2006) |
| *Bacillus cereus* | Silver | Sunkar and Nachiyar (2012) |
| *Bacillus megaterium D01* | Gold | Wen et al. (2009) |
| *Bacillus subtilis 168* | Gold | Beveridge and Murray (1980) |
| *Bacillus subtilis* | Silver | Saifuddin et al. (2009) |
| *Clostridium thermoaceticum* | Cadmium sulfide | Cunningham and Lundie (1993) |
| *Corynebacterium* sp. *SH09* | Silver | Zhang et al. (2005) |
| *Desulfobacteraceae* | Zinc sulfide | Labrenz et al. (2000) |
| *Desulfovibrio desulfuricans* | Palladium and selenium | Kessi et al. (1999), Yong et al. (2002) |
| *Desulfovibrio vulgaris* | Gold, uranium, and chromium | Kashefi et al. (2001) |
| *Desulfovibrio magneticus strain RS-1* | Magnetite | Pósfai et al. (2006) |
| *Enterobacter cloacae* | Silver and selenium | Kessi et al. (1999) |
| *Escherichia coli MC4100* | Gold | Deplanche and Macaskie (2008) |
| *Geobacillus* sp. | Gold | Correa-Llantén et al. (2013) |
| *Klebsiella aerogenes* | Cadmium sulfide | Holmes et al. (1995) |
| *Klebsiella pneumonia* | Silver | Shahverdi et al. (2007) |
| *Lactobacillus strains* | Gold | Nair and Pradeep (2002) |
| *Magnetospirillum magnetotacticum* | Magnetite | Philipse and Maas (2002) |
| *Nocardiopsis* sp. *MBRC-1* | Silver | Manivasagan et al. (2013) |
| *Pseudomonas fluorescens* | Gold | Rajasree and Suman (2012) |
| *Pseudomonas putida NCIM 2650* | Silver | Thamilselvi and Radha (2013) |
| *Rhodopseudomonas palustris* | Cadmium sulfide | Bai et al. (2009a, b) |
| *Rhodobacter sphaeroides* | Zinc sulfide | Bai et al. (2006) |
| *Serratia nematodiphila* | Silver | Malarkodi et al. (2013) |
| *Shewanella algae* | Platinum | Konishi et al. (2007a, b) |

## 4.6 Fungus- and Alga-Based Synthesis

In fungus-mediated green synthesis, extracellular enzymes produced by them transform the metal ions to their counter metal oxide nanoparticles (Durán et al. 2011). Fungal production of nanoparticles is comparatively faster than bacterial synthesis as they produce more extracellular proteins. *Saccharomyces cerevisiae* was investigated for biofabrication of $TiO_2$ nanoparticles (Vainshtein et al. 2014). Zinc oxide nanoparticles from ZnNO3 were fabricated using *Aspergillus fumigatus* TFR-8 (Raliya et al. 2013). *Fusarium oxysporum* was reported to produce bismuth oxide (Jha and Prasad 2010). Algae are autotrophic and aquatic photosynthetic organisms which are unicellular and also multicellular in nature. Microalgal polysaccharides alginate, fucoidan, and laminarin are known to be involved in the formation of nanoparticles (Pinheiro et al. 2014; Lee and Lim 2014, 2015; Kimura et al. 2013). Govender et al. (2010) proposed that the hydrogenase enzyme from *Fusarium oxysporum* was involved in the formation of platinum nanoparticles. Yan et al. (2012) investigated yeasts for fabrication of zinc phosphate nanopowders with a size between 10 and 80 nm. The nanofabrication of nanoparticles using fungi is presented in Table 4.3.

## 4.7 Actinomycete-Based Nanoparticle Synthesis

Actinomycetes can also produce nanoparticles via extracellular or intracellular synthesis (Golinska et al. 2014). It was found that extracellular synthesis was the most preferred way of formation by these groups. *Rhodococcus* sp. could form nontoxic

**Table 4.3** Green fabricated nanoparticles using fungi

| Name of the organism/biomolecule | Nanoparticle synthesized | References |
|---|---|---|
| *Candida glabrata* | Cadmium sulfide | Krumov et al. (2007) |
| *Schizosaccharomyces pombe* | Cadmium sulfide | Dameron et al. (1989) |
| *Schizosaccharomyces pombe* | Cadmium sulfide | Kowshik et al. (2002) |
| *Schizosaccharomyces pombe* | Cadmium sulfide | Krumov et al. (2007) |
| *Pichia jadinii (Candida utilis)* | Gold | Pimprikar et al.(2009) |
| *Fusarium oxysporum* | Bismuth | Bansal et al. (2006) |
| *Fusarium oxysporum* | Cadmium | Kumar et al. (2007) |
| *Fusarium oxysporum f.* sp. *lycopersici* | Platinum | Govender et al. (2009) |
| *Fusarium* spp. | Zinc | Velmurugan et al. (2010) |
| *Aspergillus* | Mercury | Das et al. (2008) |
| *Fungi* | Zinc, magnesium, and titanium | Raliya and Tarafdar (2013) |
| *Penicillium fellutanum* | Silver | Kathiresan et al. (2009) |
| *Penicillium strain J3* | Silver | Maliszewska et al. (2009) |
| *Cladosporium cladosporioides* | Silver | Balaji et al. (2009) |

gold nanoparticles with size between 5 and 15 nm (Abdeen et al. 2014). Cell wall enzymes were reported to be involved in intracellular reduction of gold and silver ions to form metallic nanoparticles (Balagurunathan et al. 2011; Chauhan et al. 2013; Prakasham et al. 2012; Sukanya et al. 2013). Karthik et al. (2014) investigated *Streptomyces* sp. LK-3 for extracellular synthesis of silver nanoparticles and the various factors which could induce the production of these nanoparticles. This study revealed that NADH-dependent nitrate reductase enzyme was essential for the formation of silver nanoparticles via an electron transfer mechanism. Similarly, for gold nanoparticles it was found that nitrate reductase enzymes played a pivotal role in the reduction process (Shah et al. 2012).

## 4.8 Viral Particles for Nanoparticle Synthesis

Viral particles are being used for the past decade for the synthesis of nanomaterials. Silicon dioxide and zinc sulfide, cadmium sulfide, and iron oxide nanoparticles along with quantum dots have been synthesized by Lee et al. (2002) and Mao et al. (2003). They are particularly attractive due to the presence of capsomeres which form the capsid protein. These capsid proteins contain many functional groups and form a good surface for the formation of metal ions (Makarov et al. 2014). Tobacco mosaic virus, a plant virus, has about 2130 capsid protein molecules which can act as attachment points for the deposition of nanoparticles (Aljabali et al. 2010; Gorzny et al. 2010; Kobayashi et al. 2012; Royston et al. 2008). They can also be used to produce three-dimensional vessels of nanoparticles for drug delivery in pharmaceutical industry (Zeng et al. 2013; Steinmetz et al. 2009). Tobacco mosaic virus was able to reduce silver and gold salts before adding plant extracts of *Nicotiana benthamiana* or *Hordeum vulgare* thus resulting in lower-sized nanoparticles (Love et al. 2014). Some other studies investigated viruses as a template for the formation of nanowires and nanotubes (Merzlyak and Lee 2006).

## 4.9 Biological Derivatives for Nanoparticle Synthesis

Biological derivatives like cellulose were used to fabricate $TiO_2$ nanoparticles between 5- and 10-nm size by Farag et al. (2016). *Cylindrotheca fusiformis* peptide R5 has been used for fabricating $TiO_2$ nanoparticles (Sewell and Wright 2006). Padalkar et al. (2012) using alpha synuclein protein reported the fabrication of titanium nanowires. Titanium nanotubes were synthesized using bacterial flagella by Li et al. (2012). The lignocellulose waste material-mediated synthesis of $TiO_2$ nanoparticles was reported by Ramimoghadam et al. (2014). Using different enzymes Chen et al. (2010) reported synthesis of $TiO_2$ nanoparticles. Glucose oxidase enzyme produced rutile $TiO_2$ nanoparticles, while the enzyme catalase formed anatase $TiO_2$ nanoparticles. Chen et al. (2009) used lysozyme for anatase $TiO_2$ nanoparticle production. Shankar et al. (2003) investigated the role of terpenoids in silver nanoparticle formation using extracts from geranium leaves. *Cinnamomum* *zeylanisum* (cinnamon) extracts with eugenol could reduce silver nitrate and aurium chloride to their respective nanoparticles (Singh et al. 2010). Flavonoids comprise of anthocyanins, chalcones, flavones, isoflavonoids, flavonols, and flavanones were also found to bioreduce metal ions into nanoparticles. *Ocimum basilicum* extracts containing flavonoids resulted in the fabrication of silver nanoparticles (Ahmad et al. 2010). Gold and silver nanoparticle synthesis with a size between 21 and 30 nm was done with apigenin glycoside from *Lawsonia inermis* (Kasthuri et al. 2009). Monosaccharides such as glucose can act as reducing agents, while keto group containing sugars can act as antioxidants. Maltose and lactose have also shown to reduce metallic nanoparticles to metals (Panigrahi et al. 2004). Glucose and fructose were reported to be involved in fabricating metal nanoparticles of various morphologies (Panigrahi et al. 2004). Magnolia vine extract could reduce gold salts to gold ions (Shankar et al. 2003). Gruen (1975) observed that lysine, methionine, cysteine, and arginine could bind to silver ions. Saikat et al. (2002) reported that aspartate can reduce gold salt, while valine and lysine could not do so.

Spectral studies indicated that nanoparticles formed using plants or plant extracts are bound to proteins (Zayed et al. 2012). Tan et al. (2010) established that tryptophan is the strongest reducer of gold ions, while histidine only binds to gold ions (Glusker et al. 1999). Tan et al. (2010) demonstrated that amino acid sequence determines the ability to bind or reduce metal ions. Glusker et al. (1999) reported that peptides with some amino acids were ineffective in reducing gold salts. Synthetic peptide GASLWWSEKL reduces metal ions to form small nanoparticles of size 10 nm, while SEKLWWGASL sequences form larger nanoparticles of 40 nm in size (Glusker et al. 1999). Biological nanoparticles can be derived from biomolecules having a size range of 10 nm–1 μm (Kumar and Lal 2014). Polysaccharides such as pectin and alginates, dextran, insulin, and chitosan are being primarily used for the fabrication of nanomaterials (Sahdev et al. 2014). Albumin, elastin, collagen, gelatin, zein, gliadin and ferritin, sericin, and fibroin have been so far investigated for the production of nanoparticle (Nitta and Numata 2013; Hazeri et al. 2012) and (Zhao et al. 2015). DNA and RNA can also be used for the production of nanomaterials where the size of nanoparticles can be controlled. They can also be tagged with fluorophores, oligonucleotides, and aptamers (Panigaj and Reiser 2016). Lipid-based nanoparticles are promising due to their longer shelf-life and stability and have good encapsulation capacity which can also be used for drug delivery (Tamjidi et al. 2013; Weiss et al. 2008; Willner et al. 2007).

## 4.10 Green Nanocatalysts

Catalysts will provide the sites for the reactants to be activated leading to the formation of the products. Nanocatalysts have a nanoscale dimension, which could be used for overcoming mass transfer limitation and reducing the cost of biofuel production. Nanoscale catalysts can be divided into various types based on carbon, graphite, and metal oxides. Nanocatalysts enhance the selectivity of the reactions at a lower temperature with higher recycling rates and less energy consumption. Hence, there are more effective alternatives when compared to conventional catalysts for the efficient production of biofuels. Wen et al. (2010) reported that the solid base nanocatalyst KF/CaO could be used for biodiesel production with higher yields (Chaturvedi et al. 2012). Wen et al. (2010) demonstrated that the nanocatalyst KF/CaO has increased catalytic activity with enhanced stability. Sidra et al. (2016) investigated the transesterification process using CaO-$Al_2O_3$ nanocatalyst for biodiesel formation using Jatropha oil. Sivakumar et al. (2013) used smoke-deposited nanocatalyst magnesium oxide for the production of biodiesel. Three to five times higher conversion ratios were seen due to enhancement of surface area than those with conventional catalyst earlier reported. Biodiesel produced from castor oil and *Pongamia pinnata* oil with methanol was studied using nano-iron catalyst by Mookan et al. (2014a, b). Gupta and Agarwal (2016) prepared and characterized CaO nanoparticles from calcium nitrate (CaO/CaN) and snail shell (CaO/SS) to estimate the performance of the catalysts in terms of biodiesel yield from soybean oil. They concluded that the nanocatalyst from snail shell showed good catalytic activity and enhanced stability for the transesterification reaction. Zhang and Misra (2007) prepared CaO catalyst which was used for the transesterification of soybean oil into biodiesel. Deng et al. (2011) designed a hydrotalcite Mg/Al (3:1) nanocatalyst for transesterification of Jatropha oil. Acid nanocatalysts can be used for low-quality oil feedstocks with high FFA content. Acid catalysts catalyze esterification of free fatty acids and also transesterification of triglycerides for biodiesel production simultaneously (Canakci and Van Gerpen 2001; Zhang et al. 2003). Wang et al. (2015) prepared a magnetic acid catalyst for biofuel production. Bifunctional nanocatalysts promote simultaneous esterification and transesterification in a single step. A bifunctional quintinte-3T nanocatalyst was prepared for transesterification and as esterification promoter by Kondamudi et al. (2011). Palladium and molybdenum phosphate nanoparticles on silica were used for phenol hydrodeoxygenation (Hess et al. 2017; Löfstedt et al. 2016; Xing et al. 2017). Enumula et al. (2017)) reported production of alkyl levulinates from lignocellulosic feedstocks using alumina/SBA-15 heterogeneous catalysts. Nanoparticle immobilization can maintain enzymatic activity efficiently at low cost and higher stability (Dinçer and Telefoncu 2007; Ho et al. 2008; Wu et al. 2005); Liao et al. 2008, (Cherian et al. 2015; Liao et al. 2010). Verma et al. (2013a, b) immobilized nanoparticles with β-glucosidase on functionalized magnetic nanoparticles for biofuel production. Nanomagnetic solid base catalyst can improve biodiesel production up to 95% (Hu et al. 2011). Ren et al. (2011) have devised a method for lipase immobilization on polydopamine-coated magnetic nanoparticles. Rhodium particles were entrapped in carbon nanotubes to enhance catalytic activity (Pan et al. 2007).

## 4.11 Bioenergy Applications of Nanoparticles

Developing clean and efficient energy technologies has become the need of the hour due to the ever-increasing energy demand. Nanotechnology application in biofuel production mainly focuses on breaking down the feedstock more efficiently, decreasing the transportation cost of feedstock, and improving the yield of biofuels. Biodiesel production improved when nanocatalysts were used from animal fats and vegetable oils were used as feedstock (Jennifer and Peter 2006). In biofuel production, economic viability can be achieved by enzyme immobilization in nanomaterials. In industry, covalent linking of an enzyme to a support is the most interesting method of immobilization which is based on activation of chemical groups to support its reaction with protein nucleophiles (Ahmad and Sardar 2015; Mateo et al. 2007). With the dwindling energy sources, the problem of energy crisis looming at large, all countries are looking for alternative sources of their energy needs. The European Union has a target to increase its biofuel energy source to 5.75% by 2010 and 20% by 2020 without any substantial breakthrough. A study has indicated that the world's fossil fuel reserves would be depleted by the year 2050. In India, the need for exploring alternate sources of energy has become the first agenda in terms of investment issues. Indians have primarily depended on conventional energy sources, such as fossil fuels such as coal and petroleum, which has caused rapid depletion of fossil fuels with significant effects on the environment. Bioenergy sources are eco-friendly and pose no serious environmental risks. There is an increasing interest on bioenergy research using nanomaterial as the potential to increase the yield. The application of nanomaterials can contribute to potential bioenergy research. Bioresources are renewable as they are self-prevalent and ubiquitous and leave no toxic end products or residues.

Energy resources are rapidly depleting, and we are facing an energy crisis due to which we are forced to look for alternative sources of energy. Biofuel is an alternative source of energy which is sustainable and environment-friendly. As of now the biodiesel and biogas production are being investigated for this purpose. For this purpose nanomaterials are also being explored to increase the production of biofuels. Various methods of nanoparticle production are being improvised for efficient and sustainable production of nanoparticles. Along with enzyme-based nanocatalysts are being used to enhance the production of biofuels. Due to increasing fuel demand and emission of greenhouse gases, biomass-based biofuels have been gaining intense attention. Farrell et al. (2006) have suggested the need to search for alternative renewable energy sources. Biomass can be divided into two types, namely, oleaginous feedstock and carbohydrates. Triglycerides make up the largest portion of oleaginous feedstock and have some of the highest hydrogen to carbon (H/C) ratios, combined with relatively low amounts of oxygen. Carbohydrates form the most of the biomass available for conversion which includes mono-, di-, and polysaccharides. Biofuels can reduce the global dependency and global warming due to petroleum (Kumar and Sharma 2014; Lee et al. 2014). The first-generation biofuels have some limitations (Naik et al. 2010); therefore, lignocellulosic materials are being investigated (Eggert and Greaker 2014; Patumsawad 2011).

*Pseudomonas cepacia* lipase bound to FeO nanoparticle was effective in the production of biofuel. Among the explored nanomaterials, $ZrO_2$ showed the highest conversion and selectivity for n-C17 alkanes. Ni/ZrO2 catalysts transformed microalgal oil to liquid alkanes. Kandel (2013) reported that aminopropyl-functionalized Ni-MSN catalysts increased octadecane yield. Ni-supported zeolites were investigated by Peng et al. (2012) for conversion of microalgal oil. Various metal oxides including $SiO_2$ and $TiO_2$ have been investigated for lignocellulosic biomass conversion apart from enzymatic degradation and ball milling method (Cherian et al. 2015; Hendriks and Zeeman 2009; Liao et al. 2010). Nanotechnology application in biofuel production mainly focuses on breaking down the feedstock more efficiently, decreasing the transportation cost of feedstock, and improving the yield of biofuels. Biodiesel production improved when nanocatalysts were used for animal fats and vegetable oils were used as feedstock (Jennifer and Peter 2006). Mahmood and Hussain (2010) used spent tea in a three-step reaction for formation of biofuels using nanomaterials with *Aspergillus niger*. Wen et al. (2010) produced biodiesel using KF/CaO nanocatalyst from tallow seed oil. Qiu et al. (2011) prepared nanocatalyst for biodiesel production from soybean oil and methanol. Immobilized cellulase on silica nanoparticles was used for efficient breakdown of lignocellulosic biomass (Lupoi and Smith 2011). Polyacrylonitrile nanofiber immobilized with polycaprolactone for biodiesel production was investigated by Sakai et al. (2010). Tran et al. (2012) investigated biodiesel production by immobilizing lipase on ferric silica nanocomposites. Immobilized enzyme nanocatalysts showed biodiesel production higher than 90 % with high methanol tolerance and reusability when compared to other types of nanocatalysts. Cho et al. (2012) immobilized cellulose onto gold nanoparticles for ethanol production. In biofuel production, economic viability can be achieved by enzyme immobilization in nanomaterials (Ahmad and Sardar 2015; Mateo et al. 2007). Immobilization of β-glucosidase and cellobiase enzymes on nanomaterials for improved bioethanol production was also reported by several workers (Ansari and Husain 2012; Datta et al. 2013; Verma et al. 2013a, b). Bioethanol production using β-glucosidase immobilized on nanomaterials was reported by other workers (Tsai and Meyer 2014; Verma et al. 2013a, b; Zhang et al. 2013). Verma et al. (2013a, b) immobilized β-glucosidase from *Aspergillus niger* on functionalized magnetic nanoparticles. Zheng et al. (2013) also reported similar results. *Trichoderma reesei* cellulase immobilized on silica was used for pretreatment of biomass (Das et al. 2011). Abraham et al. (2014) immobilized cellulase from *Trichoderma reesei* onto nanoparticles for hemp hurd biomass hydrolysis. Cherian et al. (2015) used $MnO_2$ nanoparticle-immobilized cellulase for bioethanol production. Nanocatalysts (Johnson et al. 2009) increased the conversion efficiency of lignocellulose. Carbon nanotubes coated with nano-size rhodium and manganese were used to produce ethanol from carbon monoxide and hydrogen (Pan et al. 2007). Rhodium- and manganese-loaded carbon tubes enhanced activation of carbon monoxide and improved ethanol production rate.

Laccases are extracellular enzymes produced by fungi which degrade various cellulosic material and produce sugars and phenolic products. Nanostructured enzymes have about 1000 times half-lives when compared to native enzymes and can also be reused. Laccase is used in the pretreatment of agrowastes for biofuel production. Laccase are immobilized with various nanomaterials to enhance biofuel production. Lipase enzymes are extracellular or intracellular in nature. Lipase from *Thermomyces lanuginosa* covalently bound to ferric oxide nanoparticle was investigated using sunflower oil (Xie and Ma 2010) and waste grease (Ngo et al. 2013) for the production of biodiesel. Magnetite nanoparticles are the most investigated due to their low toxicity and good biocompatibility (Lie et al. 2011). Casals et al. (2010) have used nano-iron oxide of diameter 7 nm to enhance methane production. Murahashi et al. (1992) have successfully employed the nano-iron powder as a catalyst to carry out the oxidation of cyclohexane at room temperature with a conversion efficiency of 11% and a selectivity of 70% for the formation of cyclohexanol and cyclohexanone. Kesavan et al. (1999) have reported a conversion of 40% and a selectivity of 80% using nanophased catalytic particles of iron and nickel from the catalytic mixtures of iron carbonyl, Fe(CO)5, and nickel carbonyls. Nanotubes (Martone et al. 2011) and nanoporous materials which possess high aspect ratios have made the immobilization process easier for enzyme loading, biocatalyst recovery, and reusability (Misson et al. 2015). Covalent binding of enzymes with nanostructured supports are being used for long-term enzyme stability (Ovsejevi et al. 2013). Hussain et al. (2011) have used nickel- and cobalt-based nanoparticles as catalysts for biofuel production, and they have also shown excellent improvement in terms of the biofuel yield from the process. Nickel nanoparticles when employed in combination with cobalt particles result in better catalysis (Guo et al. 1995). Spent tea is a type of solid waste. It is chiefly composed of milk and sugar. *Aspergillus niger* can biologically reduce the spent tea to give a mixture of liquid extracts, solid charcoal, and gaseous compounds (Mahmood and Hussain 2010). To improvise the current method, cobalt or cobalt oxide nanostructures were used (Son et al. 2002). Studies on nanocatalysts have reported the extensive and exhaustive use of cobalt nanoparticles for the purpose of gasification of biomass. The various factors which contributed to biomass gasification were investigated by Patra and Baek (2014). Lestari et al. (2008) have reported the active role of cobalt nanoparticles in the tea extract in their attempt to make biodiesel as esters. Kondamudi et al. (2008) have accessed the biodiesel formation and efficiency of the biofuel obtainable from tea as well as coffee. The chief advantages of incorporating cobalt nanoparticles as catalysts in this bioconversion stem from high reactivity and high surface areas which resulted in the decrease of the reaction temperature and the corresponding activation energy requirement. Suarez et al. (2009) have reported that the biodiesel produced had low sulfur and nitrogen content, which is more eco-friendly. Nanofarming technology has proved to be very effective by enabling the synthesis of biofuels with the advantage of reuse of biocatalysts. Nanobiocatalysts, which are highly selective mesoporous membranes supplemented with nanoparticle adsorption, eliminate the problem of algal biomass getting killed after the extraction process is over. Removal of water in harnessing the biofuels is important as it disturbs the ethanol extraction by formation of azeotropic mixtures. Abdelsalam et al. (2016) investigated fresh raw manure using nickel, magnetite, cobalt, and iron nanoparticles for improving biogas yields.

Similar enhancement was found by Wang et al. (2016) who utilized waste-activated sludge for methane production using nZVI and Fe2O3 nanoparticles. Inorganic nanocatalysts such as (CaO, MgO), hydrotalcites, zeolites, zirconia, and sulfated oxides are reported for the synthesis of biodiesel. Titanium dioxide (Gardy et al. 2017), strontium oxide (Liu et al. 2007), calcium oxide (Liu et al. 2008), magnesium oxide (Verziu et al. 2008), and metal oxide nanocatalysts have been developed with high catalytic performance for biodiesel production. Gardy et al. (2018) proposed that magnetic nanoparticle-based catalysts are more advantageous due to their easier separation and reuse apart from being cost effective. Yahya et al. (2016) prepared different mesoporous nanocatalysts, with enhanced catalytic activities, for biodiesel production. For biodiesel production carbon-based nanocatalysts (Guan et al. 2017), carbon nanofibers (Stellwagen et al. 2013), graphene oxide (Mahto et al. 2016), and biochar (Dehkhoda et al. 2010) were found to have potential applications for biodiesel production using a wide range of feedstocks. Enzyme immobilization during lipase-catalyzed biodiesel and cellulosic ethanol production processes was investigated by Kim et al. (2018). Trindade (2011) explained the benefits of nanostructures which include large surface area for high enzyme loading, higher enzymatic stability, enzyme reusability, and reduction of the operational costs of biofuel production. Trindade (2011) also proposed nanoparticles as fuel additives to boost fuel blend performance. Zhang et al. (2013) stated that silica nanoparticles, single-walled carbon nanotubes, metal oxide nanoparticles, and nano-clay have been studied for lipid accumulation, extraction, and transesterification processes in biofuel production. Basha and Anand (2011) have suggested utilization of alumina nanoparticles and carbon nanotubes to enhance the combustion characteristics of biodiesel-operated engines with less harmful emissions. Ganzoury and Allam (2015) have observed enhanced methane production using nano-iron oxide, nano-bottom ash, nano-zerovalent iron, nano-fly ash, and bioactive nano-metal oxides under anaerobic condition. Nickel and cobalt nanocatalyst was useful for gasification of waste animal fat (Mahmood et al. 2010). Biomass thermal decomposition with catalyst showed gases like CO, $CO_2$, $CH_4$, and soot (Davidian et al. 2006). Gates (2000) have used molecular nanocatalysts which have the advantages of improved activity and lifetime resistance to poisoning (Mahmood and Hussain 2010). Size and structure influencing the efficiency of cobalt, silicon, nickel, and magnesium nanoparticles were investigated by different researchers (Kesavan et al. 2001; Son et al. 2002; Hussain et al. 2011). Cobalt nanoparticles are inexpensive and give higher yields of products within shorter duration (Mahmood et al. 2010). For gasification purposes, nickel catalyst was widely used (Kimura et al. 2006; Hussain et al. 2011). The size and shape influence the melting point of nanoparticles. Reduction of aromatic and other carbonyl compounds can take place using nickel nanoparticles (Kidwai et al. 2006; Mahmood and Hussain 2010). Mahmood et al. (2010) have opined that Ni nanoparticles can be used as efficient catalyst for reduction of complex aromatic and carbonyl compounds. TiO2 particles were found to have unique properties, less toxicity, and more stability (Kidwai et al. 2006).

**Table 4.4** Bioenergy applications of nanoparticles

| Nanoparticles used for biofuel production | Biofuel | References |
|---|---|---|
| Ni-supported zeolites | Diesel-range alkanes | Peng et al. (2012). |
| Nanometal oxides of SiO2, TiO2 | Biogas | Hendriks and Zeeman (2009), Liao et al. (2010) |
| KF/CaO nanocatalyst | Biodiesel | Wen et al. (2010). |
| PAN nanofiber immobilized with PCL | Biodiesel | Sakai et al. (2010) |
| Lipase immobilized on ferric silica nanocomposites | Biodiesel | Tran et al. (2012). |
| Gold nanoparticles | Ethanol | Cho et al. (2012) |
| β-Glucosidase and cellobiase enzymes immobilized on different nanomaterials | Bioethanol | Verma et al. (2013a, b) |
| β-Glucosidase immobilized on nanomaterials | Bioethanol | Zheng et al. (2013) |
| Magnetic nanoparticles | Sugars | Abraham et al. (2014) |
| Immobilized cellulase onto $MnO_2$ nanoparticles | Bioethanol | Cherian et al. (2015) |
| Cobalt nanoparticles | Biodiesel | Lestari et al. (2008) |
| Ni, Co, Fe nanoparticles | Methane | Abdelsalam et al. (2016) |
| Nano-iron oxide (Fe3O4 NPs) | Methane | Casals et al. (2010) |
| Nanocatalysts and sulfonic acid cobalt spinel ferrite magnetic nanoparticles | Biogas | Johnson et al. (2009) |

Lestari et al. *(*2008*)* used cobalt for preparing biodiesel as esters. Kondamudi et al. *(*2008*)* also have reported the formation of biodiesel from tea and coffee. All applications in the area of bioenergy are illustrated in Table 4.4.

## 4.12 Prospective Applications of Green Synthesized Nanoparticles

Silver nanoparticles fabricated using *Pinus thunbergii* extracts exhibited good antibacterial activity against pathogens of *Xanthomonas oryzae*, *Burkholderia glumae*, *Pseudomonas syringae*, and *Bacillus thuringiensis* (Velmurugan et al. 2013). Green synthesized silver nanoparticles from *Tridax procumbens* (tridax daisy) extract showed similar antimicrobial activity to nanoparticles against bacteria *Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae* (Dhanalakshmi and Rajendran 2012). Silver nanoparticles synthesized in *Iresine herbstii* showed good cytotoxic activity against various tumor cell lines of HeLa cells, while silver nanoparticles from *Euphorbia nivulia* extracts were toxic to the A549 cell lines (Valodkar et al. 2012). *Nerium oleander* synthesized silver nanoparticles which demonstrated larvicidal activity (Suganya et al. 2013). Silver nanoparticles from *Cyamopsis tetragonoloba* (cluster bean) were used to make a biosensor to determine ammonia (Pandey et al. 2012). *Ocimum sanctum* extracts generated platinum nanoparticles which were shown to possess catalytic activity in hydrogen generation (Soundarrajan et al. 2012). *Sesbania drummondii* gold nanoparticles were shown to reduce aromatic nitro compounds such as toxic 4-nitrophenol to 2-amino-phenol. Green synthesized nanoparticles have a functionalized surface and have organic ligands, proteins, polysaccharides, and polyatomic alcohols which cannot be obtained using physical and chemical methods. This makes green synthesized nanoparticles as promising catalysts in organic synthesis (Sintubin et al. 2012). Table 4.5 shows the applications of green fabricated nanomaterials.

## 4.13 Advantages and Disadvantages of Green Synthesis

In green synthesis of nanoparticles, the precursors are the most expensive followed by the substrates which have to be procured (Kurakhmaeva et al. 2009). Functionalization step of the nanoparticle is eliminated when we use a bionanocatalsyt unlike in physiochemical synthesis (Zhang et al. 2011). Phytochemicals, pharmacological metabolites, and enzymes act as stabilizers in plant- and microbial-based synthesized nanoparticles. Many researchers have explained that green synthesized nanoparticles play an important role in the medical field for diagnostic applications (Gunalan et al. (2012a, b). Jayaseelana et al. (2012) concluded that green methods of nanoparticle synthesis show better antibacterial effects. Copper oxide nanoparticles fabricated using *Malva sylvestris* have antibacterial, antifungal, and antiparasitic activities (Gusseme et al. 2010; Sanghi and Verma 2009; Velayutham et al. 2012). *Lactobacillus fermentum* was investigated for biogenic silver production which was used for the removal of viruses from drinking water. Chen et al. (2005) have explored small interfering RNA (siRNA) with quantum dots for producing fluorescent nanoparticles. Elechiguerra et al. (2005) reported that nanoparticles could readily bind to HIV-1. Some researchers have clearly stated that nanoparticles may cause cell wall and membrane damage (Chaloupka et al. 2010; Gopinath et al. 2014). They can also be used for bioremediation (Njagi et al. 2011). Dhandapani et al. (2012) have opined that green nanomaterials can be used for bioremediation purposes. Nanoparticles can be exploited for cleaning hazardous waste sites and pollutants (Kalaiselvi et al. 2015; Sankar et al. 2014). *Azadirachta indica*-fabricated titanium dioxide nanoparticles were investigated for photocatalysis by Singh and Naraa (2013). Similarly, other workers have reported that iron NPs can be used for treating water and soil remediation (Mahdavi et al. 2013). Management of plant diseases has also been reported with nanoparticles rather than with pesticides (Khot et al. 2012). Bacteria and diatoms produced magnetite and siliceous material which were reported to be useful for optical coatings (Joerger et al. 1999). Biosynthesized $TiO_2$ by *Psidium guajava* showed significant antibacterial and antioxidant effect (Santhoshkumar et al. 2014). Khan et al. (2015) showed that the antimicrobial activity of CuO-NPs and ZnO-NPs against *E. coli*, *B. subtilis*, and *S. aureus* was higher when compared to chemically synthesized nanoparticles. The size and shape of the nanoparticles and the compounds involved in their synthesis are unknown (Oberdörster et al. 2006).

**Table 4.5** Applications of green fabricated nanomaterials

| Name of the organism | Name of the nanoparticle | Applications | References |
|---|---|---|---|
| *Aloe barbadensis* | Gold and silver | Cancer, optical coatings | Chandran et al. (2006) |
| *Acalypha indica* | Silver | Antibacterial activity against pathogens | Krishnaraj et al. (2010) |
| Apiin extracted from henna leaves | Silver and gold | Cancer and optical coatings | Kasthuri et al. (2009) |
| *Azadirachta indica* | Gold, silver, and silver-gold alloys | Bioremediation of toxic metals | Shankar et al. (2004) |
| *Camellia sinensis* | Gold and silver | Catalysts and sensors | Mondal et al. (2011) |
| *Coriandrum sativum* | Gold | Drug delivery, tissue imaging, photothermal therapy | Narayanan and Sakthivel (2008) |
| *Cymbopogon flexuosus* | Gold | IR-absorbing optical coatings | Shankar et al. (2005) |
| *Garcinia mangostana* | Silver | Antimicrobial activity | Veerasamy et al. (2010) |
| *Gardenia jasminoides Ellis* | Palladium | Nanocatalysts for *p*-nitrotoluene hydrogenation | Jia et al. (2009) |
| *Syzygium aromaticum* | Gold | Destruction of cancer cells | Raghunandan et al. (2010) |
| *Medicago sativa* | Iron oxide | Cancer, drug delivery | Herrera-Becerra et al. (2008) |
| *Morus* | Silver | Antimicrobial activity | Singh et al. (2017a, b) |
| *Nelumbo nucifera* | Silver | Larvicidal activity | Santhoshkumar et al. (2011) |
| *Ocimum sanctum* | Gold and silver | Biolabeling, biosensor | Philip and Unni (2011) |
| *Pear fruit extract* | Gold | Catalysis, biosensing | Ghodake et al. (2010) |
| *Sedum alfredii* | Zinc oxide | Nanoelectronics | Qu et al. (2011) |
| *Bacillus mycoides* | Titanium | Green solar cells and antibacterial activity | Aenishanslins et al. (2014) |
| *Bacillus subtilis* | Titanium | Suppress aquatic biofilm growth | Dhandapani et al. (2012). |
| *Bacteria strains NS2 and NS6* | Lead sulfide | Bioremediation | Singh and Naraa (2013) |
| *Cassia alata* | Copper | Medicinal value | Jayalakshmi and Yogamoorthi (2014) |
| *Stigmaphyllon littorale* | Silver | Antibacterial activity | Kudle et al. (2013a, b, c) |
| *Tectona grandis* | Silver | Antibacterial activity | Nalvothula et al. (2014) |
| *Phototrophic bacteria* | Silver | Antibacterial activity | Manisha et al. (2014a, b). |

(continued)

**Table 4.5** (continued)

| Name of the organism | Name of the nanoparticle | Applications | References |
|---|---|---|---|
| *Allmania nodiflora* | Silver | Antimicrobial activities | Kudle et al. (2013a, b, c). |
| *Ixora coccinea* | Silver | Antitumor and antimicrobial activity | Nalvolthula et al. (2015a, b). |
| *Justicia adhatoda* | Silver | Antitumor activity on human epithelioid carcinoma cells | Kudle et al. (2014a, b) |
| *Acacia senegal* | Silver | Antibacterial activity | Kudle et al. (2014a, b) |
| *Citrus limon* | Silver | Antitumor activity | Nalvolthula et al. (2015a, b). |
| *Coriandrum sativum* | Silver | Antimicrobial activity | Manisha et al. (2014a, b). |
| *Sterculia foetida* | Silver | Antimicrobial activity | Kudle et al. (2013a, b, c) |
| *Toddy palm* | Copper and silver | Antibacterial activity | Deshpande et al. (2018) |
| Fruit pulp of Palmyra fruit | Silver and copper | Antibacterial, antioxidant activity | Chitturi et al. (2018) |
| Toddy palm | Silver and nickel | Antibacterial, antioxidant activity | Padala et al. (2019) |
| *Cassia auriculata* | Zinc oxide | Stabilizing and reducing agent for nanoparticle synthesis | Ramesh et al. (2014) |
| *Catharanthus roseus* | Titanium | Effective against *Hippobosca maculata* and *Bovicola ovis* | Velayutham et al. (2012) |
| *Euphorbia condylocarpa* | Palladium, iron | Recyclable catalyst | Nasrollahzdeha et al. (2015) |
| *Gloriosa superba* | Copper | Antibacterial activity | Naikaa et al. (2015) |
| *Camellia sinensis* | Zinc oxide | Effective against *Klebsiella pneumoniae* | Senthilkumar and Sivakumar (2014) |
| *Gum karaya* | Copper | Antimicrobial activity | Padil et al. (2013) |
| *Humicola* sp. | Copper | Medicinal applications | Khan and Ahmad (2013) |
| *Malva sylvestris* | Copper | Antimicrobial activity | Awwad et al. (2015) |
| *Phyllanthus amarus* | Copper | Antimicrobial activity | Acharyulu et al. (2014) |
| *Oryza sativa* | Titanium | Photocatalysts | Ramimoghadam et al. (2014) |
| *Raphanus sativus* | Silver | Antioxidant and antibacterial | Koyyati et al. (2013) |
| *Amaranthus viridis* | Silver | Antibacterial activity | Koyyati et al. (2014) |
| *Pleurotus florida* | Gold | Anticancer activity | Bhat et al. (2013) |

(continued)

**Table 4.5** (continued)

| Name of the organism | Name of the nanoparticle | Applications | References |
|---|---|---|---|
| *Acalypha indica* | Silver, gold | Cytotoxic activity | Krishnaraj et al. (2014) |
| *Rosa damascena* | Silver | Anticancer activity | Venkatesan et al. (2014) |
| *Eucalyptus chapmaniana* | Silver | Antimicrobial and cytotoxic activity | Sulaiman et al. (2013) |
| *Dracocephalum kotschyi* | Gold | Anticancer, antibacterial | Dorosti and Jamshidi (2016) |
| *Inonotus obliquus* | Gold | Antibacterial, antioxidant, and cytotoxic activities | Lee et al. (2015) |
| *Quercetin and gallic acid* | Silver and selenium | Antitumor and antimicrobial activity | Mittal et al. (2014a, b) |
| *Gymnema sylvestre* | Silver and gold | Antioxidant and anticancer activities | Nakkala et al. (2015) |
| *Syzygium aromaticum* | Silver | Anticancer activity | Venugopal et al. (2017). |
| *Cissus quadrangularis* | Silver | Antimicrobial and anticancer potentials | Renugadevi et al. (2012). |
| *Ganoderma neo-japonicum Imazeki* | Silver | Antitumor activity | Gurunathan et al. (2013) |
| *Dioscorea bulbifera* | Platinum and palladium | Anticancer and antioxidant activities | Ghosh et al. (2015) |
| *Pleurotus ostreatus* | Silver | Anticandidal and anticancer activities | Yehia and Al-Sheikh (2014) |
| *Gracilaria edulis* | Silver and zinc oxide | Anticancer activity | Priyadharshini et al. (2014) |
| *Borago officinalis* | Silver | Anticancer and antibacterial activities | Singh et al. (2017a, b) |
| *Corallina officinalis* | Gold | Cytotoxic activity | Kassas and El-Sheekh (2014) |
| *Dimocarpus longan* | Silver | Antibacterial activity and anticancer | He et al. (2016) |

## 4.14 Future Directions and Conclusions

Nanotechnology can overcome many technical hurdles due to their unique properties and small size compared to macrosize particles. Carbon and graphene are the major nanomaterial sources that are being widely explored. Only few kinds of nanomaterials such as nanotubes and nanofibers, nanoplates, and nanocrystals are being studied. Multi-walled nanotubes, nanocomposites, and other kinds of nanomaterials also need to be studied to enhance the effectivity and efficiency of the process. Recyclable, highly active, and selective heterogeneous nanocatalysts should be synthesized using biological approaches. Faster methods of synthesis, kinetics of nanoparticle formation, process parameter optimization, and nondestructive characterization which is nonexpensive should be understood for the production of nanomaterials. Catalysts normally employed in the synthesis of chemicals are expensive and have several disadvantages. Green synthesized nanocatalysts with intermediate characteristics between homogeneous and heterogeneous systems can be used effectively to overcome these disadvantages. Green-based syntheses of base nanocatalysts, acid nanocatalysts, and bifunctional nanocatalysts which are advanced biocatalysts have many applications for biofuel as well as high-density biofuel production. The potential prospects of this technology are still in a nascent stage and need to be explored with biological materials so that they can be effectively used for many applications. But due to their extremely small size, they are toxic to the environment. The risks should be properly investigated before going for optimization and large-scale production and subsequent applications of these particles.

**Acknowledgement** I would also like to show my gratitude to my teachers Prof. S.M. Reddy, Emeritus Professor, Kakatiya University, and Prof. S. Girisham, Kakatiya University, for sharing their pearls of wisdom with me during the writing of this chapter. I am immensely grateful to Prof. Katta Narasimha Reddy, former Vice-Chancellor, Mahatma Gandhi University, for encouraging me to write this chapter.

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# Nanomaterials: Types, Synthesis and Characterization

# 5

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**Abstract**

Nanoparticles are generally defined as particles having one or more dimensions of sizes ranging from 1 to 100 nm. Nanoparticles can be classified into organic, inorganic and carbon-based materials. In comparison with conventional micro-size particles, nanoparticles show enhanced properties, such as high reactivity, strength, surface area, sensitivity and stability due to their nanosize. Various preparation methods, viz. physical, chemical and mechanical, have been employed to synthesize different nanoparticles. This chapter presents an overview on nanoparticles and their types, properties, synthesis methods and application in bioconversion of biomass into biofuels.

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_5

**Keywords**
Nanoparticles · Synthesis · Characterization · Properties · Biofuel application

## 5.1 Introduction

Nanotechnology has been a topical subject in the academia and industrial communities for the past years (Zhang et al. 2013; Romero and Moya 2012; Margulis-Goshen and Magdassi 2012; Moreno-Vega et al. 2012). This technology offers a new dimension to a science of working and manipulating materials having a size of 100 nm or less at least in one dimension (Zhang et al. 2013). With respect to their nanosize, these materials are recognized by their unique properties, viz. physicochemical and mechanical properties such as reactivity, tenacity, elasticity, strength and excellent electrical and thermal conductivities. These nanomaterials can easily be employed in a wide range of fields including crop production, cosmetics, drug delivery, photonic crystals, analysis, food, coatings, paints, bioremediation, catalysis and material science. The nanomaterials' size, shape and morphology play a significant role on their properties; therefore, more research has been dedicated in finding means to fine-tune these properties (Romero and Moya 2012; López-Serrano et al. 2014). For example, gold (Au), platinum (Pt), silver (Ag) and palladium (Pd) nanoparticles all with a size of 20 nm have characteristic colours, i.e. wine red, yellowish-grey, black and dark black colours, respectively (Khan et al. 2017). The overall shapes can be 0D, 1D, 2D or 3D.

Nanotechnology can simply be defined as the science of designing, synthesizing and characterizing nanomaterials as well as their applications. Since the introduction of the term "nanotechnology", there has been a lot of reports based on the classification, preparation and characterization of nanomaterials. Different synthesizing routes have been developed in order to control the size, structure and morphology of the resulting nanomaterials. These include chemical vapour deposition (CVD), sol-gel technique and mechanochemical processes. The preparation route depends on the resulting nanoparticle type and properties and thus intended application. Inorganic nanoparticles such metal oxides are often produced via wet chemistry, sol-gel, chemical microemulsion, hydrothermal, solvothermal, microwave-assisted combustion, sonochemical and direct precipitation, whereas carbon-based nanomaterials are usually produced through techniques such as arc discharge (AD), laser ablation and chemical vapour deposition (CVD). In the case of graphene nanosheets, techniques such as chemical, mechanical and thermal exfoliation are used. Despite the success that has been made on the production of various nanomaterials, the use of green methods in order to produce these materials has been one of the major research activities for the past decade (Shao et al. 2018; Logeswari et al. 2015). In this case, the plant extract or natural material is either used as reducing or stabilizing agent for nanoparticle production. Chitosan and alginate were recently reported as a suitable stabilizing and reducing agent in synthesizing silver nanoparticles (AgNPs) (Shao et al. 2018; Logeswari et al. 2015; Mokhena and Luyt 2017a; Mokhena and Luyt 2017b).

Nanotechnology application in biofuel production provides very intriguing solutions to some challenges faced in this field. Nanomaterials can be used as favourable carriers to immobilize some catalyst in order to facilitate their recovery from liquid phase by filtration or centrifugation (Zhang et al. 2013). For instance, it was demonstrated that modified nanosphere silica realizes the extraction from alive microalgae without harm which avoids recultivation by sending the microalgae back for lipid accumulation (Lin et al. 2009). Moreover, nanoparticles can be used directly as heterogeneous catalysts in order to improve the biofuel conversion yields and resultant fuel quality (Wen et al. 2010). Nanoparticles such as CaO, $Al_2O_3$ and MgO are already being recognized as heterogeneous catalysts with conversion rate of more than 99% with less amount of oil (i.e. ˂1%) (Zhang et al. 2013; Wen et al. 2010; Venkat Reddy et al. 2006). There has been quite a lot of publications, reviews and book chapters covering the use of nanoparticles in order to facilitate the biofuel production and resultant fuel quality (Zhang et al. 2013; Lee et al. 2015). This chapter presents an overview on nanoparticles and their types, properties, synthesis methods and application in bioconversion of biomass into biofuels.

## 5.2 Classification

Nanoparticles can be classified either by source (natural or anthropogenic); chemical composition (organic or inorganic); synthetic route (biogenic, geogenic, anthropogenic and atmospheric); their shape, size or structure; or their applications (Romero and Moya 2012; Margulis-Goshen and Magdassi 2012; Khan et al. 2017; Cui et al. 2018). In this chapter, we reviewed the nanoparticles based on their chemical composition as discussed below. In general, the fabrication/preparation of the nanoparticles can either be "bottom-up" or "top-down" approach as schematically presented in Fig. 5.1. Bottom-up approach as reflected by its name involves building up NPs from single molecule or atom, e.g. sedimentation and reduction techniques. In this context, precursor or source is used as simple substance to synthesize NPs either by sedimentation or reduction. Examples include sol-gel, biosynthesis, spinning and pyrolysis. On the other hand, top-down approach involves breaking down bulk materials into smaller units which are further converted into suitable NPs. Examples include milling, CVD, physical vapour deposition and decomposition processes.

### 5.2.1 Organic Nanoparticles

Organic nanoparticles feature unique properties such as biodegradability, biocompatibility and high stability in biological fluids (Romero and Moya 2012; Margulis-Goshen and Magdassi 2012; Moreno-Vega et al. 2012). These particles are available in nature as protein aggregates, lipid bodies, milk emulsions or more complex organized structures such as viruses, etc. Organic nanoparticles have been used as a part of different industrial products, such as food (e.g. creams, chocolate and cakes) and

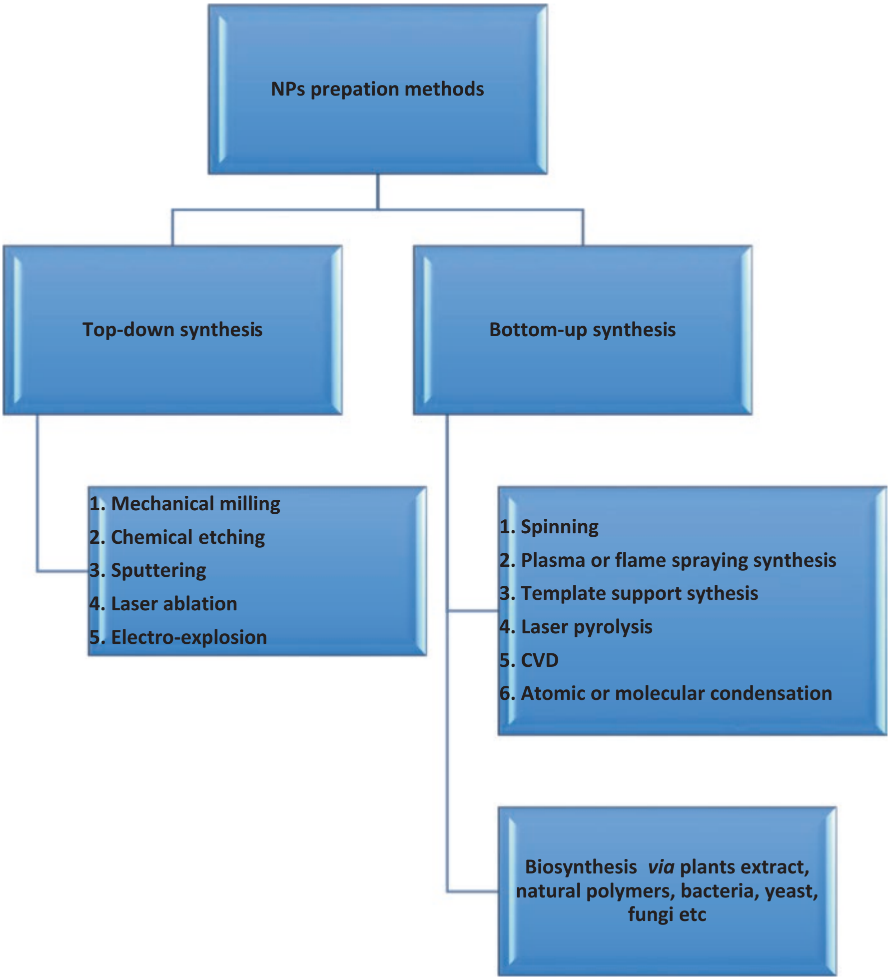

**Fig. 5.1** Schematic presentation of synthetic routes for NPs

cosmetics (as nanoemulsions), and in pharmaceutical formulations (viz. liposome vectors, polymersomes and polymer-protein and polymer-drug conjugates). Organic nanoparticles are often fabricated by both "top-down" and "bottom-up" approaches as discussed in the next sections (Romero and Moya 2012; Margulis-Goshen and Magdassi 2012; Cui et al. 2018).

#### 5.2.1.1 Synthesis of Organic Nanoparticles

The "top-down" approach such as milling, lithography and microfluidics is often used to physically reduce the size of bulk material into nanomaterials (Romero and Moya 2012; Rolland et al. 2005). Milling is the most used process especially for drug NPs made from poor water-soluble compounds. The limitations of the milling process include unavoidable contamination of the produced NPs and their wide size distribution. Elsewhere, it was demonstrated that a wet milling process can be employed to produce particles having sizes of 230 nm from poor water-soluble drug crystals (Sigfridsson et al. 2011). The obtained NPs were chemically stable for 10 months in both room temperature and when refrigerated and physically stable (particle size) for 10 months under refrigeration and 3 years at room temperature. Microfluidic and lithography are often employed in order to gain more control over the size and the shape of the resulting NPs. A very general top-down approach, particle replication in nonwetting templates (PRINT), was used to fabricate monodisperse particles with simultaneous control over structure (i.e. shape, size and composition) and function (i.e. cargo, surface structure) by Rolland et al. (Rolland et al. 2005). They prepared monodispersed NPs of poly(ethylene glycol diacrylate), triacrylate resin, poly(lactic acid) and pyrrole having sizes below 200 nm. Considering the compatibility of PRINT with other synthetic schemes (free radical polymerization, metal-catalysed high-temperature reaction, oxidative coupling using strong acids), it demonstrated its chemical flexibility and tolerance which is important for production of NPs for various applications.

Bottom-up approach is based on building NPs through physicochemical processes, starting from single molecule or atoms (Romero and Moya 2012). It involves single atom or molecule combining via synthetic chemistry and self-organization to produce various organic nanoparticles, such as micelles, vesicles and liposomes, polymersomes, polymer conjugates, dendrimers, capsules and polymeric NPs (Romero and Moya 2012).

## 5.2.2 Inorganic Nanoparticles

### 5.2.2.1 Metal Oxide and Metallic Nanoparticles

Metal and metal oxide nanoparticles include silver, silver oxide, copper oxide, magnesium oxide, ZnO, $Fe_3O_4$ and many more (Jamdagni et al. 2018). Metal oxide nanoparticles feature unique characteristics such as antifungal, antimicrobial, optical absorption, large surface-to-volume ratio, semiconducting and chemical-sensing properties. In addition, their non-toxicity and biocompatibility characteristics offer them opportunity to be applied in different fields such as biomedical, food packaging, biofuel conversion and agriculture. Several researchers have investigated the small-scale production of metal and metal oxide NPs using chemical, green, physical and a combination of these methods with some showing a great potential for large-scale production (Jamdagni et al. 2018) (Table 5.1).

#### 5.2.2.1.1 Synthesis of Metal and Metal Oxide Nanoparticles

Several methods have been applied to synthesize metal and metal oxide nanoparticles, such as physical, chemical, enzymatic and biological methods(Ahmed et al. 2017). Physical methods include plasma arcing, ball milling, thermal evaporation, spray pyrolysis, ultrathin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer-by-layer growth and diffusion flame synthesis. Chemical

**Table 5.1** Selected studies on the synthesis of nanoparticles and their properties

| Type | Precursor | Route | Techniques | Highlights | References |
|---|---|---|---|---|---|
| AgNPs | Silver nitrate ($AgNO_3$) | Biosynthesis using chitosan as stabilizing and reducing agent | UV-vis, TEM and FTIR | The optimum preparation time for AgNPs was 12 h heating at 90 °C to obtain NPs of sizes of 5–20 nm | Mokhena and Luyt (2017a, b) |
| ZnNPs | Zinc acetate dehydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) | Biosynthesis using extract from *Nyctanthes arbor-tritis* | UV-vis, FTIR, XRD, DLS and TEM | NPs had size range of 12–32 nm | Jamdagni et al. (2018) |
| ZnNPs | $ZnCl_2$ | Hydrothermal method | UV-vis, XRD, SEM, TEM, SAED | ZnO hollow spheres | Yu and Yu (2008) |
| $TiO_2$ | Tetrabutyl orthotitanate (($Ti(OC_4H_9)_4$) | Hydrothermal method | XRD, XPS, TEM, BET | Mesoporous fluorinated anatase $TiO_2$ powders having primary particles of 11 ± 2 nm | Yu et al. (2009) |
| ZnNPs | Zinc acetate | Spray pyrolysis | XRD | Polycrystalline films having hexagonal wurtzite-type crystal structure with grain size of 20 nm | Ashour et al. (2006) |
| MgO | Magnesium nitrate ($mg(NO_3)_2.6H_2O$) | Spray pyrolysis | XRD, TEM, UV-vis | Crystallite size of 9 nm was obtained | Nemade (2014) |
| ZnNPs | ZnO powder | Chemical vapour deposition | SEM, TEM, SAED, XRD | Nanowires with diameter of 5 nm and length of 50 nm | Xiang et al. (2007) |

methods include electrodeposition, sol-gel process, chemical solution deposition, chemical vapour deposition, Langmuir-Blodgett method, soft chemical method, hydrolysis, co-precipitation and wet chemical methods. One of the most widely used chemical syntheses is co-precipitation (Dobrucka and Długaszewska 2016). In this case, the metal oxide precursor is mixed with stabilizing agent to form nanoparticles. Chemical synthesis is disadvantageous because it requires hard labour and is time consuming and costly (Dobrucka and Długaszewska 2016). Moreover, the chemical used is toxic and may contaminate the resulting NPs and produces large amount of secondary wastes. In solvothermal method, two steps are involved: (i) NPs are synthesized from chemical method and (ii) then calcined at high temperatures. In this method, it is possible to control the size, shape and crystallinity of the metal oxide or metal nanoparticles by changing the experimental conditions such as temperature, reaction time, solvent type, surfactant type and precursor type.

Hydrothermal synthesis has also been utilized to fabricate metal or metal oxide nanoparticles (Yu and Yu 2008; Yu et al. 2009). Similar to solvothermal method, it involves high temperatures and pressures (in autoclave), but the solution is nonaqueous (Yu and Yu 2008). Yu and Yu (2008) synthesized hollow spheres of ZnO nanoparticles by hydrothermal approach from a mixture of sugar and $ZnCl_2$. This mixture was treated at 180 °C for 24 h and then calcined in air at 500 °C for 4 h. The average diameter of as-prepared microsphere was 10 μm (Fig. 5.2a) which drastically decreased to about 800 nm after calcination (Fig. 5.2b). The hollow spheres had shell thickness of about 60 nm (Fig. 5.2c) which were polycrystalline (inset in

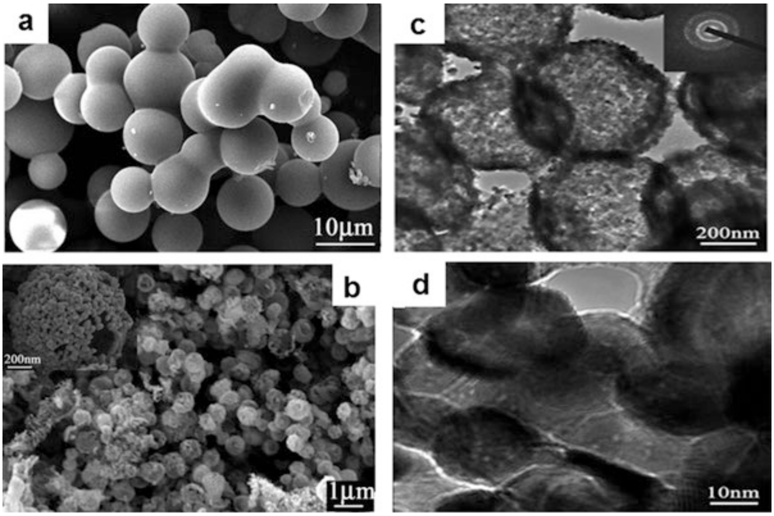

**Fig. 5.2** SEM images of the ZnO hollow spheres prepared before calcination (**a**) and after calcination (**b**) at 500 °C for 4 h. TEM image (**c**), high magnification. TEM image (**d**) and SAED pattern (inset in **c**) of the ZnO hollow spheres prepared and calcined at 500 °C for 4 h. (Reprinted with permission from Yu and Yu 2008 with permission from American Chemical Society)

Fig. 5.2a). It was observed that the hollow spheres were composed of randomly aggregated nanocrystal particles with sizes of about 18 nm.

Biosynthesis is a new area of nanotechnology in which natural materials are used to synthesize different nanoparticles (Jamdagni et al. 2018; Ahmed et al. 2017; Dobrucka and Długaszewska 2016; Matinise et al. 2017). These materials can be used as either stabilizing or reducing agent or both. The economic and environmental benefit associated with this technique opens the door for future NP production especially to overcome the challenges associated with chemical and physical methods. Natural materials such as gelatine, alginate, chitosan and many more have been studied for the synthesis of metal oxide nanoparticles as depicted in Table 5.1. In general, the natural polymer or plant extracts are mixed with precursor and then heated under certain temperature and for a given period to obtain NPs (Jamdagni et al. 2018; Matinise et al. 2017). The extracts or natural polymers may act as either reducing agent or stabilizing agent or both.

Another strategy to synthesize NPs involves sol-gel technique (Tamilselvi et al. 2013). This process is divided into two reaction steps (considered as chemical method), namely, hydrolysis and condensation. Hydrolysis is when hydroxyl groups (−-OH) are produced, whereas condensation involves polycondensation of the formed hydroxyl groups and residual alkoxy groups to form a 3D network (Mtibe et al. 2018). These steps solely depend on the presence of catalyst, type and concentration as well as the ratio of water and alkoxide. The sol-gel can further be classified into (i) hydrolytic route which involves water as ligand and solvent and (ii) nonhydrolytic route which involves ethers, alcohols and ketones as oxygen donors (Mtibe et al. 2018). The sol-gel technique offers the processor the opportunity to control the properties of the resulting nanoparticles through large number of parameters such as pH, temperature, hydrolysis and condensation rate of the metal oxide precursor, the nature and concentration of anions, the method of mixing and the mixing rate (Mtibe et al. 2018). The advantage of this technique includes a control on particle-size distribution, choice of precursor, high yield and low overall production cost (Tamilselvi et al. 2013).

Chemical vapour deposition (CVD) is known as a parent to a family of processes used to coat almost any metallic or ceramic compound, including elements, metals and their alloys and intermetallic compounds (Guo et al. 2011; Carlsson and Martin 2010; Wang 2011). It involves a solid material being deposited from a vapour by a chemical reaction occurring in the vicinity of a normally heated substrate. The resulting solid materials include thin films, powder or single crystal. It is recognized that by varying the experimental conditions, substrate material, substrate temperature and composition of the reaction gas mixture, total pressure gas flows' different materials having a wide range of physical, tribological and chemical properties can be produced (Guo et al. 2011; Carlsson and Martin 2010; Wang 2011). The CVD process includes chemical vapour deposition, metal-organic chemical vapour deposition, low-pressure chemical vapour deposition, laser chemical vapour deposition, photochemical vapour deposition, chemical vapour infiltration, chemical beam epitaxy, plasma-assisted chemical vapour deposition and plasma-enhanced chemical vapour deposition. The most used methods are thermal chemical vapour deposition, plasma-enhanced chemical vapour deposition and laser chemical vapour deposition (Carlsson and Martin 2010). This technique has been employed in the production of oxides, nitride and carbide nanopowders.

Spray pyrolysis involves the particle formation through atomization of liquid feed in a spray nozzle, combustion of the precursor, evaporation and decomposition of the metal precursor molecules and then nucleation of the particle clusters and growth of particles by condensation and coagulation (Nemade 2014; Meland et al. 2006; Patil 1999). Flame pyrolysis can be utilized to produce metal oxide nanoparticles with large surface through flame conditions and the burner design. The properties of the resulting NPs can further be modified by changing the conditions during the synthesis. For instance, the rutile phase appeared when the preparation temperature was increased and surpassed anatase when temperature was above 1000 °C; however, below 800 °C rutile was not observed (Seo et al. 2006). In addition, the average sizes of the crystallite increased with an increase in preparation temperature. This method is capable of yielding high-purity homogeneous samples, and up-scaling is possible (Meland et al. 2006).

## 5.2.3 Carbon-Based

### 5.2.3.1 Graphene

Graphene is recognized as a monolayer of graphite consisting of a honeycomb network of $sp^2$-hybridized carbon atoms (Goriparti et al. 2014; Kucinskis et al. 2013). The carbons are bonded into 2D sheets having nanometre thickness (single-atom thickness). It has distinctive properties which include high electrical conductivity, good mechanical properties, large surface area and high values of charge mobility, hence receiving unprecedented interest from various fields such as chemical, physical, biological and engineering sciences (Goriparti et al. 2014; Kucinskis et al. 2013). Graphene can be fabricated using various methods, such as micromechanical exfoliation of highly oriented pyrolytic graphite with or without previous processing surface, epitaxial growth, chemical vapour deposition (CVD) and reduction of graphene oxide (GO) (Goriparti et al. 2014; Kucinskis et al. 2013). The most preferable route is the reduction of GO because this process is the most suitable for large-scale production.

#### 5.2.3.1.1 Synthesis of Graphene

Natural graphite also known as expandable graphite is one of the carbon-based material used to produce graphene by various exfoliation processes such as mechanical, chemical or electrochemical and thermal exfoliation techniques. These techniques can be used alone or in combination in order to produce graphene of high-quality and fine-tuned morphology or structure. Mechanical exfoliation or micromechanical cleavage is the most used method to obtain graphene layers. This is the first method which was reported for graphene layers' production using scotch/adhesive tape by repeated peeling of graphite crystal (Novoselov et al. 2004). Another mechanical technique involves rubbing multilayer graphene against a flat surface to obtain a single graphene layer (Mtibe et al. 2018; Bonaccorso et al. 2012). The use of atomic force microscopy (AFM) tip by gluing graphite on it and scratching on Si substrate also offers the production of 2D graphene layers (Bonaccorso et al. 2012). This method, however, offers minimal control over separation and number of resulting graphene layers; hence, it is still difficult for most of these mechanical techniques to be realized as scalable and cost-effective (Mtibe et al. 2018; Bonaccorso et al. 2012).

Chemical exfoliation involves the use of strong oxidants and acids to oxidize graphite through Hummers, Staudenmaier and Brodie methods or by electrochemical oxidative exfoliation of bulk natural graphite (expandable graphite) to yield expanded graphite (EG) or graphite oxide (GO) (Mtibe et al. 2018; Cooper et al. 2014; Zhong and Swager 2012). The oxygen functionality on GO facilitates the dispersion of the sheets in aqueous and organic solvents, however at the expense of electronic conductivity of graphene. The disadvantage of chemical process is the use of harsh chemicals, which are not environment friendly and not easy to handle, and the long production periods associated with this process (Mtibe et al. 2018; Cooper et al. 2014; Zhong and Swager 2012). Thermal exfoliation involves the exposure of expandable graphite to rapid heat, and thus, forcing the graphite sheets apart results in an enormous increase in volume and high surface area material with low bulk density. The chemical and thermal processes can be used together to afford maximum exfoliation of the expandable graphite.

X-ray diffraction (XRD), Raman spectroscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are usually used to establish the extent of exfoliation or the properties of the resulting graphene layer. Among these techniques, TEM is the most reliable technique to elucidate the properties of resulting material. For instance, the difference between the products obtained from graphite electrode using ionic liquid-assisted electrochemical exfoliation was reported by Lu et al. (2009). They studied time dependence of products exfoliated from graphite in three different stages in the electrochemical exfoliation (Fig. 5.3) using high-resolution TEM. Each stage resulted in different products as reflected by colour change, and their structure is analysed by TEM. Stage I led to hexagonal-shaped water-soluble and fluorescent carbon nanocrystals sized 8–10 nm (Fig. 5.4a). The nanocrystals had a lattice spacing of 0.21–0.25 nm comparable to the (100) facet of graphite (Fig. 5.4b). In stage II, fluorescent nanoribbons of 10 nm × (60 ± 20) nm size with flat rectangular edges with some graphene were obtained. The nanoribbons had a lattice distance of 0.34 nm that corresponds to the (002) plane of graphite (Fig. 5.4c, d), while ultrathin graphene sheets (200 nm × 500 nm) were obtained as shown in Fig. 5.4e, f.

#### 5.2.3.2 Carbon Nanotubes (CNTs)

Carbon nanotube features unique characteristics such as high electrical and thermal conductivities and extremely large surface because of their 1D tubular structure (Goriparti et al. 2014; Liu et al. 2012; Mubarak et al. 2014; Fang et al. 2016). CNTs are recognized as cylinders consisting of rolled-up graphene sheets with hollow central core having diameters on nanometer scale and end caps with a hemisphere

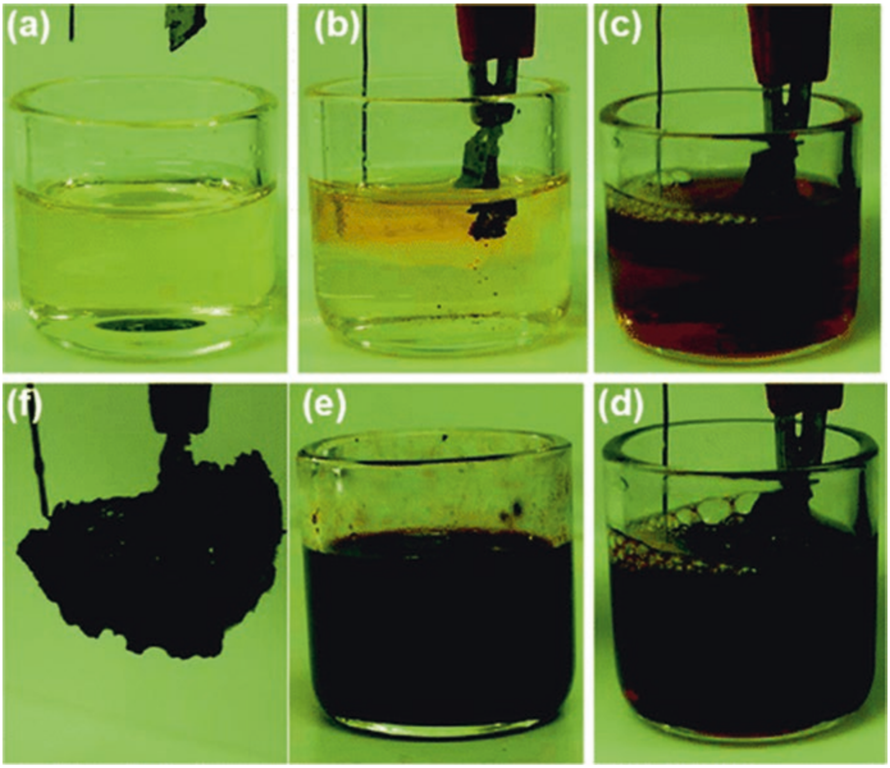

**Fig. 5.3** Time evolution of IL electrolyte and highly oriented pyrolytic graphite (HOPG) anode during exfoliation in 60 wt % water/[BMIm][$BF_4$] electrolyte. Stages I, II and III are shown correspondingly in panels b, c and d. Heavily expanded HOPG is obtained in panel f. (Copied from Lu et al. 2009 with permission from American Chemical Society)

of fullerene structure. They are categorized into single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs) based on the number of graphene layers. As reflected by their names, SWCNTs consist of a single graphene layer, while MWCNTs consist of two or more graphene layers with van der Waals forces between these layers (Goriparti et al. 2014; Liu et al. 2012). In general, CNTs are often synthesized using various techniques, such as arc discharge (Mubarak et al. 2014; Fang et al. 2016; Zhao et al. 2006), laser ablation (Choi et al. 2016; Bota et al. 2015; Vander Wal et al. 2003), gas-phase catalytic growth from carbon monoxide and chemical vapour deposition (CVD) from hydrocarbons. Among these methods, gas-phase methods such as CVD have a potential for large-scale production at fairly low cost. Chemical bonding of CNTs consists of entirely $sp^2$ carbon-carbon bonds which are stronger than the $sp^3$ bonds found in diamond, thus providing CNTs with such remarkable mechanical properties (Young's modulus of 1.2 TPa and tensile strength of 50–200 GPa) (Goriparti et al. 2014; Liu et al. 2012).

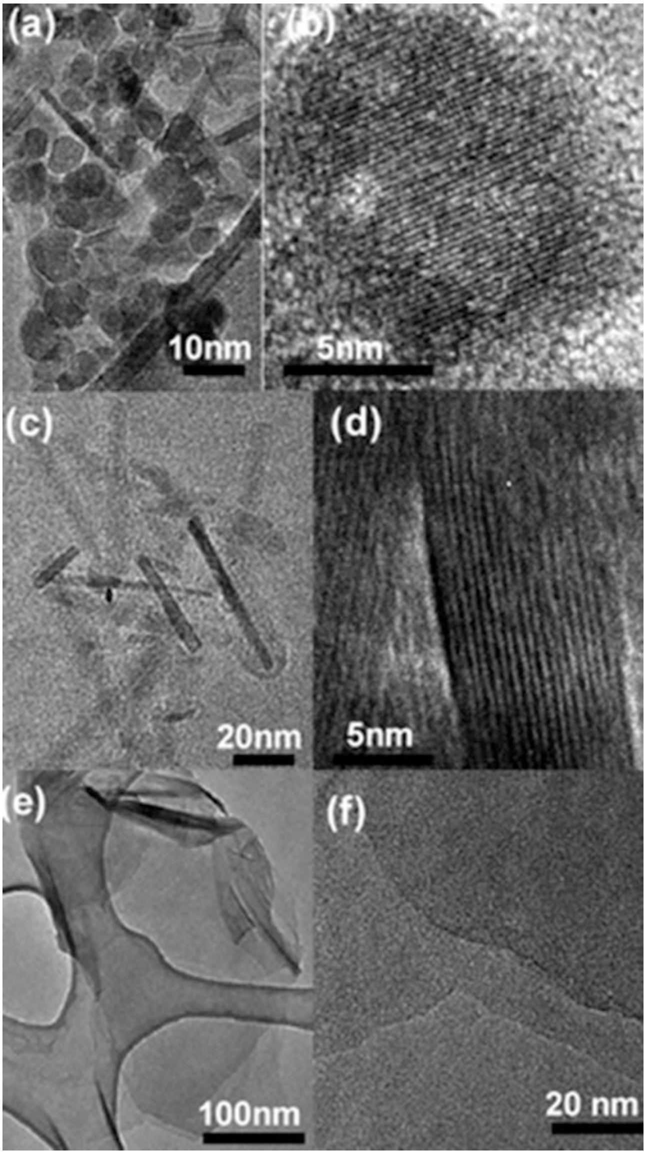

**Fig. 5.4** TEM images of carbon nanoparticles (**a**, **b**), carbon nanoribbons (**c**, **d**) and graphene sheets (**e**, **f**) produced in the one-pot electrochemical exfoliation (Reprinted from Lu et al. 2009 with permission from American Chemical Society)

#### 5.2.3.3 Synthesis of CNTs

Different types of CNTs can be produced using various techniques which include electric AD, laser ablation and CVD. These techniques require carbon source, catalyst and energy to produce CNTs. In the case of electric AD method, electric discharge is used as energy source with electric current ranging from 40 to 125 A and potential difference ranging from 20 to 50 V (Fang et al. 2016; Zhao et al. 2006; Kim and Kim 2006; Berkmans et al. 2015; Maria and Mieno 2015; Su et al. 2011, 2014). The graphite electrodes are placed close to each other (1 mm) under inert atmosphere (i.e. at a pressure of 500 Torr) (Mtibe et al. 2018). Moreover, the metal catalyst such as cobalt, nickel, yttrium or iron and high temperatures (2000–3000 °C) are required in order to evaporate the carbons from the electrodes (Mubarak et al. 2014; Fang et al. 2016; Zhao et al. 2012). The evaporated carbon is then condensed in the cathode to form CNTs with diameters of 0.6–1.2 nm. The processors can vary experimental parameters such as metal catalyst concentration, electrode gap, current flow rate, temperature distribution gas type and gas pressure in order to afford the production of either MWCNTs or SWNTs (Mubarak et al. 2014; Fang et al. 2016; Zhao et al. 2006; Kim and Kim 2006; Berkmans et al. 2015; Maria and Mieno 2015; Su et al. 2011). By varying the ratio of catalyst (yttrium and nickel) and carbon source concentration, different carbon-based materials were obtained (Berkmans et al. 2015). It was revealed that a combination of SWCNT having a diameter of ~5.7 nm and single-walled carbon nanohorn (SWCNH) bundles sized about 25 nm can be produced using arc discharge technique in an open air atmosphere which makes this method cheaper and effective for hybrid carbon materials. However, low yields and the quality of CNTs produced via this method limit its industrial production (Mubarak et al. 2014; Berkmans et al. 2015; Su et al. 2011, 2014). The effect of the ratio of gases, viz. carbon monoxide (CO) and helium, and pressure was investigated by Su et al. (Su et al. 2011). It was reported that impurities increased with an increase in the concentration of CO in the gas mixture; however, the gas pressure ˃ 4 kPa can result in smaller diameters. Laser ablation uses the same principle as AD but uses laser pulses as source rather than heat (Choi et al. 2016; Bota et al. 2015; Vander Wal et al. 2003; Chrzanowska et al. 2015). It is recognized that the yield and quality of the resulting CNTs depend on the laser power, catalyst and temperature (Bota et al. 2015; Chrzanowska et al. 2015). In a similar manner, this technique suffers from low yield and low quality of resulting material and requires high level of purification process (Bota et al. 2015; Chrzanowska et al. 2015).

CVD merits special interest due to its capability for large-scale production of high-quality and well-oriented CNTs (Ji et al. 2017; Marchand et al. 2013; Meysami et al. 2013a). In this case, the carbon sources are the hydrocarbons methane, ethylene and benzene and metal catalysts Ni, Co and Fe. Furthermore, high temperature is also required for the production of CNTs. In this regard, the hydrocarbons' catalytic decomposition on monolayer metal catalyst is carried out at temperatures 700–900 °C to form CNTs. The quality of CNTs, yield and structure can be improved by using a single or more metal catalyst. Quite a lot of studies have been carried out to modify CVD in order to improve growth rate, quality and yield as well as to produce different types of CNTs. These modified systems include aerosol-assisted chemical vapour deposition (AACVD) (Marchand et al. 2013; Meysami et al. 2013a, b, 2015), plasma-enhanced chemical vapour deposition (PECVD) (Ji et al. 2017), hydrogen-free spray pyrolysis CVD (Ionescu et al. 2011), catalytic CVD (Almkhelfe et al. 2017; Maruyama et al. 2016; Miura et al. 2018), low-temperature CVD (Maruyama et al. 2002) and alcohol combustion CVD (Hou et al. 2017).

## 5.3 Characterization

Chemical composition, structure, size and shape play significant role on the properties of resulting NPs; thus, several analytical techniques were utilized to characterize these properties (Hoo et al. 2008).

### 5.3.1 Size Determination

NPs are often characterized based on their size, morphology and surface charge using various microscopic techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), scanning tunnelling microscopy (STM) and atomic force microscopy (AFM) (Hoo et al. 2008; Binnig and Rohrer 1983). TEM and SEM are essential for providing information based on the NPs' surface, crystal structure, elemental composition, size and shape. It is recognized that the treatment of the sample before the analysis (coating, drying, staining, etc.) and vacuum conditions in the chamber may result in imaging artefacts. Field emission scanning electron microscopy (FESEM) can overcome these limitations by imaging samples which are not pretreated; however, in most cases, the samples are still treated. On the other hand, AFM provides information based on the size, morphology and surface texture and roughness (Hoo et al. 2008). However, AFM may overestimate the dimensions of the NPs especially when the geometry of the tip is larger than the NPs themselves. In the case of STM, atoms and molecules made up of NPs can be identified by alternating the environment that the specimen is observed, such as vacuum, liquid environment or gaseous environment (Binnig and Rohrer 1983).

Light scattering techniques (e.g. dynamic light scattering (DLS)) are mostly employed to establish the NPs' size (Hoo et al. 2008; Glatter 2018). Dynamic light scattering (DLS), also known as photon correlation spectroscopy, is the most used technique to determine the size of the particle in colloidal suspensions. The suspended particles undergo random Brownian motion; hence, the light scattered off the particles in suspension renders calculation of particle size. The calculation is obtained from analysing fluctuation intensities of the light scattered by the particles during irradiation by a laser beam (Glatter 2018; Ramos 2017).

X-ray-based methods include X-ray absorption (XAS), X-ray fluorescence (XRF), X-ray photoelectron spectroscopy (XPS), energy-dispersive X-ray spectroscopy (EDS), small-angle X-ray scattering (SAXS) and X-ray diffraction (XRD) (Balasubramanian et al. 2014; Dao et al. 2015). These techniques are often used to provide information about surface properties and coatings, crystallographic structure, bonding environment or elemental composition. EDS and XPS are usually combined with either SEM or TEM to elemental assessment and quantitative analysis (Dao et al. 2015). X-ray absorption is used to characterize unoccupied electronic states, the chemical composition and the bonding environment in NPs (Balasubramanian et al. 2014; Dao et al. 2015; Mottana 2014). XRD is used to establish the crystal phase and crystallinity of the NPs (Balasubramanian et al. 2014). The comparison between patterns of the as-synthesized nanoparticles with the standard (JCPDS data) gives information based on the structure and properties of the resulting NPs (Balasubramanian et al. 2014). Spectroscopic techniques are used in NPs with plasmon resonance by collective oscillations of their conduction band electrons in response to electromagnetic waves (Dutta and Ganguly 2012; Begum et al. 2018; Okitsu 2013). UV-visible is utilized to characterize the properties of metal NPs especially the size and shape as well as surface property in the case of functionalized NPs (Begum et al. 2018; Okitsu 2013). This results from plasmonic NPs absorbing radiations of visible to near-infrared region (NIR) depending on their size and shape. This character could be related to collective oscillation of surface electrons of nanoparticles and known as surface plasmon resonance (SPR) (Begum et al. 2018; Okitsu 2013). Because of the SPR property of the nanoparticles, dispersed plasmonic NPs in suspension give one or more peaks that can be used to extract valuable information with regard to the shape, size and size distribution of the nanoparticles (Begum et al. 2018; Okitsu 2013).

Nanoparticle tracking analysis (NTA) is one of commonly used systems for particles of sizes below ~20–1000 nm, with the lower detection limit being dependent on the refractive index of the NPs (van der Pol et al. 2014; Zhou et al. 2015; James and Driskell 2013). It uses a highly sensitive CCD video camera to capture scattered light from each NPs and track the Brownian motion of individual NPs to yield independent size measurements (James and Driskell 2013). Yet another technique is hyperspectral imaging which relies on scattering the obliquely incident visible and near-infrared light in an enhanced dark field. It provides information based on spatial distribution and spectral characteristics depending on NP type, at a sensitivity of a single nanoparticle (size ˂10 nm) (Pena et al. 2016; Badireddy et al. 2012).

### 5.3.2 Quantification

NP quantification is another important step to understand the fate, behaviour and occurrence of NPS in different environments. Different sensitive analytical techniques have been used to detect and characterize nanoparticles as discussed in the next section.

ICP spectrometry is commonly employed during NP synthesis because it is capable of determining the total element concentration of the colloidal solution (Scheffer et al. 2008). Nowadays, it has been further extended to the size characterization of NPs. ICP mass spectroscopy (ICP-MS) was recently used to establish the size distribution of nanoparticles. In this regard, the determination of discrete ion clouds generated from the atomization of a single nanoparticle in the plasma causes an intensity signal at the detector associated with the size of the particle (Scheffer et al. 2008; Degueldre et al. 2004; Degueldre and Favarger 2004). Single-particle ICP (SP-ICP) is one of the analytical techniques used to determine NP concentration, size and size distribution. It measures the intensity signal produced after ionization of an individual particle rather than a continuous flow of ions reaching the plasma (de la Calle et al. 2017). ICP optical emission spectroscopy (ICP-EOS) has been also utilized for NP analysis (Scheffer et al. 2008; Fischer et al. 2007).

Liquid chromatography mass spectroscopy (LC-MS) is commonly used to analyse fullerenes in environmental matrices (Chen et al. 2008a). Since this technique relies on the rate at which the NPs elute from the stationery phase over a mobile phase gradient, the extraction of C60 has been exploited by its solubility in toluene (Chen et al. 2008a).

Fourier transform infrared (FTIR) and Raman spectroscopy are also used for identification of NPs. The most important range for NPs is the fingerprint region which provides signature information of the sample. Surface-enhanced Raman spectroscopy (SERS) is considered the new innovative method due to its signal-enhanced capability via SPR phenomenon (Muehlethaler et al. 2015; Ma et al. 2011).

## 5.4 Applications of the Nanoparticles in Biofuels

Nanomaterials can be employed in biodiesel production from biomass as substrate for lipid accumulation, lipid extraction and transesterification process as either catalyst or catalyst support. Due to their high surface area, NPs have been applied as immobilizing agent for lipids; thus, they can be easily recovered from liquid phase by either filtration or centrifugation. Since organic solvents such as hexane and methanol were mainly used in lipid extraction due to their strong affinity towards lipids, immobilization of organic solvent-like chemical onto NPs provides easy recovery and extraction of the lipids. Elsewhere, it was demonstrated that the modified nanosilica afforded the extraction of alive algae which can be sent back for further lipid accumulation again (Lin et al. 2009). Some studies also revealed that NPs enhance the growth of microalgae which improves lipid accumulation (Gao et al. 2010). However, selection of the NPs has to be carefully considered in order to avoid any negative effect on the microbes (Jin et al. 2007; Magrez et al. 2006; Williams et al. 2006).

Transesterification is one of the most reliable and simple technologies applied in biodiesel production (Lee et al. 2015). In this context, various oils from different sources such as animal, plant or oleaginous microorganisms react with alcohol to produce fatty acid methyl esters (FAMEs or biodiesel) (Lee et al. 2015). There are two conditions in which the reaction takes place, viz. either severe high temperatures and pressures or mild conditions at the presence of catalyst. In the latter, acids ($H_2SO_4$ and HCl) and base (NaOH and KOH) are often employed as catalyst for biodiesel production in the lab and industrial scale, respectively (Xu et al. 2006; Vicente et al. 2009; Ullah et al. 2009). The corrosiveness of the acids and soap formation for base catalyst opened door for enzymatic biocatalyst lipase which is environmentally friendly and efficient (Vicente et al. 2009; Ullah et al. 2009). However, the cost associated with the use of this enzymatic biocatalyst has been recognized as the major limitation that hurdles its success (Du et al. 2004; Pizarro and Park 2003; Noureddini et al. 2005). Different propositions have been made to reduce the overall cost, such as reducing lipase production cost, enhancing its efficiency and reusing it for more than once. The latter has been considered as a feasible route to reduce the overall cost. In this regard, lipase has been immobilized on different carriers, which include fibre cloth, acrylic resin, silica gel, hydrotalcite and micro- and macroporous materials (Noureddini et al. 2005; Yang et al. 2006; Bai et al. 2006). In recent years, nanomaterials received tremendous interest due to their unique attributes such as large surface-to-volume ratio which can provide enormous surface area for lipase immobilization. Different nanomaterials were reported to be employed for lipase immobilization and had the capability of retaining the enzyme and improving its efficiency, selectivity and stability as summarized in Table 5.2.

It was demonstrated that the activity of the lipase immobilized onto nanosized silica was 93% after 7 months of storage as compared to 40% activity for free lipase (Kwon et al. 2007). Elsewhere, the activity was further enhanced by immobilizing lipase onto functionalized zirconia (~214%) and remained high as initially after reusing for eight times (Chen et al. 2008b). Recent study by Li et al. (Li et al. 2018) also demonstrated that other nanoparticles such as carbon nitride can also be utilized for lipase immobilization. Carbon nitride ($C_3N_4$) as one of the types of two-dimensional nanomaterials has the capability of immobilizing lipase to improve its efficiency. It was reported that nanosheets for immobilization of lipase exhibited satisfactory enzyme loading (44.76 mg/g), pH flexibility, thermostability (after 180 min at 50 °C, 67% of the initial activity remained) and recyclability (after 10 runs, 72% of the initial activity). The concern with regard to immobilization of biocatalyst onto nanomaterials is the recovery of these materials which can end up being cumbersome. In some studies, centrifugation has been used as a recovery technique; however, it consumes a lot of energy and time. In order to easily recover both nanomaterials and immobilized biocatalyst, the use of magnetic nanomaterials has been a major subject in research in the past decades (Tsang et al. 2006; Konwarh et al. 2009; Dyal et al. 2003; Solanki and Gupta 2011; Amirkhani et al. 2016; Netto et al. 2009; Xie and Ma 2009). The magnetic nanoparticles can be easily recovered by using external magnet, thus improving biocatalyst recovery and reusability. However, the aggregation and the nature of chemical activity of magnetic nanoparticles have been the major drawbacks (Amirkhani et al. 2016). One of the proposed methods in order to solve these problems involves coating the magnetic NPs with inorganic layers such as carbon and silica (Amirkhani et al. 2016; Lu et al. 2007; Horak et al. 2007). Moghaddas and co-workers synthesized magnetic silica aerogel support by iron oxide nanoparticles and sodium silicate precursors in a sol-gel process followed by chemical modification and ambient pressure drying (Amirkhani et al. 2016). A maximum adsorption capacity of lipase through physical adsorption was about 81.9 mg/g.

Apart from enzymatic catalyst, heterogeneous catalysts (viz. calcined Li-CaO, Mg-Al hydrotalcites, calcium oxides, magnesia-rich magnesium aluminate spinel, Mg/Zr), which are solid acid or base, have been investigated in biofuel production (Wen et al. 2010; Venkat Reddy et al. 2006; Wang et al. 2009; Sree et al. 2009). Among these, nanocatalyst renders high catalytic efficiency and eases separation from products. Biofuel production through transesterification using nanocatalyst

**Table 5.2** Selected studies on the immobilization of lipase

| Lipase source | NP type | Synthesizing route and highlights | Activity remaining (%) | Reusability | References |
|---|---|---|---|---|---|
| *Candida rugosa* | MWCNTs | MWCNTs were commercially procured having diameters of 30 ± 5 nm | 97 | – | Shah et al. (2007) |
| *Candida rugosa* | $Fe_3O_4$ | Co-precipitation method to obtain NPs having sizes of 10 ± 2 nm | 80 | 4 | Solanki and Gupta (2011) |
| *Candida rugosa* | $ZrO_2$ | Chemical method followed by calcination to obtain $ZrO_2$ nanoparticles having diameter of ~20 nm | 214 | 8 | Chen et al. (2008b) |
| *Candida rugosa* | Carbon nitride ($C_3N_4$) | Thermal exfoliation (oxidation) to obtain 2D graphene-like nanosheets | ~100 | 10 | Li et al. (2018) |
| *Candida rugosa* | $\gamma$-$Fe_3O_4$ | Sonication of Fe(CO) in decalin followed by annealing to obtain NPs of average size of 20 ± 10 nm | $^{<}100$ | – | Dyal et al. (2003) |
| *Candida antarctica* | $Fe_3O_4$ | Co-precipitation resulted in nearly cubic particles of sizes ranging from 5 to 10 nm | 200 | 4 | Netto et al. (2009) |
| *Thermomyces lanuginosus* | $SiO_2$ | Sol-gel process resulted in mesoporous structure having a pore size of 15 nm satisfying lipase-chitosan conjugation | 93 | – | Kwon et al. (2007) |
| *Candida antarctica* | $Fe_3O_4$ | Co-precipitation to produce almost spherical NPs having diameters of ~11.2 nm | 70 | 4 | Xie and Ma (2009) |

from various oils, which include plant oils and waste oils from selected studies, is summarized in Table 5.3. It is recognized that the required nanocatalyst is 30% of that of conventional catalyst to obtain similar reaction conditions. In addition, the reaction was found to be not dependent on moisture and the free fatty acid content (Venkat Reddy et al. 2006; Boz et al. 2009).

**Table 5.3** Selected studies on heterogeneous nanocatalysts

| Catalyst | Size and synthesizing route | Oil type | Catalyst:oil ratio (%w/w) | Reaction time (hours) | Yield (%) | References |
|---|---|---|---|---|---|---|
| MWCNTs | Commercially procured and impregnated with KOH and calcined | Canola | 12 | 4 | 94.2 | Omraei et al. (2013) |
| CaO-NaY-Fe3O4 | Grind and then calcined resulted in NPs of average particle size of 200–500 nm with surface area of 359.42 $m^2/g$ | Canola | – | 4 | 95.37 | Firouzjaee and Taghizadeh (2017) |
| CaO-AuNPs | Impregnation by deposition of AuNPs onto CaO | Sunflower | – | 3 | 94 | Bet-Moushoul et al. (2016) |
| TiO2-ZnO | Combustion resulted in spherical (34.2 nm) particles | Palm | – | 5 | 92 | Madhuvilakku and Piraman (2013) |
| CaO | Commercially procured NPs with diameters of ˃160 nm | Soybean | 16 | 6 | 93.5 | Luz Martínez et al. (2010) |
| CaO | Commercially procured with size ranging from 20 to 40 nm | Poultry oil | 0.6 | 12 | 99 | Venkat Reddy et al. (2006) |
| $Cs_2Mg(CO_3)_2$ | Co-precipitation to yield NPs with crystallite size of 17.5 ± 5.3 nm and surface area of 70 $m^2/g$ | Butter | – | 3 | 100 | (Montero et al. 2010) |
| $KF/Al_2O_3$ | Commercial procured | Soybean | 3 | 8 | 99.84 | Boz et al. (2009) |
| KF/CaO-MgO | Co-precipitation resulted in mesoporous structure with pore size of about 34 nm and the grain size in the range of 100–300 nm | Rapeseed | 3 | 3 | 95 | Wang et al. (2009) |
| KF/CaO-Fe3O4 | Co-precipitation followed by impregnation resulted in NPs having average diameter of 50 nm and surface area of 20.8 $m^2/g$ | Stillingia | 4 | 3 | 95 | Hu et al. (2011) |
| $K_2O/\gamma\text{-}Al_2O_3$ | Grinding calcination method to afford spherical NPs of about 50 nm | Rapeseed | 3 | 3 | 94 | Han and Guan (2009) |

(continued)

**Table 5.3** (continued)

| Catalyst | Size and synthesizing route | Oil type | Catalyst:oil ratio (%w/w) | Reaction time (hours) | Yield (%) | References |
|---|---|---|---|---|---|---|
| Li-CaO | Impregnation method afforded a mixture of rod-shaped and hexagonal particles having sizes ranging from 50 to 70 nm | Karanja and jatropha | 5 | 1 | 100 | Kaur and Ali (2011) |
| MgO | Hydrolysis followed by supercritical solvent removal and then dehydrated to form monocrystalline particles of 1–3 nm with lattice spacing of 2.1 Å and surface area of 435 $m^2/g$ | Sunflower and rapeseed | 1.5 | 6 | 90 | Verziu et al. (2008) |
| MgO | Decomposition of $mg(OH)_2$ | Palm | 0.5 | 4 | 51.3 | Yacob et al. (2009) |
| MgO | Commercially procured having average of 60 nm | Soybean | 2 | 17 | 99 | Wang and Yang (2007) |

## 5.5 Conclusion and Future Remarks

In this chapter, different fabrication methods for various nanomaterials and their characterization as well as classification were presented. It can be concluded that nanoparticles received tremendous interest towards biofuel applications due to their inherited properties, such as particle size, larger surface area, tuneable porosity, crystallinity, shape, compositions and many more. A wide variety of methods, such as microscopic, X-ray-based, light-scattering, spectroscopic and other methods, have been employed to establish these properties because they are of significance with regard to their application. From microscopic viewpoint, TEM is the most reliable technique to provide information on the size distribution and shape of nanoparticles, whereas other quantification methods can also be used to complement TEM especially towards the intended application.

Besides the fact that the utilization of the nanomaterials for advancement of biofuel production is being in its infancy, there has been progress on applying nanomaterials instead of organic solvent with regard to safety and health issues. In addition, nanomaterials can enhance lipid production (by providing stress on biomass) and lipid extraction (even without harming microalgae) and can be used as biocatalyst carriers and/or as heterogeneous catalyst in oil transesterification to biodiesel (Zhang et al. 2013). Further studies are, however, required to further our understanding with regard to cost-effectiveness recycling and impact of these NPs on the environment in order to avoid possible negative consequences on human and other organism.

**Acknowledgements** The authors would like to thank the financial support from the National Research Funding (NRF) and Department of Science and Technology (DST) Biorefinery Program in South Africa.

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# Nanotechnology: An Application in Biofuel Production

# 6

Veer Singh, Vipul Kumar Yadav, and Vishal Mishra

**Abstract**

Nanotechnology has been extensively studied for biofuel production. Fossil fuels are available in a limited amount on earth; therefore, it is needful to build up a new alternative to fulfill the requirement of energy sources in the near future. Biofuel such as biodiesel, biohydrogen, biomethanol, and bioethanol may replace the need for fossil fuel in the future. These fuels are produced from cost-effective and renewable sources such vegetable oils that come from different biological sources such as algal biomass, microbial biomass, and plant biomass. Nanomaterials have emerging applications in biofuel production due to their unique structural behavior such as small size (nanoscale size) of nanomaterials. These nanomaterials enhance biofuel production by improving the biosynthesis pathways. This manuscript mainly focuses on the various types of nanoparticles, synthesis methods of nanoparticles, and the emerging application of nanoparticles in biofuel production.

**Keywords**

Nanoparticles · Biofuel · Biodiesel · Biohydrogen · Bioethanol

## 6.1 Introduction

Fossil fuels such as coal, petroleum ions, and liquid petroleum gases are available in nature in a limited amount because these sources of energy are nonrenewable. These fuels have towering cost and cause several types of environmental pollutions such as air, soil, and water pollution. When fossil fuels burn, it produces energy and

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_6

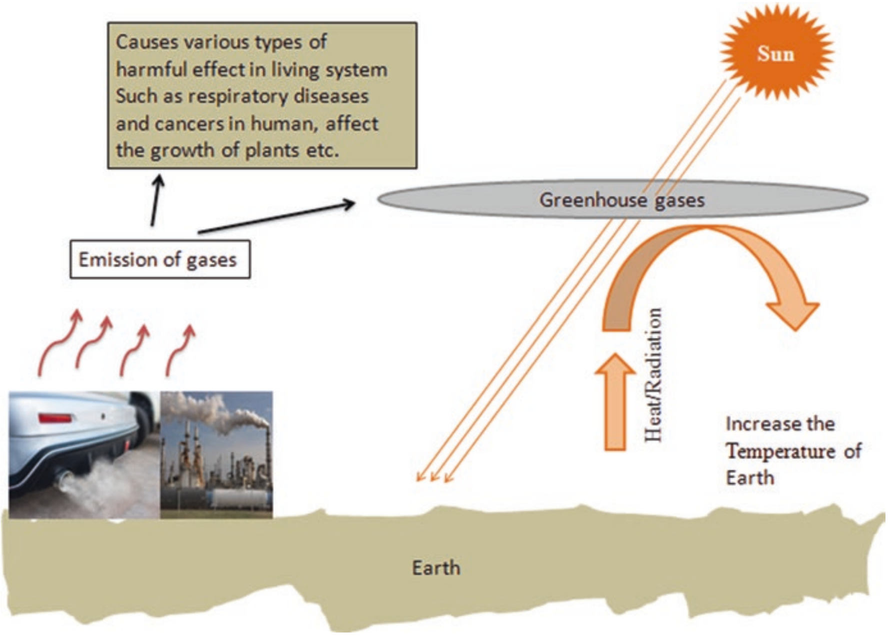

**Fig. 6.1** Diagrammatic representation of the emission of gases during the burning of fossil fuel and the role of these gases in environmental pollution

various types of gases including carbon dioxide, sulfur dioxide, and other various types of gases. Carbon dioxide is the main greenhouse gas which maintains the earth temperature. Increase in the level of $CO_2$ causes increase in the temperature of the atmosphere which affects the distribution of living organisms (Fig. 6.1). Air pollution is main component of pollution which causes the depletion of the ozone layer. The ozone layer protects the earth from ultraviolet radiations. UV radiation causes various types of health effects in humans and other living organisms. Water pollution by these fuel waste materials is responsible for the addition of many types of toxic substances in the water sources which affect the distribution and life of living aquatic organisms. These wastes generated from these fuels also cause the loss of fertility in the soil. The availability of fossil fuel is limited, and after a certain time, these sources may be exhausted, making biofuel a potential candidate as an energy source. Therefore, biofuel production is very needful in the future as an alternative energy source (Palaniappan 2017).

Biofuel is referred to as energy sources generated from different types of biomass. These biomasses come from living organisms such as algae, bacteria, plants, and agricultural waste. Biofuel can terminate the demand for the fossil fuel in the future and minimize the environmental effects generated during the combustion of fossil fuel (Voloshin et al. 2015; Dragone et al. 2010; Demirbas 2009).

A recent approach for biofuel production is based on various types of microorganisms such as microalgae or cyanobacteria. Microalgae contain a huge amount of lipid which is used as raw material for biofuel production. The past few decades also

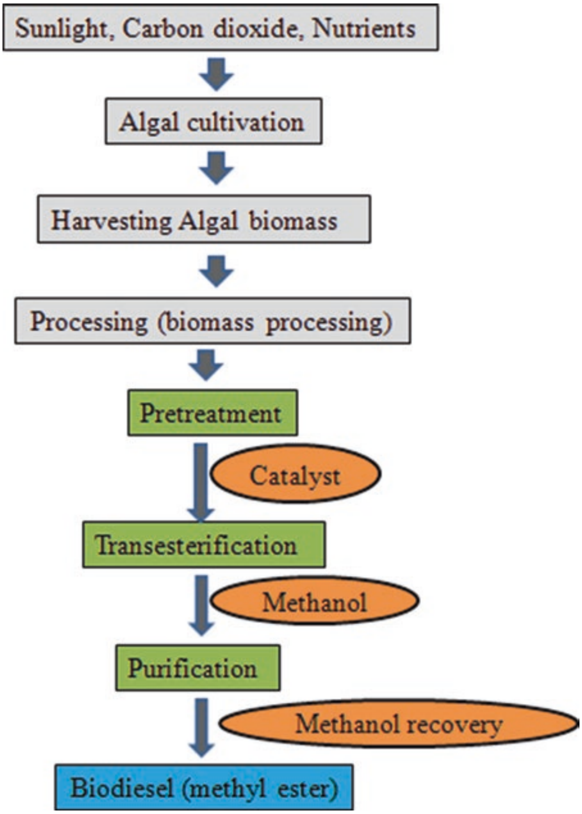

**Fig. 6.2** Biodiesel production from algal cultivation

represent the production of biofuel from plant materials. Present research mainly focuses on the production of biofuel from microalgae. Plants and algae can synthesize sugar molecule by the process of photosynthesis. Photosynthesis is a process in which green plant and algae synthesize the carbon-based materials in the presence of environmental $CO_2$ and sunlight. Photosynthetic products require the growth of plant and production of biomass (Fig. 6.2). Plant and algal biomass are used as raw sources for the production of biofuel (Demirbas 2009; Heiman 2016; Babel and Kurniawan 2004; Barakat 2011).

Nanoscience and nanotechnology have also a great potential for remediation of various types of pollutants from contaminated water (Eccles 1999; Leung et al. 2000; Kurniawan et al. 2005; Rickerby and Morrison 2007; Brumfiel 2003). Nanostructures have more efficiency and fast adsorption rate compared to other conventional methods. Nanoparticles have a broad surface area due to the nano-range (small) particle size. Varieties of nanoparticles have been considered as an inexpensive and environmentally safe adsorbent material for adsorption of several types of contaminants such as toxic metallic pollutants and azo dyes. The few applications of nanotechnology in bioenergy production and wastewater treatment are given in Fig. 6.3 (Sadegh et al. 2016; Theron et al. 2008; Dil et al. 2017; Savage and Diallo 2005).

Nanotechnology has prospective applications in biofuel manufacturing industries. Biofuel is the best alternative option of energy source in the future. Nanoparticles have an important role in the production of biofuel such as enhanced production of biohydrogen methane, biodiesel, etc.

## 6.2 Classification of Biofuel

Biofuel is classified by the raw material used in its production and by its efficiency. Biofuel is mainly classified into two groups, namely, primary and secondary. The primary category of biofuel is classified as a natural biofuel source derived from plants, animal waste, forest, and crop residues. The second type of biofuel is directly synthesized from microorganism such as microalgae. The second type of biofuel is divided into four generations (the fourth generation is basically correlated with the third generation) (Fig. 6.3). The first generation of biofuel is produced from starch-rich materials such as sugarcane, wheat, oats, potato, sweet potato, corn, and animal fats. The second generation of biofuel is produced from various types of plant species, grass, and wood of plant. The third generation of biofuel is an advanced type of biofuel which is produced from microalgae and few microbial species. Many researchers are working in the field of biofuel production and are trying to improve the production of biofuel by the genetic modification in third-generation fuel-producing organism, known as the fourth-generation biofuel. The yield of biofuel production depends on the type of microorganism which has been selected for

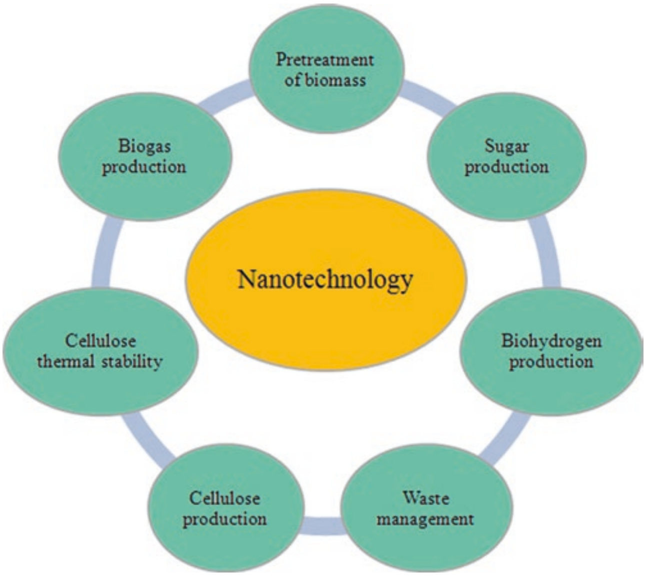

**Fig. 6.3** Diagrammatic representation of application of nanotechnology in various fields

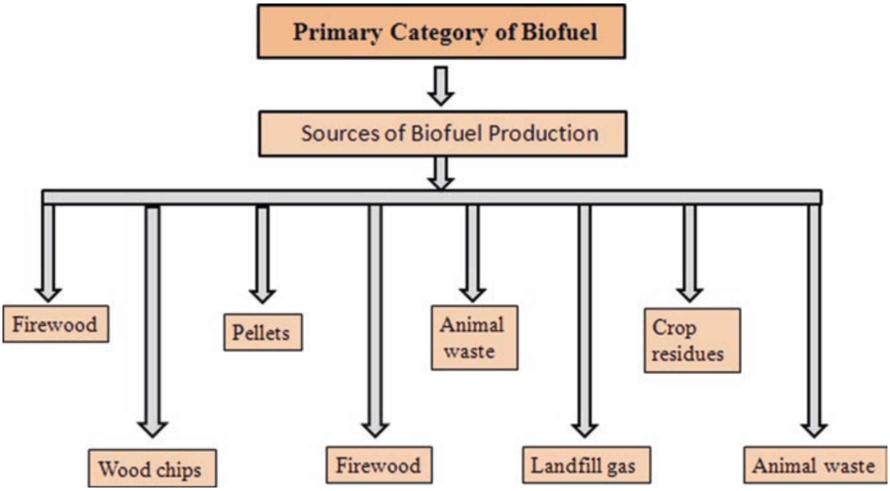

**Fig. 6.4** (**a**) The primary category of biofuel and their sources of production. (**b**) The schematic diagram represents the second category of biofuel, generations of biofuel, and sources of biofuel production

biofuel production and optimum conditions required for the growth of the microorganism. Media compositions also affect the production of biofuel. Genetic engineering techniques are also used for the modification in genetic level which may be responsible for enhancing the production of biomass. These biomasses are used for the production of biofuel (Dragone et al. 2010; Abdelaziz et al. 2013). Various types of biofuels are shown in Fig. 6.4 (a and b).

## 6.3 Production of Biofuel

Biomass such as plant, algae, microbial biomass, and other waste materials is considered as suitable raw material for biofuel production. A variety of algal and microbial (fungal and bacterial) species are used for the production of bioethanol and biohydrogen. These products are partially mixed with fossil fuel (4–5%) in diesel and petrol engines. In the next few years, biofuel may be used as an additive in petroleum industry. Hence, biofuel can be considered as the best alternative of petroleum by-products (Ong and Bhatia 2010; Chanakya et al. 2013; Swain 2014). Pacific biodiesel is the first biodiesel plant commissioned in the United States in 1996, and this plant mainly focuses on biodiesel production from cooking oils. Biodiesel production was boosted after 2001 due to the price hike of petroleum oils. Biodiesel has many advantages like emission of less toxic waste, inexpensive, and independence from fossil fuels (Chanakya et al. 2013; Swain 2014).Various types of techniques and unit operations involved in biodiesel production (Fig. 6.5) are shown in Fig. 6.5.

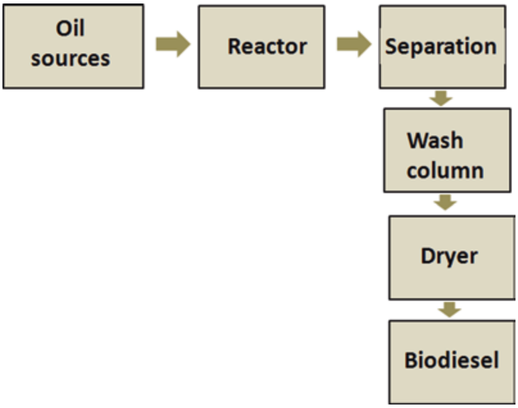

**Fig. 6.5** Schematic diagram represents the stages of biodiesel production

### 6.3.1 Production Techniques for Biofuel

Biofuel production methods depend on the materials used for the production of biofuel, reactor types, reactor volume, and reactor parameters such as temperature, pH, mixing, etc. A variety of biological materials and production methods have been used for the production of biofuel processes. A few examples have been given in Table 6.1. Hydrotreatment is a technique in which vegetable oil and other lipid materials are used for biodiesel production purposes. It is a complex reaction process in which various types of reaction are involved (Swain 2014; Torres-Ortega et al. 2017).

### 6.3.2 Algal Biodiesel

Algae are the photosynthetic organisms which fix gaseous $CO_2$ in the form of algal biomass (Maeda et al. 1995). Algal biomass is considered as raw material for the bioenergy production. Microalga is the best alternative option for the production of diverse types of biofuel such as biohydrogen, bioethanol, biodiesel, etc. due to its high yield, fast growth rate, and high lipid and sugar contents and is easy to grow (cultivated on both arable and nonarable land) (Reddy et al. 2008). However, biodiesel production from microalgae is much expensive due to the tremendous requirement of energy source and maintenance of the growth condition for the cultivation of algal species. Algal biofuel production is a very complex process. A variety of mechanisms are involved in the production process (Fig. 6.6). Many researchers are working for the reduction of production cost and enhancement of the production of biofuel (Chanakya et al. 2013; Harayama 2012).

**Table 6.1** Techniques of biofuel production and used raw materials for production processes

| Raw material | Technique | Product | References |
|---|---|---|---|
| Vegetable oils | Transesterification | Biodiesel | Alptekin et al. (2014) |
| Animal fat waste | Transesterification, gas chromatography, bench-scale reactor | Ethylic biodiesel | CunhaJr et al. (2013a) |
| Lard oil | Transesterification, methanolysis | Biodiesel | Ezekannagha et al. (2017) |
| Microalgae | Full-scale photobioreactor | Biodiesel | Faried et al. (2017) |
| Bacteria (*Clostridium tyrobutyricum* JM1) | Immobilization and packed bed bioreactor | Biohydrogen | Singh et al. (2010) |
| Mixed culture (*Clostridium butyricum* and *Rhodopseudomonas faecalis* RLD-53) | Combined dark and photofermentation process | Biohydrogen | Singh et al. (2010) |
| Lignocellulosic materials and animal manure | Pretreatment techniques such as milling and extrusion and followed by microbial reaction in batch and continuous reactor | Biogas | Alptekin et al. (2014) and CunhaJr et al. (2013a) |

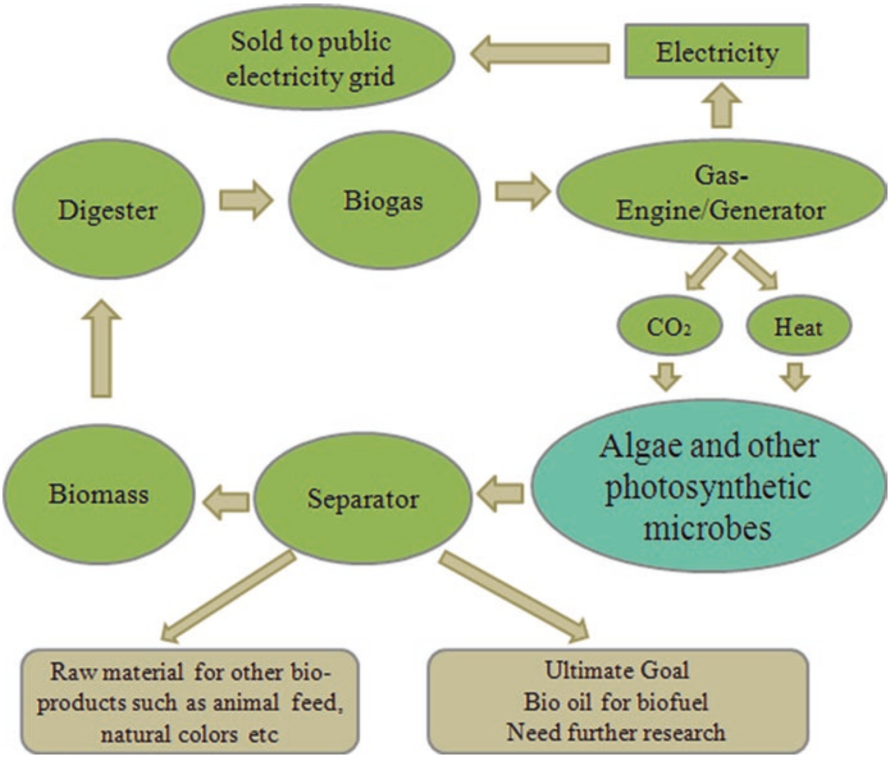

**Fig. 6.6** The schematic diagram represents the processing of algae for the production of biofuel

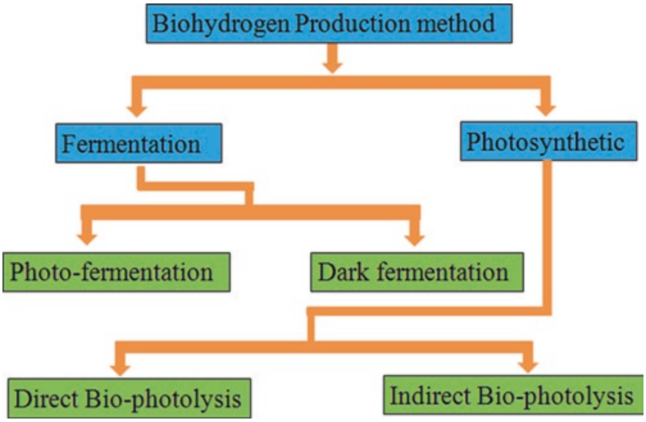

**Fig. 6.7** The schematic diagram represents the various methods of biohydrogen production

### 6.3.3 Biohydrogen

Biohydrogen can be the best option of energy source in the future. It is an eco-friendly and cost-effective biofuel. Biohydrogen has many advantages compared to other fuels. It has high efficiency due to its high octane number. Nowadays, about 99% of hydrogen is generated from fossil fuels (Shaishav et al. 2013; Jo et al. 2006). Various conventional methods are available for hydrogen production such as electrolysis of water and coal gasification (Hsia and Chou 2014). These conventional methods have few disadvantages like requirement of high temperature (more than 840 °C) and environmentally unsafe processes (Shaishav et al. 2013; Hsia and Chou 2014). Electrolysis of water is an eco-friendly process but electricity for the production of hydrogen adds extra cost. Therefore, this process is possible where electricity is inexpensive because electricity covers the major cost (about 80%) of hydrogen production (Karthic and Shiny 2012). This manuscript is mainly focused on inexpensive and environmentally safe methods of biohydrogen production.

Biohydrogen is generated during the photolytic reaction in photosynthetic microorganisms such as microalgae and plants (Fig. 6.7). It also generated by fermentation process in anaerobic microorganisms. Various types of microorganisms have been studied for biohydrogen production such as *Chlamydomonas moewusii*, *Scenedesmus obliquus*, *Rhodobacter sphaeroides* (photosynthetic bacteria), and *Enterobacter aerogenes* (fermentative bacteria) (Fabiano and Perego 2002; Kars et al. 2006).

## 6.4 Synthesis and Properties of Nanomaterials

It is most important to know about the synthesis mechanism and basic properties of nanomaterials before discussing their use in the production of biofuel and waste management processes. A range of nanomaterials are available such as nanoparticles, nanotubes, nanosheets, etc. Two major methods have been used for the formation of different types of nanomaterials. The first is the top-down approach. In this method, bulk materials such as iron, gold, etc. are broken down into nanoscale-size particles. The second method is the down-up approach. In this approach, small-size particles come together and form nanomaterials (Biswas et al. 2012; Serrano et al. 2009).

Other methods are also used for the synthesis of nanomaterials. These methods include coprecipitation, laser ablation, self-assembly, and phase separation methods. These methods produce various types of nano-size particles. Nanomaterials produced as dry powder materials are used for suitable application (Yu and Xie 2012). A nanoporous gold particle from Au and Ag alloy foil dealloys in nitric acid at 25 °C (Qiu et al. 2008). This is considered as dealloying and thermal alloying method, respectively. In the coprecipitation method, nanomaterials are synthesized by precipitation in the presence of gravitational forces and magnetic field (Kalantari et al. 2012). Carbon nanotubes (CNTs) are synthesized by various methods. The arc discharge method is one of the best methods for the synthesis of good-quality nanotubes which require electricity and carbon sources (De Volder et al. 2013). The chemical vapor deposition method requires heat and carbon source for the synthesis of nanotubes also (Saifuddin et al. 2013). The electro-spinning technique is used for the production of nanofibers. This method requires a polymer solution, high-voltage electric supply, spinneret, and collecting plates (Bhardwaj and Kundu 2010; Sill and Recum 2008).

Nanosheets mainly are produced by thermal exfoliation technique. Nanographene sheets produced from graphite powder react with hard acids such as $HNO_3$, $H_2SO_4$, and HCl and heating at high temperature (1050 °C). Argon gas is also required in this process (Kishore et al. 2012).

After the synthesis of nanomaterials, another important process is surface functionalization. Surface functionalization is a process which enhances the property of nanomaterials. This process also provides many other properties to nanomaterials such as stability and biocompatibility. It also enhances the binding capacity of nanomaterials with various types of enzymes (Pavlidis et al. 2010).The functionalization method requires addition of functional groups with nanomaterials. The resources used for functionalization are biopolymers such as gelatin, carbohydrates, polypeptides, lipids, etc. A variety of synthetic polymeric materials are also used as functionalization materials (Wang et al. 2012). Adding the functional groups with nanomaterials responsible for the enhancement in the properties of nanomaterials provides different surface charges, provides a link between functional groups of more than one nanomaterial, and minimizes the pore entrance size which is also applicable for enzyme immobilization (Lee et al. 2009).

Nanomaterials have many different properties compared to other bulk materials. They have different electrical, heating conductivity, light reflection, and catalytic properties. These advanced properties of nanomaterials are responsible for the various applications of nanomaterials in drug delivery system, environmental engineering, and bioenergy production (Savolainen et al. 2016).

## 6.5 Application of Nanotechnology in Biofuel Production

Nanoparticles enhance the production of biofuel by increasing the catalytic reaction in the production process. Various types of nanomaterials have been reported for biofuel production (Ramsurn and Gupta 2013; Gordon and Seckbach 2012). This section of the chapter is mainly focused on the application of several types of nanoparticles and their role in the production of biodiesel, biohydrogen, bioethanol, biogas, and bioethanol.

### 6.5.1 Biohydrogen Production

Biohydrogen is produced from various types of microorganisms such as anaerobic bacteria, microalgae, etc. These microorganisms generate molecular hydrogen through various metabolic routes (Das et al. 2008). The biohydrogen production is extremely dependent on several ranges of parameters such as pH, temperature, retention time, and media compositions (Lukajtis et al. 2018; Nagaragan et al. 2017). It is also reported that nanoparticles enhance the production of biohydrogen in the microbial system by increasing the electron transfer rate in the microbial cell (Serrano et al. 2009; Ali et al. 2017; Zhang and Shen 2007).

#### 6.5.1.1 Dark Fermentation for Production of Biohydrogen

Dark fermentative biohydrogen production is an inexpensive and eco-friendly process in which production of biohydrogen depends on various renewable feedstock and microbial species (Nagaragan et al. 2017). Different types of nanomaterials have application in the field of biohydrogen production. Gold (Au) nanoparticles (5 nm) enhance the substrate utilization efficiency by 56% and increase the yield of biohydrogen production up to 46% (Zhang and Shen 2007). Gold nanoparticles enhance the biohydrogen production due to their small size and large surface area which are responsible for the binding of microbial cells to active sites. Gold nanoparticles also enhance the activity of enzymes of biohydrogen synthesis machinery which is responsible for the production of biohydrogen.

Nanoparticles are beneficial to the microorganism when it uses the optimum concentration of nanoparticles. High concentration of silver nanoparticles inhibits the growth of microorganisms and decreases the production of biohydrogen. Silver nanoparticles are reported as an inducer for microbial biohydrogen production. It increases severalfolds the hydrogen production yield. Addition of silver nanoparticles enhances the substrate utilization up to 62% which is responsible for enhancing the biohydrogen production up to 2.48 mol $H_2$/mole substrate. These nanoparticles reduce the lag phase of bacterial and algal growth and activate acetic reaction. The acetic reaction is the main pathway for biohydrogen production (Zhao et al. 2013; Yang and wang 2018).

Silica nanoparticles have emerging application in the dark fermentation process for the production of biohydrogen. Silica nanoparticles combine with iron oxide and produce nanocomposite ($Fe_3O_4$@$SiO_2$) (detailed synthesis of composites is explained in Fig. 6.8). These newly synthesized nanocomposites/nanoconjugates

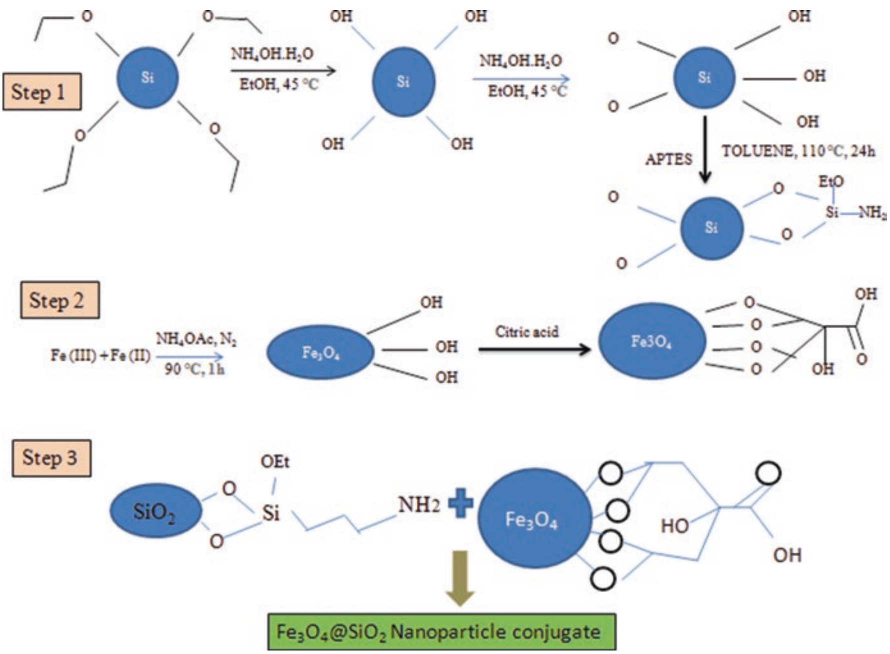

**Fig. 6.8** The schematic diagram represents the synthesis of Fe3O4@SiO2 nanoparticle conjugate

have emerging application in biohydrogen synthesis due to more catalytic activity and stability. These nanomaterials are stable at high temperature and show a low level of toxic effects (Venkata et al. 2008; Abbas et al. 2014; Kunzmann et al. 2011).

#### 6.5.1.2 Biohydrogen Production by the Photofermentation Process

Nanoparticles enhance the production of biohydrogen in photosynthetic microorganisms. The photosynthetic microorganisms used for the production of biohydrogen are microalgae, photosynthetic bacteria, etc. Addition of nanoparticles in growth medium enhances the growth, physiological process, photosynthetic efficiency, protein synthesis, lipid synthesis, and nitrogen metabolism in microbial species (Eroglu et al. 2013). Nanoparticles also enhance the activity of various enzymes which are involved in various applications such as glutamate dehydrogenase, glutamine synthetase production, etc. These microbial enzymes are also required for the metabolic activity of microorganisms (Yang et al. 2006; Mishra et al. 2014).

A variety of nanoparticles have been applied for biohydrogen production. The optimum concentration of Ag and Au nanoparticles enhances the photosynthetic activity of *Chlorella vulgaris* (Eroglu et al. 2013). Zerovalent nanoparticles also increase the activity of biohydrogen production by transfer of electrons from Fe° (Eq. 6.1) (Liu et al. 2012).

$$Fe + 2H^{+} \rightarrow Fe^{2+} + H_2 \tag{6.1}$$

### 6.5.2 Biogas Production

Biogas is an inexpensive and environmentally safe biofuel. Biogas is produced through various methods in which digestion of organic material takes place due to a variety of microorganisms (Romero-Guiza et al. 2016; Aryal et al. 2018). Biogas production occurs in many steps. The four important steps are hydrolysis (breakdown process), acidogenesis (alcohol production), acetogenesis (acetic acid production), and methanogenesis (production of methane gas) (Mao et al. 2015; Buitron et al. 2014; Sekoai et al. 2016). The hydrolysis step of biogas production contains conversion of large biomolecules (carbohydrates and proteins) into small sugar molecules and amino acids. The acidogenesis step produces hydrogen, $CO_2$, and alcohols from the products of the first hydrolysis step. Various types of pathways are involved in this process (Lochynsha and Frankowski 2018; Angelidaki et al. 2018). Acetogenesis pathways of biogas synthesis produce acetic acid and other constituents. The final step of these pathways generates methane gas by various methanogens. The growth of methanogens requires low hydrogen concentration in the growth medium (Khan et al. 2017; Leonzio 2016; Kadam and Panwar 2017).

Addition of nanoparticles has led to emerging results in the anaerobic condition. Nanoparticles act as an electron donor or acceptor and also enhance the activity of various enzymes which is responsible for biogas production (Romero-Guiza et al. 2016; Liu et al. 2013; Yang et al. 2013; Abdelsalam et al. 2016). Nanomaterials enhance the substrate utilization rate at various steps. Su et al. (Su et al. 2013) reported that adding the zerovalent iron nanoparticles ensures to improve the production of biogas from waste materials (Su et al. 2013; Karri et al. 2005).

### 6.5.3 Biodiesel Production

Biodiesel has much promising application in the future due to the emission of fewer pollutants, is eco-friendly, and is produced from edible as well as nonedible oils (De Araujo et al. 2013; Mohammadshirazi et al. 2014). Nonedible oils are considered as microbial oil and plants oil converted into biodiesel through various mechanisms. This process is considered as very beneficial because these nonedible oils are waste and are present in huge amount in nature (Rathore and Madras 2007; Bankovic Ilic et al. 2012).

Nanomaterials have promising results in biodiesel production. Nanoparticles enhance the catalytic reaction during transesterification, thereby improving the production of biodiesel (Chen et al. 2018; Lee et al. 2015). Chen et al. (2018) have reported that the biodiesel production yield was enhanced in the presence of $Fe_3O_4$/ZnMg(Al)O nanoparticles (Chen et al. 2018). $Ca^{2+}$doping in nanomaterials such magnetic nanoferrites has an emerging effect in biodiesel production. This process enhances the biodiesel production yield up to 85% from soybean cooking oils (Dantas et al. 2017).

Various researchers have reported that biodiesel production is also enhanced by using carbon-based nanoparticles. Carbon-based nanoparticles have been used as catalyst in various reactions such as transesterification of vegetable oils (Konwar et al. 2014; Zhang et al. 2014). Ion nanoparticles were incorporated on carbon nanotubes which are responsible for enzyme immobilization. Immobilization improves the efficiency of biofuel production and reduces the production cost. Nanostructure provides emerging immobilization support due to nanoscale size and large surface area. Microbial enzymes such as lipase from *Pseudomonas cepacia* are immobilized on the surface of nanoparticles and enhance the production of biofuel due to enhanced transesterification reaction (Goh et al. 2012).

Functionalization of the nanoparticle process also increases the production of biodiesel. Ion-silica nanoconjugates such as $Fe_3O_4/SiO_2$ have emerging application in biodiesel production. Nanoconjugates can increase the production of biodiesel up to 97.1%. In this process, various types of cooking and algal oils have been used. Algal oils have a high yield of production in the presence of these ion-silica nanocomposites (Chiang et al. 2015).

### 6.5.4 Bioethanol Production

It is one of the most important alternatives to fossil fuel. It is considered as an inexpensive and eco-friendly biofuel. Bioethanol has various advantages such as high evaporation enthalpy and high octane number (Saini et al. 2015; Sarkar et al. 2012; Limayen and Ricke 2012). Currently, bioethanol is produced from edible and nonedible vegetable oils, waste materials, and algal and bacterial biomass. Microalgae produced a large amount of bioethanol. Genetic engineering in microorganisms is also applied to improve production of bioethanol (Kuhad and Singh 1993; Balan 2014).

Various types of nanomaterials have emerging application in the production of bioethanol. Cherian et al. (2015) investigated that $MnO_2$ nanoparticles increase the bioethanol production by using sugarcane leaves. It catalyzes the mechanism of bioethanol production during various steps. In this process, sugarcane leaves are converted into bioethanol. $MnO_2$ nanoparticles provide the broad surface area which is responsible for the binding of enzymes on their active sites and causes the improvement in ethanol production (Cherian et al. 2015).

Bioethanol production using immobilized microbial cells on the surface nanoparticles has been reported in many literatures. Ivanova et al. (Ivanova et al. 2011) have investigated that yeast cells produced additional ethanol when immobilized on the surface of magnetic nanoparticles (Ivanova et al. 2011; Lee et al. 2011).

Lee et al. (2011) also reported that immobilized *S. cerevisiae* cells are responsible for more ethanol production. *S. cerevisiae* cells were immobilized on calcium alginate. These immobilized cells enhanced the bioethanol production yield up to 100% but production yield of suspended cells was 88%. In immobilization process, the cells can be reused up to several times and there are less chances of contamination. Therefore, immobilization is an emerging method used in microbial bioethanol production. Its application with nanotechnology can give better results at commercial-level bioethanol production (Galazzo and Bailey 1990).

## 6.6 Conclusion

This concludes that nanomaterials may play an important part in the enhancement of bioenergy production. Nanomaterials have promising application in biofuel production due to their small size, large surface area-to-volume ratio, and good catalytic properties which are responsible for enhancing the production of various types of biofuel such as biohydrogen, biodiesel, and bioethanol. This manuscript has described the synthesis of various types of nanomaterials and their application to enhance the production of biofuel. Recent trends on the use of nanotechnology in biofuel production were discussed in this chapter. From an up-to-date study in nanotechnology, the authors conclude that these approaches can be used in biofuel industries to enhance biofuel production and minimize the cost of biofuels due to unique structural properties of nanomaterials.

**Acknowledgment** The authors of this manuscript would like to give thanks to the IIT (BHU), Varanasi, for providing financial and technical support.

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# Nanomaterial Synthesis and Mechanism for Enzyme Immobilization

7

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**Abstract**

Enzymes play a significant role in different industries related to food, pharmaceutical, agriculture, and other sectors. Although enzymes find tremendous application in all sectors, their limited reusability and higher cost of production possess hurdle in their wide commercial acceptance. By using different immobilization strategies, this problem can be overcome; however, conventional strategies used for enzyme immobilization, viz., entrapment, encapsulation, and adsorption, are not efficient enough to meet the industry demands. Nowadays, the application of nanotechnology is quite a useful tool in different applications specifically in drug delivery and tissue engineering. The nanoparticle can be effectively and efficiently utilized in the enzyme immobilization to enhance its reusability while retaining its activity prevailing in the free form. This chapter focuses on the application of the novel nanotechnological tools currently used in the enzyme immobilization at the commercial level.

**Keyword**

Nanomaterial · Nanomaterial synthesis · Enzyme immobilization · Biofuels

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_7

## 7.1 Introduction

Nanomaterials have become the most active field of research in recent years due to its novel characteristics as compared to traditional materials, thus providing opportunities for new technological innovations and biomedical applications. These nanostructured materials have wide applications in numerous fields like electronics, optics, electrochemistry, biomedical science, and magnetism. These miniature particles are known to possess enhanced magnetic, optical, electronic, and mechanical properties as compared to larger counterparts of the same chemical composition. Also, the need to synthesize fine materials to enhance the speed of information transmission, decrease costs, and increase the storage of nonmaterial has stimulated their development in this field (Fernández-Pacheco et al. 2006). Table 7.1 enlists typical nanomaterials (Rao and Cheetham 2001). There has been explosive growth in nanotechnology field owing to the accessibility of new techniques of

**Table 7.1** Methods of synthesis of nanomaterials

| Synthesis method | Main principle | References |
|---|---|---|
| Sol-gel process | Nanoparticle synthesis by hydrolysis and condensation of inorganic salts or alkoxides in the presence of catalyst (mineral base or acid), followed by aging, drying, and calcinations | Rahman and Padavettan (2012) |
| Hydrothermal synthesis | Nanoparticle synthesis in an autoclave under controlled temperature and pressure conditions. Solvent used is aqueous | Qiu et al. (2011a) |
| Facile microwave-assisted hydrothermal synthesis | Nanoparticle synthesis in microwave synthesizer under controlled temperature and pressure conditions. Solvent used is aqueous | Qiu et al. (2011b) |
| Solvothermal synthesis | Nanoparticle synthesis in microwave synthesizer under controlled temperature and pressure conditions. Solvent used in non-aqueous | Xia et al. (2003) |
| Gas-phase combustion synthesis | Synthesis of nanoparticles in flame | Aruna and Mukasyan (2008) |
| Sonochemical synthesis | High-intensity ultrasound waves are used for nanomaterial synthesis | |
| Microwave synthesis | Nanoparticle synthesis in microwave reactors with optimum irradiation strength, reaction temperature, and inner pressure of the vessel | Bilecka and Niederberger (2010) |
| Arc-discharge synthesis | Single step for the formation of well-coated and homogenous particles in a chamber where a product is deposited on the cathode due to temperature gradients | Arora and Sharma (2014) |
| Solution combustion synthesis | The initial reaction mixture is an aqueous solution | Mukasyan and Dinka (2007) |
| Condensed-phase combustion synthesis | The initial reactants are in the solid state | |
| Chemical vapor condensation (CVC) | Nanoparticles are synthesized by decomposition of metal-organic precursors in high-temperature flame | Rahman and Padavettan (2012) |

nanomaterial synthesis in addition to characterization of these nanomaterials. The synthesis involves organic, biological, and inorganic systems with control of size, structure, and shape.

Recently, synthesis of nanomaterials has been given much attention, particularly in terms of its shape and size as these two factors primarily influence the performance of nanomaterials. Size of nanomaterials mainly affects its distribution in the biological system, while its shape influences its interaction with the target molecule. Besides shape and size, surface area, morphology, and surface functionality of nanomaterials must be taken into consideration as these factors help to inhibit microbial growth. In order to synthesize nanomaterials with desired characteristics, it is essential to select proper synthesis routes. For example, gas-phase routes like laser ablation, arc discharge, chemical vapor deposition, and plasma torch have been used in bulk synthesis of specific nanoparticle morphology like nanorods and nanotubes (Jiang et al., 2014). Also, control over process parameters like applied voltage, temperature of the reactor, gas composition, nature of starting material, and input power helps in determining the shape and size of nanomaterials.

Several approaches are used for the synthesis of these nanomaterials. Some of these are template-based synthesis, arc discharge synthesis, microwave synthesis, microfluidic synthesis, solution combustion synthesis, sol-gel synthesis, hydrothermal synthesis, solvothermal synthesis, sonochemical synthesis, mechanical milling, microemulsion synthesis, chemical vapor condensation, one-pot synthesis, etc.

The synthesized nanomaterial is used for various applications. Enzyme immobilization is an essential application of the nanomaterial. Various methods like adsorption, cross-linking, entrapment, and membrane confinement are utilized to develop immobilized enzymes. Immobilized enzymes have broad industrial applications in the bioenergy industry and are therefore of substantial commercial value.

This chapter aims to discuss various methods of synthesis of nanomaterials and their characterization techniques in details. It also aims to explore the various enzyme immobilization techniques used to immobilize the enzymes using synthesized nanomaterials. The chapter also highlights the uses of immobilized enzymes in the bioenergy industry.

## 7.2 Different Methods of Nanomaterial Synthesis

### 7.2.1 Sol-Gel Synthesis

The sol-gel process has become an essential area of recent research as it has found an interesting aspect in the synthesis of nanomaterials. However, this process is largely dependent on various experimental conditions that induce condensation reactions and hydrolysis rate. These conditions are time of reaction, catalyst's nature, concentration and type of starting materials, temperature and pH, concentration and nature of the solvent, water-alkoxide ratio, drying method, and aging (Jafarzadeh et al. 2009). The chief merit of this process is that it controls the size,

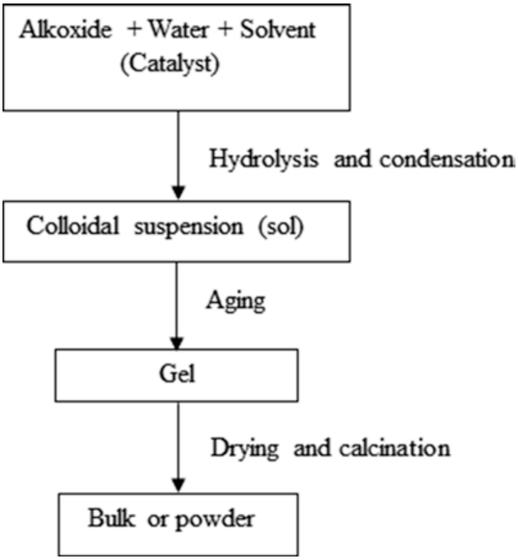

**Fig. 7.1** Sol-gel process. (Chen and Mao 2007)

morphology, and distribution of particle by carefully monitoring and manipulating the parameters of the reaction process. It also has the ability to produce homogenous and pure products at mild conditions. The process involves hydrolysis and condensation of inorganic salts or alkoxides in the presence of a catalyst (mineral base or acid). This leads to the formation of the colloidal suspension. After complete polymerization, there is a loss of solvent, and liquid sol turns into a solid gel. Further drying forms the fine, porous, and low-density material (Fig. 7.1) (Chen and Mao 2007). Each parameter of reaction is optimized, and a combination of optimal conditions is set to produce small, homogenous, and monodispersed nanoparticles. Drying leads to solid material formation. The different processes used for drying are freeze-drying, supercritical drying, thermal drying, and spray drying. Drying leads to agglomeration that is governed by coalescence and collision of nanoparticles. In addition, aging occurs during drying leading to complex agglomeration due to polycondensation reactions. These reactions increase in the presence of a catalyst and water; hence, it is important to remove water during the drying process. In addition to condensation reactions, hydrodynamic effect, capillary drag, and Brownian motion during drying also lead to agglomeration in an aqueous system (Rahman and Padavettan 2012). Alcohol dehydration reduces agglomeration in the synthesis of silica nanoparticles (Rahman et al. 2008). Out of all the chemical synthesis processes, sol-gel offers better homogeneity, phase-pure powder at low temperature, controlled stoichiometry, high purity, and high flexibility (Gnanam and Rajendran 2011).

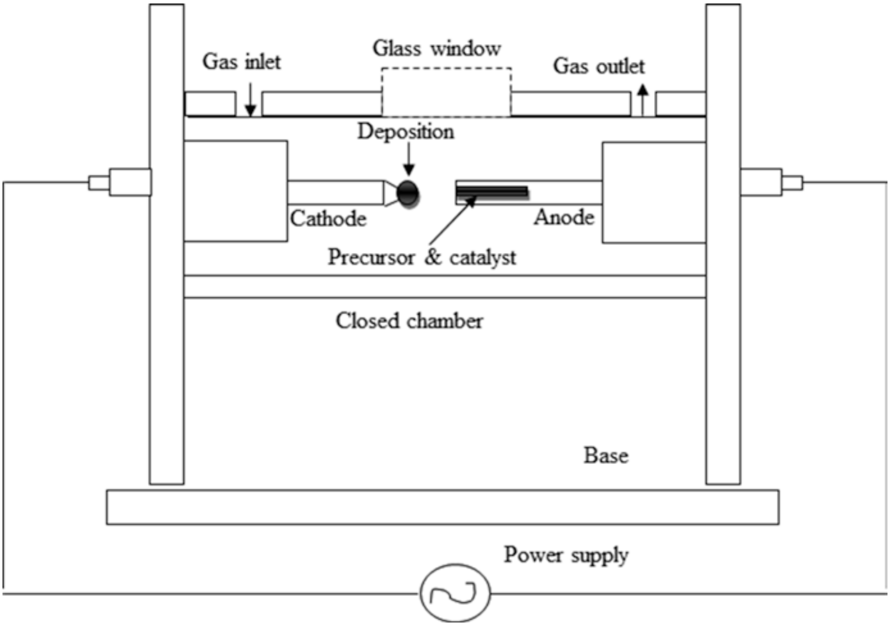

**Fig. 7.2** Arc-discharge setup

### 7.2.2 Arc-Discharge Method

Arc-discharge method, developed by Kratschmer-Huffman, involves a single step in the formation of well-coated and homogenous particles. The apparatus comprises of an evacuated cylindrical chamber filled with helium gas and two electrodes: a stationary anode and a movable cathode (Fig. 7.2). The anode is filled with precursor along with a catalyst. The lifetime of catalyst plays a crucial role in arc-discharge synthesis as they are easily poisoned due to amorphous carbon accumulation (Su and Zhang 2015). The cathode is usually a pure graphite rod in a water cooling system. The pressure inside the chamber is adjusted to reduce fluctuations during the synthesis. This is because any fluctuation in pressure will affect the product to be synthesized. Initially, the anode and cathode are in contact, and on applying a constant current between the two, gradual separation occurs until an arc is formed, which leads to helium plasma ionization. A gap of 1–2 mm is maintained between the electrodes for a steady discharge. The precursor on the anode sublimes and condenses on the cooler parts of the system. The condensed particles are collected on the cathode due to temperature gradient. During the process, the temperature of the system reaches 3000 °C or 4000–6000 K due to the arc current. The arc-discharge method depends on various parameters such as catalysts, type of power supply, electrode geometry, environment, temperature, and pressure (Fernández-Pacheco et al. 2006; Arora and Sharma 2014).

### 7.2.3 Hydrothermal Synthesis

Hydrothermal synthesis is carried out in autoclaves and steel pressure vessels, under controlled conditions (temperature and pressure) in the absence or presence of Teflon liners. The amount of solution in the autoclave and its temperature largely determine the pressure produced internally. The temperature above the boiling point of water is applied achieving a pressure of vapor saturation (Chen and Mao 2007; Qiu et al. 2011a). In facile microwave-assisted hydrothermal synthesis, a microwave synthesizer is used to carry out a reaction rather than an autoclave. This equipment is maintained at a definite temperature with 5 to 30 min of holding time (Qiu et al. 2011b).

### 7.2.4 Solvothermal Synthesis

Solvothermal synthesis uses a non-aqueous solvent, unlike hydrothermal synthesis. A variety of organic solvents can be used so the temperature can be raised much higher as compared to the hydrothermal method. This method has better control over size, shape, and crystallinity of nanoparticles which can be used for its synthesis with narrow dispersity and distribution of size (Chen and Mao 2007). The solvothermal method involves the addition of a reagent (like amines) to a solvent in appropriate ratios. The catalyst regulates the growth of crystals in the solvent. The mixture is then kept to react in an autoclave at appropriate temperature and pressure conditions. The main advantage is the solubility of materials by the application of heat and pressure at a critical point. However, this method gives a poor yield and non-uniform-sized materials, which have less purity and poor morphological uniformity. In addition, the solvents used are not environmentally friendly, making the process complex. The use of autoclaves also hinders the study of mechanisms of growth (Xia et al. 2003).

### 7.2.5 Combustion Synthesis (CS)

Combustion synthesis (CS), also known as self-propagating high-temperature synthesis (SHS), is a cost-efficient and useful technique for nanoparticle production. The combustion synthesis is divided into further categories on the basis of physical nature of initial reaction medium. In conventional SHS, the initial reactants are in the solid state so it is also known as condensed phase combustion. The heterogeneity scale for reactants ranges from 10 to 100 μm, and the reaction temperature is quite high (less than 2000 K). Due to this, SHS cannot be used for the synthesis of large surface area nanostructures (Aruna and Mukasyan 2008). The conventional SHS is characterized by ignition of the reaction mixture by external thermal source and propagation of high-temperature reaction wave through the mixture. It leads to solid material formation without using any additional energy (Mukasyan et al. 2007). To overcome the drawback of SHS, it is combined or modified using a number of other processes such as intensive milling, mechanical activation, and chemical dispersion and using additives, carbon, and reaction fuel. Chemical dispersion is the method of dissolving the SHS powder in dilute acid to remove the impurities and defects in crystals. When an additive is used, it is termed as alkali metal molten salt-assisted combustion. The metal particles are protected from agglomeration and growth as their nucleation occurs in molten salt (NaCl). The salt melts (1083 K) due to the heat generated by the combustion reaction between reducing metal and transition metal oxide of the alkali metal salt. In carbon combustion synthesis, carbon is employed as a fuel in the reaction unlike metals in conventional SHS. This makes the reaction gaseous, and due to the release of carbon dioxide at a high rate, highly porous powders of particle size ranging from 50 to 800 nm can be synthesize easily. In gas-phase combustion synthesis, nanomaterials are synthesized in the flame (Aruna and Mukasyan 2008).

Volume combustion synthesis (VCS) is another mode of CS, which involves the uniform heating of the entire sample until the self-initiation of reaction through the entire volume. However, this method cannot be used for weakly exothermic reactions requiring preheating before ignition since the mode is less controllable. Therefore, conventional CS and reactive solution are combined to form a process called solution combustion synthesis (SCS). SCS is an attractive, effective, novel, rapid, and versatile process for nanomaterial synthesis. The main features of SCS, which make it an effective method, are that firstly, the initial reaction medium is an aqueous solution, thereby allowing reactant mixing at the molecular level and permitting uniform and accurate formulation of composition as desired on the nanoscale. Secondly, the reaction temperature ($T_c$) is high ensuring high purity and crystallinity of the product. Finally, it has a short duration process and allows the rapid synthesis of nanosize materials as it does not require high-temperature product calcinations. It favors the synthesis of nanosize powders having a high surface area due to the formation of various gases, which also inhibits the growth of particle size (Mukasyan et al. 2007; Mukasyan and Dinka 2007).

### 7.2.6 Microwave Synthesis

The microwave synthesis is a fast technique for nanomaterial synthesis and has great potential in case of nanomaterials whose growth is highly affected by slight changes in the reaction conditions. This technique allows the controlled and efficient heating by manipulating the microwave irradiation conditions. The setup mainly involves the microwave reactors available commercially for the chemical synthesis and use of microwave irradiations (frequency −0.3 to 300 GHz; wavelength −1 nm to 1 m). The matter is heated using microwave dielectric heating that involves the absorption of microwave energy by a specific material and its conversion to heat energy. This forms the principle of microwave synthesis. The microwave photon energy is too low, i.e., $1.0 \times 10^{-5}$ eV (1 J $mol^{-1}$) at 2.45 GHz frequency to break the chemical bonds. Thus, the process depends upon the effective heating of material and not on chemical reaction induction. The microwave heating mechanism involves dipolar polarization and ionic conduction. When the samples are irradiated with microwaves, ions or dipoles try to reorient in the electric field continuously as EM radiation produces an oscillating field. This causes molecular friction and dielectric loss, depending on orientation and disorientation time scales, leading to the production of heat. If the dipole fails to realign, there is no heating. Dielectric loss is due to the lagging of polar molecular rotations behind the electric field in liquid at 2.45 GHz frequency. The ionic conduction process involves the production of heat due to the collision of dissolved charged particles, oscillating back and forth under microwave irradiation effect, with the surrounding molecules. This process produces more heat as compared to dipolar polarization. The advantage of microwave synthesis is that it provides uniform internal heating as compared to other synthesis processes. Other advantages include control of reaction parameters for better product quality, selective heating, high product yield, high rates of heating to speed up the reaction rate, high-throughput synthesis, indirect reactant, and heating source contact and atomization. The limitations include the use of costly microwave reactors and scale up the problem as microwave irradiation has a short depth of penetration in a liquid medium (Bilecka and Niederberger 2010).

### 7.2.7 Experimental Tools and Characterization of Nanomaterials

Characterization of individual nanostructure requires extreme accuracy, sensitivity, and high level of resolution at the atomic level. The various structural characterization techniques used widely are electron microscopy (EM), scanning electron microscopy (SEM), transmission microscopy (TEM), small-angle X-ray scattering (SAXS), X-ray diffraction (XRD), and scanning probe microscopy (SPM). The chemical characterization techniques include ionic spectrometry, optical spectroscopy, and electron spectroscopy.

### 7.2.8 Structural Characterization

The structural characterization of nanostructures involves the techniques that analyze the surface properties of these nanomaterials. The following techniques are used in the structural characterization of nanomaterials.

### 7.2.9 X-Ray Diffraction (XRD)

X-ray diffraction is a strain-sensitive and nondestructive technique that has been used widely for determining the lattice parameters, geometry, composition, identification of unknown materials, thickness, crystallinity, and also the defects and stresses of nanoparticles and nanowires (Cao 2004). In this technique, a monochromatic X-ray beam of wavelength range of 0.7–2 Å generated by bombardment of electrons on a metal in a tube is incident on the surface of the crystal. The electron

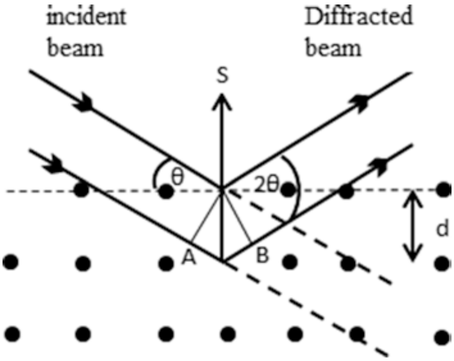

**Fig. 7.3** X-ray diffraction. *S* scattering. (Adapted from Moram and Vickers 2009)

cloud of the atoms of crystal scatters these rays, and constructive interference occurs which follows Bragg's law as path difference (nλ) equals 2d sin θ (Fig. 7.3).

According to Bragg's law, "the spacing between the planes of atoms from which diffraction is occurring (d) to the angle (θ) at which the incident monochromatic beam must probe the plane to give constructive interference." The 2θ angle, corresponding to the intensity of X-rays, can be measured experimentally, and the crystal acts as a 3-D diffraction surface. d is interplanar spacing in the crystalline phase and λ is the wavelength of X-rays. The real space-associated crystal planes produce reciprocal space-associated diffraction spots, forming a diffraction pattern. The position and shape of spots are related inversely to the size of crystallites and spacing of crystal planes (Moram and Vickers 2009). The main advantage of XRD is that it does not involve the preparation of large samples, as it is a nondestructive technique. The disadvantage is the low intensity of X-rays and small diffraction, which makes it less sensitive. However, XRD measures diffraction peak positions accurately making it suitable for both homogeneous and inhomogeneous strain. Homogenous strains shift the diffraction peak positions leading to change in d-spacing which corresponds to lattice constant change under strain. In case of inhomogeneous strain, broadening of a diffraction peak occurs which increase with sin θ within a single crystallite. The broadening of peak also occurs due to finite crystal size, but it is independent of sin θ (Cao 2004).

### 7.2.10 Small-Angle X-Ray Scattering (SAXS)

SAXS is an incredible asset for nanomaterial characterization at large distances or small angles and has become popular worldwide. In this technique, the X-rays are scattered which produces strong diffraction peaks from constructive interference. However, the scattering in SAXS is due to the inhomogeneity in size ranging from several nanometers to ten nanometers (Jeng et al. 2010). It is an effective tool for determination of size and shape of the object, surface structure, and size distribution. It also determines the relative position of a particle from which pair potential and equation of states can be inferred. It is a nondestructive technique and provides structural data for large volumes of samples. According to SAXS theory, the intensity scattered by a number of noninteracting nanoparticles having a uniform electron density in a homogeneous medium (Li et al. 2016) is given by

$$I(q) = I_0 N(P - P_0)^2 F^2(q)$$

where $I_0$ is the intensity of incident X-ray, $I(q)$ is SAXS intensity, $N$ is the number of noninteracting nanoparticles, $P$ is uniform electron density, $P_0$ is electron density of the homogeneous medium, and $F(q)$ is form factor which is expressed as

$$F(q) = 4\Pi R^3 \frac{\{\sin(qR) - qR\cos(qR)\}}{(qR^3)}$$

where $R$ = radius of sphere, $q$ = [4Π sin ($\theta$/2)]/$\lambda$, $\lambda$ = wavelength of X-ray, and $\theta$ = angle between primary and scattering beam of X-ray (Cao 2004).

### 7.2.11 Electron Microscopy (EM)

Electron microscopy helps to characterize and visualize the nanomaterials due to its high resolving power as it uses a low wavelength electron beam, which is below the nm range. There are two main techniques of EM, namely, scanning EM and transmission EM.

### 7.2.12 Scanning Electron Microscopy (SEM)

This technique employs low energy electron beam ranging from 1 to 30 keV, which scans the sample surface and detects the scattered electrons using detectors which select unique ranges of energy from the scattered signal (Dudkiewicz et al. 2011). In this technique, the electron source is focused into a beam and is incident on the sample mounted on aluminum or carbon stubs. The electrons strike and penetrate the sample resulting in various reactions leading to photons and electron emission from the sample. The cathode ray tube (CRT) collects the electrons and photons emitted and produces the images. SEM produces three types of images, namely, secondary electron images, backscattered electron images, and elemental X-ray maps. The interaction between an atom and a high-energy primary electron results either in elastic scattering with nucleus or inelastic scattering with electrons. During inelastic scattering, energy is transferred from the primary electron to another electron, and if the transferred energy is large, then another electron present in the sample emits it. If the energy of emitted electron is less than 50 eV, it is known as a secondary electron. The electrons, which are scattered elastically and have the same energy as the primary electron, are called backscattered electrons, and it increases with atomic number (Cao 2004). The secondary electrons (SEs) having lower frequency provide data on topological surface, and high-energy backscattered electrons (BSEs) provide information mapping contrast which depends upon the atomic number of elements in a sample. The image produced has a visible contrast of light and heavy elements. The BSE detectors give contrast, when the atomic number difference is equal to or less than 0.1, and thus have superiority of simple data interpretation over other techniques. The best resolution was reported for platinum in which 6–9 nm was achieved at 25 keV (Brodusch et al. 2013). BSE imaging is the main feature of SEM (Dudkiewicz et al. 2011). The resolving power of SEM

$$R = \frac{\lambda}{2NA}$$

where $\lambda$ = electron wavelength and $NA$ = numerical aperture of objective and condenser lens.

### 7.2.13 Transmission Electron Microscopy (TEM)

This technique uses electrons with energy ranging from 100 KeV to 1 MeV, which is projected onto the sample with the help of a condenser lens. The thickness of the sample should be less than 200 nm for proper penetration. As the electron beam penetrates, the electrons experience scattering in the sample, which is generally of two types, viz., elastic and inelastic scattering. Depending on the type of scattering, various types of data are acquired. In the case of elastic scattering, there is no loss of energy, and hence diffraction patterns are obtained, whereas inelastic interactions lead to spatial variation in transmitted electron intensity. Inelastic interactions occur due to heterogeneity, viz., dislocations, defects, density variations, boundaries, etc., and it causes complex absorption. TEM has high magnification due to the application of small electron wavelength λ (Cao 2004). This resolution largely depends upon sample thickness and voltage at which the electron beam is accelerated. The higher the voltage at which the electron beam is accelerated, the better the resolution of the image (Dudkiewicz et al. 2011). The electron wavelength is given by de Broglie relationship:

$$\lambda = \frac{h}{\sqrt{2mqv}}$$

where $m$ = mass and $q$ = charge of electron, $h$ = Planck's constant, and $v$ = voltage at which the electron beam is accelerated.

The advantage of TEM is its high resolution ranging from 50 to $10^6$ and greater electron penetration due to high-energy electron which interacts weakly with sample matter as compared to low-energy electrons. It makes TEM suitable for operation at high voltage with thicker samples. Another advantage offered by TEM is that it provides both diffraction information and image from the same sample by altering the intensity of the intermediate ocular lens. The only shortcoming is the limited depth resolution of TEM as the scattering information from a 3D sample is projected on a 2-D detector (Cao 2004).

### 7.2.14 Scanning Probe Microscopy (SPM)

Scanning probe microscopy is a recently developed tool for the characterization of nanomaterials. This technique has revolutionized in the last few years due to its wide applications at nanoscale characterization. This technique provides three-dimensional real space images, which give it an edge over other characterization techniques. It uses two types of probing forces depending on which two members of SPM, viz., scanning tunneling microscopy (STM) and atomic force or friction force microscopy (AFM/FFM). Both techniques provide three-dimensional topographic images in real spaces with high atomic resolution. It helps to characterize all types of solid surfaces whether electrically conductive or not or hard or soft (Alex and Gruverman 2004).

Scanning tunneling microscopy, developed by Binning and Rohrer in 1981 (Bhushan 2008), is based on the phenomenon of electron tunneling. The sample electrons cannot move through the insulator due to the energy barrier. On applying the voltage across the surfaces, energy barrier is changed, and the electrons move across the surfaces by tunneling under the influence of a driving force. The movement of electrons produces a small amount of current given by

$$I = e^{-2kz}$$

where $z$ = distance between two surfaces or insulator thickness and $k$ is given by

$$k = \sqrt{2m}\left(V - E\right)/h$$

where $m$ = mass of electron, $E$ = energy of electron, $V$ = insulator potential, and $h$ = Planck's constant.

The conductive tip is placed on the top of the sample at some height, and as the tip moves over the sample, its position is used to obtain a topographic map (Fig. 7.4). The tunneling current is kept constant by adjusting the distance between the conductive tip and sample. The limitation of STM is that it can only be used for a surface, which is electrically conductive as it depends on tunneling current. STM can be operated in two different ways (modes). Constant voltage mode involves keeping the position of the tip constant, and constant current mode involves keeping the distance between the tip and sample constant. Generally, the height is maintained between 0.2 and 0.6 nm, producing a current of 0.1–10 nA. The image has a resolution of 0.01 and 0.002 nm in XY and Z direction, respectively, and thus produces a 3-D image (Cao 2004; Xu et al. 2009).

Atomic force or friction force microscopy, developed in 1985, was introduced as a modification for STM as it can be used for dielectric materials (Bhushan 2008). Both long-range and short-range forces operate in AFM involving van der Waals forces between the tip and sample. It has three components, namely, induced dipole, permanent dipoles, and electronic polarization. The principle is based on the deflection of cantilever by a photodiode which records the laser beam position reflected off the top of cantilever (Fig. 7.5) (Bhushan 2001; Cao 2004).

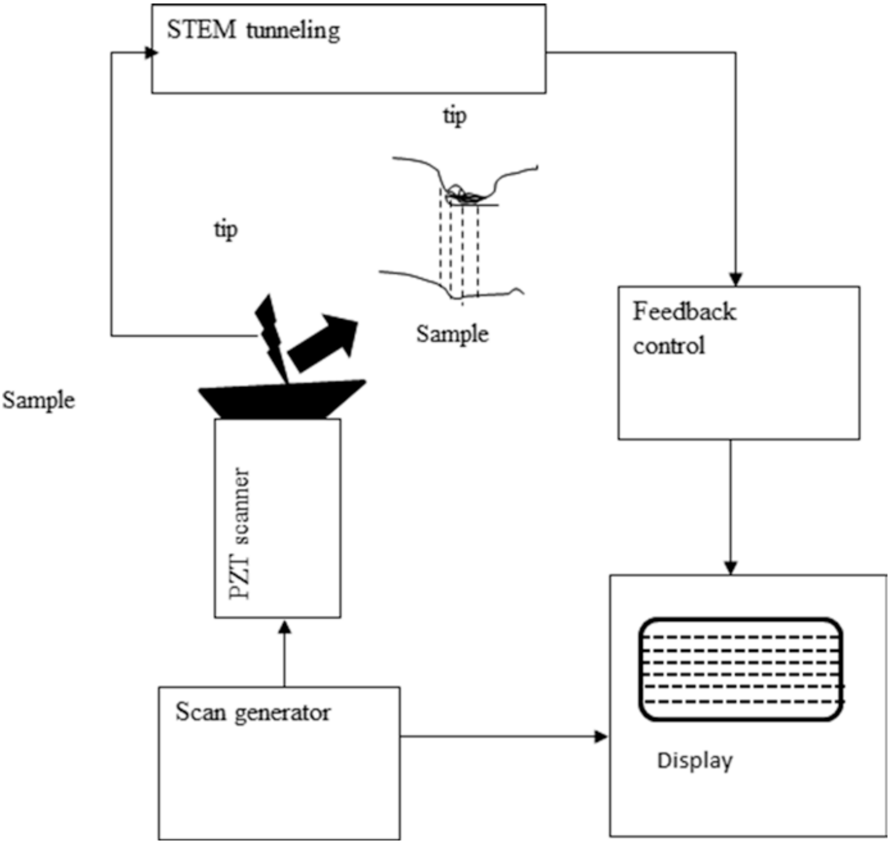

**Fig. 7.4** Structure of scanning tunneling microscope

Near-field scanning optical microscopy (NSOM) is another member of SPM that involves the irradiation of sample through a tapered sub-wavelength aperture in probe. This leads to the formation of waves corresponding to the wavelengths of the diameter of aperture only. These waves are unable to spread in free space and are called evanescent waves. However, these transfer the radiative energy on the other side of the screen by winding around the aperture. Three zones of energy density are formed depending on the distance from the screen. At a distance of 2–5 nm, the intensity is constant and is known as evanescent wave regime. At a distance of 5–500 nm, the intensity decreases proportionally to $s^{-3.7}$ (distance from aperture). This is known as near-field regime and has a very small energy density ranging from $10^{-4}$ to $10^{-6}$. In this regime, the evanescent waves evanescent. The energy density decreases by $s^{-2}$ at a distance larger than wavelength and is known as far-field regime. Hence, it is important to keep the sample-tip distance in the nm range (near-field regime) so the intensity is sufficient for detection by the system. During the process, two types of images are produced: topographic image by scanning force

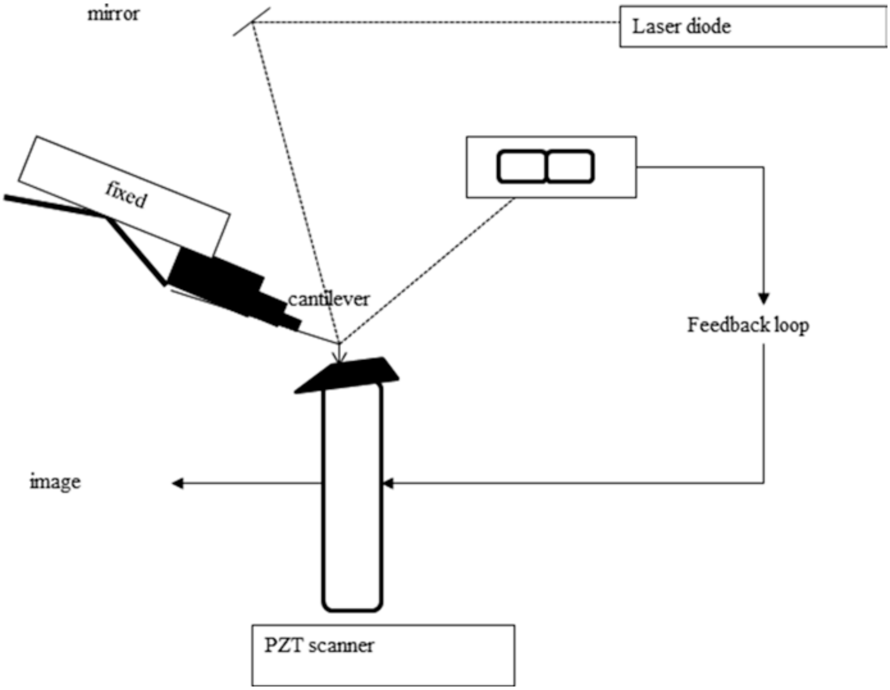

**Fig. 7.5** Principle of atomic force microscope. (Adapted from Cao 2004)

microscopy and optical image by near field microscopy. The setup is similar to AFM and allows access to sub-wavelength scales ranging from 50 to 100 nm by breaking the diffraction limit (Cao 2004; Rao and Cheetham 2001).

Magnetic force microscopy (MFM) is another member of SPM and was discovered in 1987 by Martin and Wickramasinghe. It is based on the measurement of force between the magnetized tip and magnetic or diamagnetic sample. In MFM, a large volume of the sample interacts with a larger volume of sample, so the resolution is poor (20 nm) as compared to STM or AFM. It helps in the magnetic characterization of nanomaterials involving the orientation and magnitude of every spin in the sample and its 3-D location (De Lozanne 2006).

### 7.2.15 Chemical Characterization

The chemical characterization of nanostructures involves the techniques that are used to determine the atoms and compounds present inside and at the surface of the sample and their spatial distribution. The most popular methods are described below.

### 7.2.16 Optical Spectroscopy

The techniques of optical spectroscopy are divided into absorption spectroscopy, emission spectroscopy, and vibrational spectroscopy. In absorption spectroscopy, the excitation of electrons occurs from ground state to excited state (absorption) and in emission spectroscopy; the relaxation of electrons occurs from the excited state to ground states (emission). These techniques help in determining electronic structures of molecules, ions, and atoms of crystals of sample. In vibrational spectroscopy, the photons interact with the sample particles, and energy is transferred between them through vibrational excitation and de-excitation. This technique determines the types of chemical bonds in the sample.

#### 7.2.16.1 Photoluminescence and UV/Vis Spectroscopy

Photoluminescence is a phenomenon of light emission by a material through the relaxation of electrons as they shift back from excited states to ground state by the emission of some energy in the form of light. When light strikes the sample, electrons are excited which produces luminescence that can be collected and passed by a lens through the optical spectrometer to a photon detector. The major benefit of this technique is high sensitivity which allows the analysis of very small quantities, ranging from nanograms to parts per trillion (Kumar 2013).

#### 7.2.16.2 Raman Spectroscopy

The technique is an effective tool for determining the scattering of light by photons and to analyze the electronic and structural properties of the sample. It is a type of vibrational technique. It involves a combination of high-frequency radiations with chemical bond vibrations. This technique is more sensitive for determining the strength, length, and distribution of chemical bonds excluding the chemical composition. When the photon is incident on the sample, it interacts with the chemical bonds in the material leading to the excitation of chemical bond to higher energy level. Two types of scattering occur: Rayleigh scattering and Stokes scattering. The former is due to the reradiation at a frequency same as that of incident light used for excitation. The latter is due to the excitation of vibrational modes due to the energy transfer. Further reradiation will have lower frequency as compared to incident light. A third type of scattering, known as anti-Stokes scattering, occurs when the existing exciting vibrations combine and add their energies to incident beam (Kumar 2012).

### 7.2.17 Electron Spectroscopy

This technique involves the energy levels of emitted photons or electrons from the atoms. When the photon strikes, there is release of energy either in the form of X-rays or fluorescent X-rays or Auger electrons. Every element possesses a peculiar and characteristic electronic structure with predefined energy levels. Also, the Auger spectral lines and X-rays are specific for each element in the periodic table. Thus, the determination of composition and chemical character of a sample can be done by measurement of X-rays and Auger electron energies emitted by them. On this basis, types of electron spectroscopy are discussed below.

#### 7.2.17.1 Energy Dispersive X-Ray Spectroscopy (EDS)

The fundamental principle of this technique is the X-ray emission when an outer shell electron dissipates its energy to complete the inner shell electron vacancy. The excess energy is released in the form of an X-ray. When an electron or photon is incident on an unexcited atom, electron vacancy is created by the ejection of an electron from the inner shell of the atom (Cullity and Stock 2014).

#### 7.2.17.2 Auger Electron Spectroscopy (AES)

Pierre Auger discovered the Auger electron spectroscopy and it is a nonradiative process. The Auger process occurs when the Auger electron, a third electron from a further electron shell, is ejected to fill the electron vacancy in the inner shell. These Auger electrons have very small mean free path in solids. This Auger effect largely influences the fluorescence yields and X-ray line width broadening by shortening the vacancy state lifetime (Carlson 2013).

#### 7.2.17.3 X-Ray Photoelectron Spectroscopy (XPS)

It involves the use of low-energy X-rays for the electron ejection from an atom through photoelectric effect:

$$E_E = hv - E_B$$

where $E_E$ = energy of ejected electron, $hv$ = energy of incident electron, and $E_B$ = bound electron state.

The ejected electron energy by photoelectric effect is known as photoelectron energy, and as the binding energy is element specific, the chemical composition can be determined by measuring the photoelectron energies.

### 7.2.18 Ionic Spectrometry

#### 7.2.18.1 Rutherford Backscattering Spectrometry (RBS)

This technique is discovered by Rutherford for the characterization of thin films, and the basic principle involves the use of beams of high energy, generally in MeV, of ions with low mass. In this technique, the collimated alpha particles are incident on a thin film, and almost all these incident particles reappear on the far side of thin film with small reduction in energy and slight alteration in direction. This implies that the incident beam is transmitted with minor losses. Very few alpha particles interact with the nucleus of the atoms of the thin film, and as a result, they are lost with large changes in energy and direction. In the case of thick samples, the particles are scattered backward at an angle of 90°, and they are detected. The backward scattering is due to the repulsion between ion and nucleus called coulomb repulsion, and this is called Rutherford backscattering (RBS). The collision is elastic and unresponsive to chemical bonding of atoms of sample. By measuring the backscattered ion energy and number, the atomic data and its concentration can be determined as the backscattered energy depends on energy and mass of incident ion and mass of target atom. It also depends upon the scattering angle (Chu 2012).

#### 7.2.18.2 Secondary Ion Mass Spectrometry (SIMS)

This technique is used for analyzing exceedingly lesser concentration of elements or atoms present in a solid which is a major limitation of other analytical techniques. It can detect all the elements of periodic table, unlike RBS which can only detect those elements whose spectra do not overlap. In this technique, ions are incident on the sample surface, and it scatters most of the neutral atoms and a few positive as well as negative ions from the outermost shell. These ions have mass which is analyzed to determine the type and concentration of the atomic species in the sample. There are generally two types of SIMS, viz., static and dynamic. Static SIMS involves data collection before the incidence of surface bombardment and thus determines the surface structure. Dynamic SIMS depends on scattering rates of ions and thus helps in-depth analysis (Cao 2004).

## 7.3 Enzyme Immobilization

Enzymes play a vital role in biocatalysis owing to their high substrate specificity and simple production and posing lesser threats to the environment. For significant development, the utilization of enzymes becomes finite, as they are not as cost-effective because of their high cost and low recyclable factor. Throughout the biochemical reaction, in the presence of an organic solvent at a higher temperature and acidic or alkaline pHs, these enzymes are unable to reuse as these cannot conserved from the reaction medium and affects the molecular structure of enzymes (Naqash et al., 2019). To overcome this, immobilization of enzymes with practical potency and enhanced reliability is more straightforward despite their high cost. Whole cells and enzymes are used as immobilized biocatalysts frequently (Kawaguti et al., 2006). Immobilized enzyme refers as a catalyst that constricts to a certain extent; however, their catalytic activities are preserved (Brena and Francisc 2006). Immobilized enzymes are essential, as they can be reused and able to sequester an enzyme, enhance stability and favorable variations in temperature and pH, and maintain activity for an extended duration (Soozanipour and Taheri-Kafrani 2018). Immobilized enzyme can be attained by binding the enzyme enclosed by a carrier matrix. The enzyme molecule and the product formed ought to move naturally in and out of the part to that enzymes were controlled. Biocompatible carrier matrices that are insoluble in water used ought to have a vast surface area with functional groups (Naqash et al., 2019). Carrier matrices used for enzyme immobilization should have the following properties (Singh 2009) as shown in Fig. 7.6.

Selection of carrier matrices for the enzyme immobilization should have properties as mentioned earlier. These carrier matrices can be categorized as organic (natural and synthetic) and inorganic. Natural organic carrier matrices include agar,

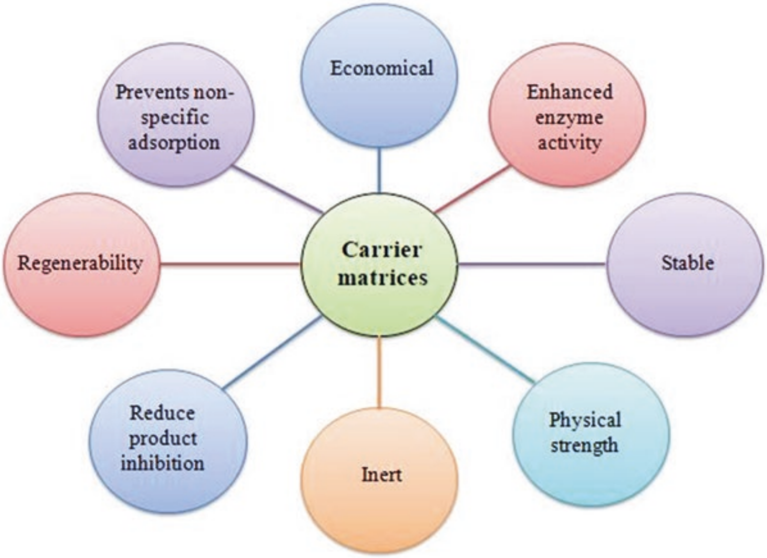

**Fig. 7.6** Properties of carrier matrices

cellulose, agarose, dextran, chitin, albumin, alginate, and collagen, while synthetic organic carrier matrices include polynorbornenes, polystyrene, polymethacrylate, polyacrylate, polyacrylamide, polyamide, polyisobutylene, and polyglycerol. Inorganic carrier matrices include bentonite, silica, glass, metals, and hydroxyapatite. Popat et al. (2011) reported that eco-friendly carrier matrices are biocompatible, biodegradable, efficient, and economical and prevent ethical issues. Eco-friendly carrier matrices such as coconut fibers show increased cation exchange property and better water-holding capacity; kaolin shows increased retainability of the enzyme upon acetylation; cellulose has the irreversible binding ability, and mesoporous materials have functionalized thiol group.

Immobilization of enzymes onto carrier matrices results in higher productivity due to their long-term reusability, with further definite control of the degree of reaction, the potential of mechanization, and steady operation and abolition of enzyme inactivation (Pundir 2015). An immobilized enzyme has potential merit: (i) easy to handle, (ii) low contamination, (iii) productive recovery of enzyme, (iv) reusable, (v) minimum chances of allergenicity, (vi) increased enzyme stability, and (vi) higher productivity and low cost. Immobilized enzymes also have some demerits that restrict its application such as high cost of carrier matrices, reduced enzyme activity, and steady mass transfer by the carrier matrices. At present, immobilized enzymes like lipase, glucose isomerase, and lactase contribute to extensive production (Gutarra et al., 2016). Expansion in enzyme immobilization thus leads to the use of nanomaterials. These nanomaterials enhance the immobilization because of their increased surface area in comparison to the conventional carrier matrices. Enzyme immobilization may be implemented either reversibly or irreversibly.

Reversible immobilization allows adsorption directly to the surface by encouraging the interaction through hydrophobic interactions, hydrogen bonding, electrostatic affinity interactions, and van der Waals forces, thus enabling the recovery of carrier matrices. In irreversible immobilization, enzymes are covalently attached to the surface or entrapped within a carrier matrix to halt its leaching; however, the carrier matrices cannot be reused. Specific properties of carrier matrices are essential to attain an optimal enzyme immobilization. Up-to-date advancement in nanotechnology has enhanced multiple alternatives for immobilized enzyme. Nanomaterials embellish better substitutes because of their small surface area, increased diffusivity, and low mass transfer rate. The amalgamation of nanomaterials with enzymes forms nanobiocatalyst, which contributes to green technology by replacing the use of chemical catalysts (Naqash et al., 2019). Enzyme immobilization on nanomaterials is an encouraging abstraction for its application in the area of bioenergy.

Moreover, a basic perceptive of biomolecular actions among the enzyme and the nanomaterial surroundings is necessary for its accomplished application. These enzymes show a necessary attribute, by facilitating specific three-dimensional configurations intended for their catalytic action. Enzyme active site is the primary aspect of stabilizing the conversion among the substrate and its product. Varying the whole conformation of the molecule affects biological activity. Usually, the freeze-dried enzyme is used which shows low activity and stability.

Conversely, immobilization of the enzyme on a carrier matrix may provide benefits like elevated catalytic activity, recovery of enzyme, and continuous operation. A study by Chibata (1978) on proteins resulted in 60% better stability on immobilization with 24% unaltered and 16% low stability. Nowadays, the use of nanomaterials is intended for elevating enzyme retention with increased biocatalytic activity and efficiency. For enzyme immobilization, nanomaterials such as nanofibers, mesoporous media, nanorods, carbon nanotubes, and nanocomposites are used resulting in increased stability and quantity of immobilized enzymes owing to their enhanced surface area and biocompatibility. Production of active biological systems by the interaction of enzymes and nanomaterials involves the expansion and characterization of nanomaterial and assessment of biological activity (Andreescu et al. 2008).

## 7.4 Techniques for Enzyme Immobilization

For immobilization of enzyme, various methods are reported. These techniques can be categorized into either reversible or irreversible or based on their chemical reaction. Each immobilization methods have definite merits and demerits. Characteristics of enzyme and carrier matrices are essential for the assortment of a suitable technique for enzyme immobilization (Adhikari 2019). There are four techniques of enzyme immobilization:

(i) Carrier-binding method or adsorption which uses synthetic polymers, polysaccharide derivatives, and glass as a carrier matrix

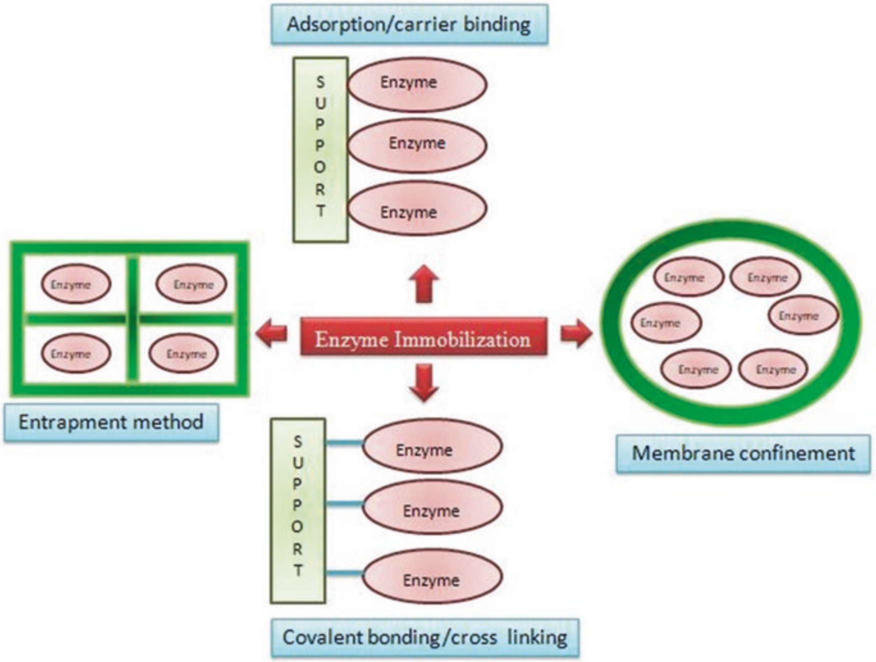

**Fig. 7.7** Various methods of enzyme immobilization

(ii) Covalent bonding or cross-linking method which uses glutaraldehyde, hexamethylene diisocyanate, and bis(diazo)benzidine
(iii) Entrapment method which uses polymers like collagen and κ-carrageenan for entrapping enzyme
(iv) Membrane confinement method which uses microcapsules as carrier matrix

Figure 7.7 represents the different mode of enzyme immobilization

1. *Adsorption/carrier-binding method*

Adsorption or carrier-binding method of enzyme immobilization is a simple method where an adsorbent is being mixed with the enzyme to be immobilized under the appropriate environment and is kept for incubation to wash off loosely bound proteins. A carrier matrix bounds the enzymes through different types of bonding such as hydrogen, ionic, covalent, and van der Waals forces. Selection of the carrier matrix depends on its affinity with the enzyme molecule, as not all enzymes can bind every carrier matrices. It also relies on the particle size of the carrier matrix, active group present on the surface area, and pore size. Carrier matrices are assembled as void porous fiber membrane for adsorption of the enzyme to be immobilized. This method for the production of immobilized enzyme becomes a reversible process as soon as the hydrogen ion concentration, temperature, ionic potency, and polarity of the solvent are reformed (Sneha et al., 2019). Thus, this helps in the noninvasive and straightforward recovery of the enzyme (Qi et al., 2011). This method helps to prevent the physical and chemical modification of enzyme by joining the enzymes inward or outward to the surface of the carrier matrix. Silicone is used to prevent the leakage of the enzyme adsorbed to the customized adsorbent. For instance, *Candida antarctica* lipase B (novozyme 435) prevents leakage of the enzyme by coating the enzyme surface by a silicon polymer (Sneha et al., 2019). Immobilization of lipase by using octyl-agarose and octadecyl-sepabeads has been studied resulting in 97% immobilization. As compared to free lipase, octadecyl-sepabeads attained tenfold increased enzyme stability, whereas the enzyme stability of octyl-agarose is decreased (Cunha et al., 2007). Immobilization of *Candida rugosa* lipase with biodegradable poly(3-hydroxybutyrate-co-hydroxyvalerate) revealed that after 4 h at 50 °C, the residual activity is 94% with reusability till 12 cycles (Cabrera-Padilla et al., 2012). Mateo et al. (2000) observed that coating of polyethylenimine covalently on various adsorbent carrier matrices like silica, agarose, and polymeric resins resulted in effective and strong D-amino acid oxidase β-galactosidase and lipase immobilization. The adsorption between the carrier matrices and enzyme molecule is not stable, whereas enzyme immobilized by covalent bonding is a limitation of this method of immobilization. Sabbani et al. (2006) observed that during the process of biocatalysis, the enantiomeric ratio and reaction rates are higher with the reduction in particle size of carrier matrix Accurel. Mishra et al. (2011) studied the adsorption of urease with 1,4-butenediol diglycidyl resulting in high pH stability with 50% retention activity.

### 2. *Covalent-bonding/cross-linking method*

Enzyme immobilization by covalent bonding results in strong bonding of active groups on the enzyme with the carrier matrices. Chemical properties of carrier matrices and the active groups present on the enzyme molecules are an important factor for enzyme immobilization by covalent bonding (Agyei et al., 2015). Functional groups on the surface of the enzyme molecules are amino group, carboxylic group, hydroxyl group, and sulfhydryl group (Adhikari 2019). Immobilization of enzyme by cross-linking binds the enzyme to the carrier matrices by forming a three-dimensional structure and prevents desorption of the enzyme molecule. This method is an irreversible method that is advantageous, as the hydrogen ion concentration and substrate potency do not reverse. However, the limitation is that this method of immobilization is relatively expensive, and at the time of immobilization, it may block the active site of the enzyme molecule of which the enzyme rigidifies. With an increase in hydrogen ion concentration, the enzyme activity decreases. To overcome this, researches continue to produce a stable and robust immobilized enzyme for the food sector. For example, immobilization of plant protease enzyme (cardiosin A) on agarose-glutaraldehyde has been used as rennet enzyme for cheesemaking and processing of whey in the dairy industry (Agyei et al., 2015). Enzyme immobilization by chitosan and mesoporous silica as carrier matrices resulted in higher thermal stability and half-life of immobilized enzyme. When modified silica gel is used as a carrier matrix, the covalently immobilized enzymes act as highly stable and hyperactive biocatalysts. The process of silanization modifies silica gels where it eliminates the aldehyde groups. Carrier matrices used for immobilization may lead to a decrease in enzyme activity which might be overcome by immobilization by enzyme cross-linking. This immobilization process does not require carrier matrices, which are advantageous over carrier-bound immobilized enzyme system (Agyei et al., 2015). For example, the formation of a bond with an amino group immobilizes (Poulsen 1984). Cross-linking method of immobilization can be classified as (i) cross-linked dissolved enzymes (CLEs), where the enzymes to be immobilized cross-linked together; (ii) cross-linked crystalline enzymes (CLECs), where the crystals of enzymes cross-linked together; and (iii) cross-linking aggregated enzymes (CLEAs), where aggregates of enzymes are cross-linked together. CLECs and CLEAs are used commonly for the immobilization of enzyme because of their easy handling and reliable nature. CLEA method immobilizes enzymes such as chymotrypsin, trypsin, lipase, subtilisin, naringinase, and cellulases, which results in high enzyme stability and improved enzyme activity. This method is inexpensive as the carrier matrices are not used (Agyei et al., 2015). Enzymes cross-linked to electrospun nanofiber increases the porosity and surface area by increasing its residual activity. Cross-linking agents help to sustain the function and structural characteristics of enzymes. Glutaraldehyde is used as a bifunctional cross-linker as it is soluble in aqueous solvents (Datta et al., 2013).

**Case Study**

Cross-linked aggregates of enzymes can form in two steps: (i) protein precipitation and (ii) coagulation. First, the enzyme (5–25 mg of protein/ml) was mixed with ammonium sulfate to attain 60–90% of salt saturation. Second, the mixture was homogenized by using a magnetic stirrer for 2 h at 4 °C. Third, glutaraldehyde was added and mixed using a magnetic stirrer for 24 h at 4 °C. Fourth, centrifugation of the mixture was done at 14,000 rpm for 10 min to separate the enzyme aggregates. And lastly, the aggregates were washed using a buffer and stored at 4 °C (Gutarra et al., 2016).

### 3. *Entrapment method*

Enzymes are immobilized by entrapping it in a polymeric network. This network allows the substrate to pass through and preserves the enzymes. The carrier matrices create a barrier for mass transfer. Entrapment refers to entrapping the enzymes within a fiber or gel matrix. There are different methods of entrapment like temperature-induced gelation (like agarose and gelatin), ionotropic gelation of macromolecules with alginate (multivalent cation), and organic polymerization (like polysaccharide), for instance, entrapping amyloglucosidase or α-amylase with a polyacrylamide gel. Immobilization by entrapping reduces the blockage of enzyme active site (Adhikari 2019). Entrapment of enzyme also controls the thickness of the carrier matrix (Andreescu et al. 2008). The demerit of this process of immobilization is that the enzymatic reactions are retarded because of diffusional control of the product and the substrate and a loss of enzyme from the matrix pore. Enzymes are immobilized by confining it within the insoluble matrix. Thus, it prevents the leaching of the enzymes, but if the carrier matrix is not uniform or the pore size is increased, then leaching may occur. To overcome this, enzymes are premixed with the carrier matrix and form a gel with desired particle size. This method helps to maintain the structural integrity of the enzyme. Studies on the entrapment method of immobilization show that silica can be used as an entrapment material. Forsyth and Patwardhan (2013) reported that entrapment of *Candida antarctica* lipase in bio-inspired silica increases the efficiency of immobilization, enhances the enzyme activity, and betters thermal stability and pH (Agyei et al., 2015). Enzymes are immobilized by using activators like quinoline derivatives, organic salts, tetrathiafulvalene, and ferrocene that help transport the electrons prevailing at the enzyme active site to the surface of electrode (Andreescu et al. 2008). Commonly used carrier matrix for entrapment is alginate as it forms beads which can entrap various macromolecules (e.g., calcium-alginate beads). Shen et al. (2011) reported that use of alginate-gelatin-calcium hybrid carriers for the entrapment provides efficient encapsulation by hampering the leaching of enzyme and increasing the mechanical stability. Electrospun nanofibers when used as carrier matrix for entrapment show a significant impact on immobilization of enzyme (Sneha et al., 2019). Entrapment of *Candida rugosa* lipase in chitosan prevents the leakage and friability of enzyme and augments the entrapment efficiency (Datta et al., 2013). Ispas et al. (2009) studied the entrapment by mesoporous silica which provides uniform pore distribution, higher surface area, and enhanced adsorption capacity. Magnetite and lipase nanoparticles entrapped at the same time in biomimetic silica increase the enzyme activity. Entrapment of *Candida rugosa* lipase done in sol-gel matrices enhances their selective binding (Datta et al., 2013). κ-carrageenan used for entrapment of lipases showed high thermal stability.

4. *Membrane confinement*

Immobilization of enzymes by membrane confinement refers to encapsulation of enzyme in a carrier matrix. This method of immobilization is easy and low cost. Encapsulation can be done by two methods, viz., encapsulation in the matrix and gel technology. Natural or synthetic gels are used as a carrier matrix. Natural gels can be obtained from alginate, agar, or κ-carrageenan and synthetic gels from acrylamide. Gels obtained from acrylamide are used as carrier matrices for entrapment due to their high molecular weight which is cross-linked in the polymer network. These gels absorb the solvent by the process of osmosis or by capillary forces. The internal structure of the gel relies upon the cross-linker. Choice of cross-linkers should be accurate as they modify the pore size. When an optimized gel is used for entrapment, it separates the carrier matrices from diffusional resistances. Agar consists of agarose and agaropectins found in red algae cell wall. It can be degraded enzymatically in two types of agarases by hydrolysis, specifically β- and α-agarases which hydrolyze β-1, 4-linkages and α-1,-3 linkages in agarose, respectively. The α-agarase segregates the α-1-(1,3) bonding of agarose to yield oligosaccharides such as 3,6-anhydro-1-galactopyranose at the reducing end of the sugar (Datta et al., 2013). In contradiction, β-agarase segregates the β-d-(1,4) bonding of agarose to yield neoagro-oligosaccharides with d-galactopyranoside residues at the reducing end of the sugar. Neoagro-oligosaccharides obtained from β-agarase are of great interest due to their various biological functions, like inhibiting the growth of bacteria, retarding starch degradation, and improving the quality of food as low-calorie additives. Thus, agar-derived oligosaccharides have a large area of application in industries such as biodiesel (Kim et al., 2018).

## 7.5 Application of Nanomaterial-Immobilized Enzyme in Biofuel Production

Enzymes play an essential role in different industrial processes focusing on the improvement of enzyme's activity along with its efficiency and stability. Different applications of the nano-immobilized enzyme on bioenergy-based industry have been studied. Table 7.2 represents the immobilized enzyme application in the bioenergy-based industry.

**Table 7.2** Immobilized enzyme application in bioenergy industry

| S no | Immobilized enzyme | Nanomaterial | Carrier matrix | Application |
|---|---|---|---|---|
| 1. | Cellulase | Activated magnetic nanoparticle | Activated magnetic nanoparticle | Enhancing enzymatic saccharification of pretreated hemp biomass |
| 2. | Cellulase | Silica nanoparticle | Microcrystalline cellulose | Production of ethanol and glucose |
| 3. | β-Glucosidase (*Aspergillus niger*) | Iron oxide nanoparticle | Cellobiose | Production of glucose |
| 4. | β-Glucosidase (*Trichoderma reesei*) | Magnetic nanoparticle | Wheat straw, *Eucalyptus globules* pulp | Production of glucose |
| 5. | Lipase (*Burkholderia* sp.) | Ferric silica nanocomposite | Olive oil and methanol | Biodiesel |
| 6. | Lipase (*Rhizopus miehei*) | Silica nanoparticle | Triolein and methanol | Biodiesel |
| 7. | Lipase (*Pseudomonas cepacia*) | Nanoporous gold | Soybean and methanol | Biodiesel |
| 8. | Lipase (*Candida rugosa*) | Carbon nanotubes | Multi-walled carbon nanotube (MWCNT) | Enhanced enzyme activity |
| 9. | Lipase (*Candida rugosa*) | Nanofibers | Polyvinyl alcohol | Equivalent esterification activity similar to Novozym 435 |

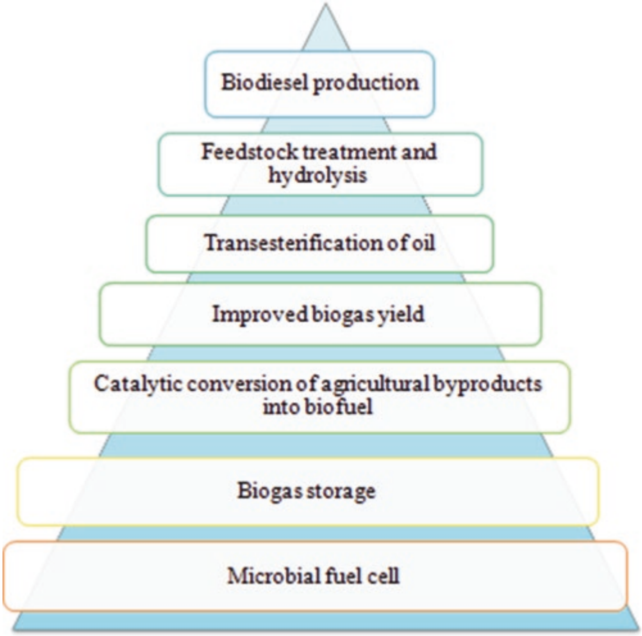

**Fig. 7.8** Schematic view of the application of nano-immobilized enzymes in the bioenergy industry

In recent times, using nanomaterials as a substrate for enzyme immobilization shows an increasing trend for production of biodiesel. This nano-immobilized enzyme contributes to enhancing the stability and activity of the enzyme and reduces the expenses of the enzyme used. Figure 7.8 represents the schematic view of the probable application of nano-immobilized enzyme in the bioenergy industry.

There are reports on application of immobilized enzymes in biofuel production such as covalent lipase immobilization on magnetic nanoparticles for biodiesel production (Wang et al., 2009). The conversion rate of 100% was seen in the first three cycles during the conversion of soybean oil to biodiesel using immobilized lipase (*Pseudomonas* cepacia). This high conversion indicates that immobilized lipase (*Pseudomonas* cepacia) preserves stability and reuse of enzyme during the transesterification of soybean oil. Raita et al. (2015) stated that biodiesel produced from palm oil by using immobilized lipase (obtained from *Thermomyces lanuginosus*) on magnetic nanoparticles showed 97.2% yields with 80% enzyme activity retention (Fig. 7.9).

Karimi (2016) reported the conversion of waste cooking oil into biodiesel using immobilized *Burkholderia cepacia* lipase using superparamagnetic iron oxide nanoparticles with a yield of 91% in 35 hours. Fan et al. (2016) reported that immobilization of *Rhizomucor miehei*-derived lipase onto polyamidoamine grafted with magnetic MWCNT resulted in the conversion of waste cooking oil to biodiesel with a yield of 94%. Nano-immobilized enzyme resulted in increased production of

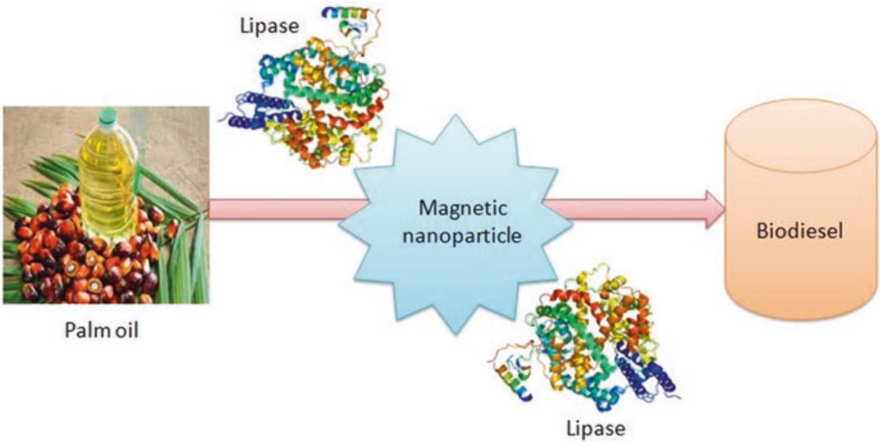

**Fig. 7.9** Use of nano-immobilized enzyme for biodiesel production

biodiesel; for instance, immobilized lipase has shown high biodiesel production obtained from different sources. Soybean oil (Xie and Ma 2010) and waste grease (Ngo et al., 2013) for the production of biodiesel using immobilized lipase obtained from *Thermomyces lanuginosus* covalently bound to magnetic nanoparticles were previously reported. Nano-immobilized enzymes are efficient for the transesterification of the oil. For example, immobilized lipase (*Burkholderia* sp.) adsorbed on ferric-silica nanocomposites is used for the transesterification of olive oil (Tran et al., 2012). Cellulase immobilized by physical adsorption on silica nanoparticles resulted in enhanced ethanol production (Lupoi & Smith 2011). Nano-immobilized enzymes are used to perk up the yield of biogas. Studies have shown the increased and improved yield of biogas by using nanomaterial (Palaniappan 2017). Nano-immobilized enzyme application for the conversion of agricultural by-products to biofuels results in enhanced biodegradability and eco-friendly biofuels. Nanosized molybdenum balls are used for the conversion of palm by-products to biofuels. Various applications of nano-immobilized enzymes in the bioenergy industry are, for the storage of biogas, to recover the performance of microbial fuel cell using nanotechnology (Zhang et al., 2010).

## 7.6 Conclusions

In the modern era, nanotechnology has gained profound attention owing to its vast application in all the fields including pharmaceutical, agriculture, food, and biotechnology. Nanomaterials have been widely used in biomedical and bioprocess applications such as synthesis of biosensors, aerodynamic devices, and also enzyme immobilization. There is extensive research going on the synthesis of various nanomaterials for enzyme immobilization. Although significant efforts have been made in the nanomaterial synthesis and its characterization, it is still in the nascent phase. There is a need to look for an innovative approach for nanomaterial synthesis which should be efficient, less complicated, and economical. The nanomaterial-based enzyme immobilization has shown significant improvement in the enzyme activity and stability. This technology has been quite successful in the different enzyme-based applications as desired in detergent, pharmaceutical, drug, and agricultural-based products. The nanomaterial-based enzyme application has also shown tremendous application in bioenergy industry where it has been successfully used for biodiesel production along with feedstock treatment, biogas storage, and catalytic conversion of lipids into biodiesel. There is further scope of improvement in the approach adopted for nanomaterial synthesis so that it can be widely used at commercial level and should be cost-effective too.

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# 8

# Nanomaterial Synthesis and Mechanism for Enzyme Immobilization: Part II

Ankit Kumar Singh and Ida Tiwari

**Abstract**

Free enzymes do not possess properties of recovery and reusability, and also they are not stable at wide pH and temperature range. Therefore, new ways which can enhance enzyme stability and reusability should be developed, and hence, the immobilization technique is one such approach. These immobilization techniques offer such materials which have the ability to be active in the much wide range of pH and temperature, and also they are more stable than the free enzymes. Immobilization is carried out on the nanosized material either by adsorption, covalent coupling, entrapment, encapsulation or cross-linking. These nanomaterial-immobilized enzymes show several advances over the free enzymes because of large surface area-to-volume ratio, lower mass transfer resistance and high mobility. Several nanomaterials are used for immobilizing the enzymes; however, their recovery from the reaction mixture is very poor. Therefore, the magnetic nanomaterials are more attractively used in immobilization because the enzyme immobilized through magnetic nanomaterial has the tendency to be easily separated out from the reaction mixture. These nanomaterial-immobilized enzymes show wide range of applications in biotechnology, bioanalysis, biomedicine, pathology and biosensors.

**Keywords**

Nanomaterial · Enzyme · Immobilization · Biotechnology · Biomedicine · Nanocarrier

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_8

## 8.1 Introduction

Enzymes are protein molecules that enhance the rate of biochemical reactions, but they are not used up during the reactions. Enzymes are biological catalyst and show a lot of dominance over any chemical catalyst. Besides having high catalytic efficiency, enzymes are also chemoselective, regioselective and stereoselective, and hence, they are widely used in the industries (Gupta et al. 2011). Enzyme can catalyse several chemical and biochemical reactions. Mankind has used enzyme in the processing of food from several millennia. Various raw materials such as oils, fats, carbohydrates, proteins, vitamins, lignin and amino acids are used for the production of several biorenewables. These compounds are also important in our daily life in the form of wood, paper, starch, rubber, fabrics and some other biorenewable materials. However, the enzymatic actions on these raw materials are needed for their transformation into biorenewables under some mild and sustainable conditions (Franssen et al. 2013).

The present-day development and success widely depend on the synthesis of chemicals and their production at industry level in the presence of enzymes. This success is due to the increased number of enzymes and their applications in a wide range of processes (Hanefeld et al. 2013). Enzymes show diverse applications in dairy products: in the preparation of wine, beer, fruit and vegetable juices and in the preparation of paper and pulp. Enzymatic pathways are cost-effective, sustainable and environment friendly. However, the free enzymes have very poor stability towards high temperature and pH, and hence, there are only restricted possibilities of their recovery as well as reusability. In order to remove these limitations and to turn the enzyme activity into industrial applications, numbers of approaches have been tried, and immobilization technique is one such tool for this purpose. These increase the stability of enzyme, its selectivity and also its reusability with the emerging tools. A number of immobilization techniques are available that can utilize newly developed materials that offer high biocatalytic activity and favourable environments that are necessary for the action of immobilized enzymes. In the presence of these favourable environments, activities and stability of enzymes are improved for any applications in comparison to free enzymes because of much wider range of pH and temperature available (Mateo et al. 2007; Goldberg and Kolibas 1990; Iyer and Ananthanarayan 2008; Brady and Jordaan 2009; Stepankova et al. 2013; Chibata 1978). A powerful approach for immobilization of enzymes is by the use of nanomaterials (Franssen et al. 2013). Materials that range from 1 to 100 nm in particle size and show different characteristics from their bulk precursors are called nanomaterials. According to ISO (2015), nanomaterials are defined as materials having external dimension in nanoscale, or materials having internal structure or their surface structure in the nanoscale. Nanomaterials have extremely small dimensions, and hence, they allow us to take advantage of unique physical, chemical, optical, electronic and mechanical properties that are present at nanoscale. For the immobilization of enzymes, nanomaterials are used because they can make up a novel and attractive matrices. Enzymes immobilized on these matrices are highly impactful in the today's research due to the high surface-to-volume ratio of nanomaterials and their Brownian motion. Carbon nanotubes, superparamagnetic nanoparticles and some mesoporous materials are used as some important category of matrices.

There are number of reports of micro- and nanomaterials that have various applications in biotechnology and biomedicine due to their incredible potential in delivery systems and targeting, and it also shows advantages in biosubstance binding (Inès and Dhouha 2015; Kuthati and Kankala 2015; Wang et al. 2015; Löhr et al. 1998). There are some examples of different processes occurring on industrial scale that are using immobilized enzymes including high-fructose corn syrup production (Gupta et al. 2011). A number of substrates such as fatty acids, carbohydrates, proteins, rubber and their building blocks are utilized in the creation of biorenewables in the presence of any immobilized enzymes. The renewable produced in this way shows a wide range of applications, that is, they are acting as building blocks for several industries such as in dairy, textile industries, pharmaceutical and polymer industry and the preparation of additives for food industries. They are also showing several bioanalytical and biomedical applications because they are utilizing immobilized antibodies or antigens. They may make use of some immobilized receptors or ligands and also a variety of immobilized cells. Immobilized antigens or antibodies are used in affinity chromatography, and immobilized receptors or ligands are used in biosensors. In the last few decades, biocatalysis has emerged as an important tool in order to meet the demand for green as well as sustainable synthesis of chemicals that mainly involves the production of pharmaceuticals, vitamins, flavours, fragrances and some other specific chemicals. Additionally, the system of the immobilized enzyme is useful either in aqueous or media with low water content in the field of biocatalysis or in resolution of racemates (Wang 2006; Chiang and Sung 2006; Mikhaylova et al. 2004; Kim et al. 2003; Gao et al. 2003; Dyal et al. 2003; Gardimalla et al. 2005; Zhang et al. 2008; Wang et al. 2009; Lee et al. 2008; Netto et al. 2009).

## 8.2 Synthesis of Nanomaterials

Nowadays, researchers and scientists are working together to develop some new materials having some superior properties, more functionality and lower cost than the on-hand materials. Several physical, chemical and physiological methods have been tried to boost up the performance and behaviour of nanomaterials. Several approaches have been tried for synthesizing nanomaterials in order to get control over distribution of nanoparticles and their particle size (Shibata et al. 1998).

### 8.2.1 Different Approaches for the Synthesis of Nanomaterials

There are two general approaches that are widely involved in the nanomaterial synthesis and the manufacture of different nanostructures. These two main approaches are represented in Fig. 8.1.

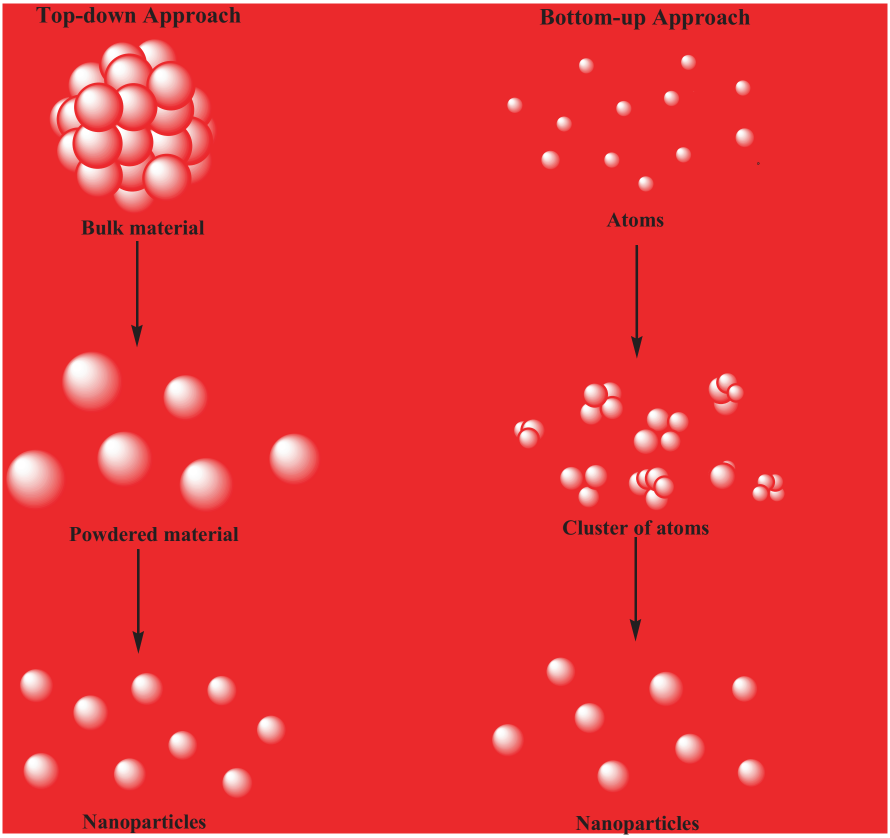

**Fig. 8.1** Scheme showing top-down and bottom-up approaches that are used in the synthesis of nanoparticles

#### 8.2.1.1 Top-Down Approach

This route generally entails breaking of larger particles into smaller ones, and this can be achieved by using several forces like crushing, milling or grinding. These approaches use larger or macroscopic structure as the initial material that is controlled during the processing of nanostructures from the outside. Although this approach is used in the synthesis of nanomaterials, this route is generally not appropriate for the preparation of materials having uniform shape. However, the major problem with this approach is the limitation in the surface configuration. Such limitations or imperfection would have a considerable impact on physical properties and surface chemistry of nanostructures and nanomaterials. These approaches are also slow and hence are not suitable for the production at large scale.

#### 8.2.1.2 Bottom-Up Approach

Bottom-up approach involves the synthesis of material from the bottom: atom-by-atom, molecule-by-molecule or cluster-by-cluster. In these approaches, material components are firstly miniaturized up to their atomic level which later on construct the nanostructures by self-assembling. During the self-assembly process, numerous substantial forces are operating at nanoscale that is used for uniting the fundamental units into the larger established structure. This route is mostly used for the preparation of the nanoscale materials, and it shows the ability for the generation of a uniform size, shape and distribution of the nanomaterials. It has the ability to control the reaction to inhibit the growth of particles further. However, the bottom-up approach is not showing any novelty, but the fabrication and processing of nanostructures and nanomaterials can be easily achieved by these approaches. Researchers always feel challenging to control the particles size distribution, purity, morphology, quality and quantity to a better extent during the synthesis of nanomaterials through environment friendly and economical processes (Cao 2006).

### 8.2.2 Methods Involved in Nanomaterial Synthesis

The top-down and the bottom-up approaches that are used in the synthesis of nanomaterials offer several diversities in the synthesis methods which result in the diversities in the product. There are several methods, namely, physical, chemical, biological or hybrid, that are widely used in the synthesis of nanomaterials such as nanoparticles, nanotubes, colloids, thin films, quantum dots or nanorods. These different methods are shown in Fig. 8.2.

#### 8.2.2.1 Physical Methods

Nanomaterials can be synthesized via physical methods through evaporation and condensation or by applying mechanical pressure and high-energy radiation or by using thermal or electrical energy for the abrasion of the material. These methods are generally based on the top-down approach. Physical methods that are generally used for the synthesis of the nanomaterials are laser pyrolysis, spray pyrolysis, high-energy ball milling, physical vapour deposition, melt mixing, laser ablation, sputter deposition, electric arc deposition, ion implantation, etc. These methods show several advantages as they can generate uniform monodisperse nanoparticles, and also they are free from the contamination through solvent. However, during the synthesis processes, profuse amount of waste is produced; hence, the physical methods may be treated as uneconomical (Dhand et al. 2015).

##### 8.2.2.1.1 High-Energy Ball Milling (HEBM)

HEBM was first time used by John Benjamin in 1970 for the synthesis of alloys strengthened with the oxide dispersion. This method is capable for synthesizing nanoparticles having a lot of variation in their shape and sizes (Dhand et al. 2015; Xing et al. 2013). The HEBM route involves the transfer of kinetic energy from the moving balls to the milled material. Due to this transfer of kinetic energy, bond breaking will take place, and hence, the new surface will be generated by the breakdown of milled materials into the smaller particles (Fig. 8.3a). HEBM process is

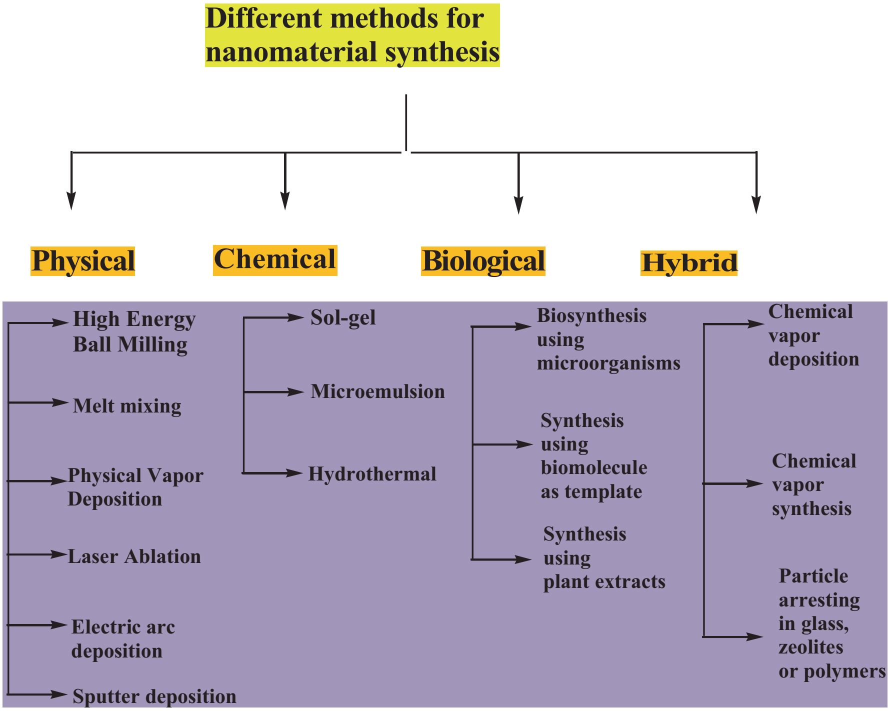

**Fig. 8.2** Flowchart representing different methods for nanomaterial synthesis

also considered a mechanochemical process of synthesis because this method is based on the conditions of temperature and pressure. Nowadays, nanomaterial synthesis using HEBM method is carried out in either the presence or absence of surfactant (Fig. 8.3b) (Dhand et al. 2015; de Carvalho et al. 2013). However, the synthesis of nanoparticles through HEBM method in the presence of surfactant is widely used because in the presence of surfactant, we can get precise particle size and their morphology (Dhand et al. 2015; de Carvalho et al. 2013) (Fig. 8.3).

#### 8.2.2.1.2 Melt Mixing

In this method, nanomaterial synthesis can be carried out by mixing the modified form of nanofillers with the polymer mechanically (Karak 2009). Melt mixing method is widely used because this method is highly compatible for different industrial processes and is also environment friendly (Lin et al. 2006). Melt mixing technique was used by Zuhal et al. for the synthesis of polypyrrole with polypropylene nanocomposite nanoparticles (Sevil and Zuhal 2010). Weiss et al. synthesized hybrid nanoparticles by mixing hydroxybenzoate (HBA) and hydroxynaphthanoate with the zinc salts of sulfonated ionomers of polystyrene. This involves the chemical bond formation between the polyester and the residual zinc acetate as it is carried out at high temperature (Lee et al. 2005).

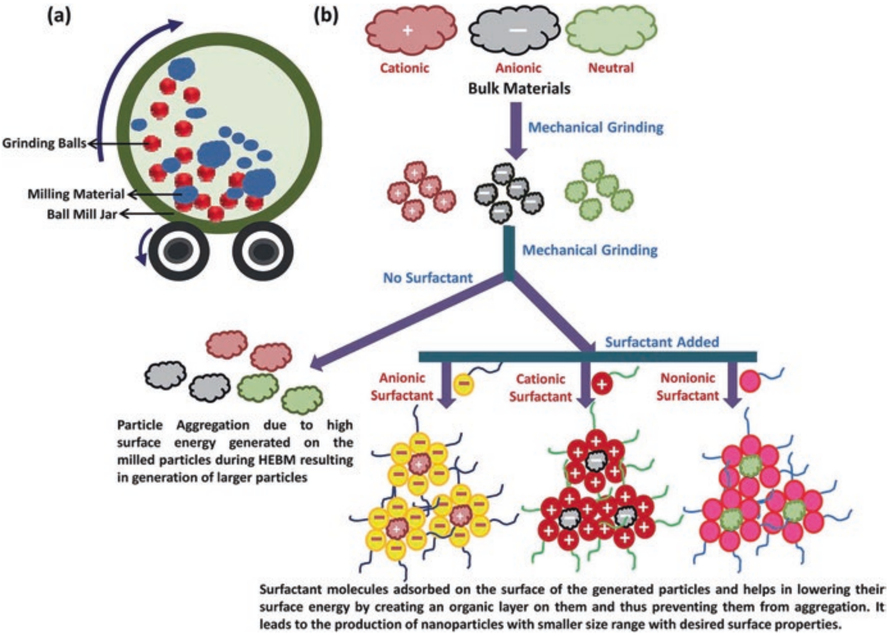

**Fig. 8.3** (**a**) It represents the high-energy ball milling scheme of nanomaterial synthesis and (**b**) representation of nanoparticle synthesis through HEBM system in the presence and absence of surfactant. (Dhand et al. 2015)

#### 8.2.2.1.3 Laser Ablation

In laser ablation method, vaporization of material is carried out by using laser light (Chen and Yeh 2002). After vaporizing the material, nanomaterial can be obtained in the colloidal solution form. It is a very easy and fast method for synthesis of the nanomaterials. It is also environment friendly and hence considered a green method. In this method, we are also able to introduce some organic moieties on the surface of the nanoparticles (Dhand et al. 2015; Singh and Gopal 2007).

#### 8.2.2.1.4 Physical Vapour Deposition

Physical vapour deposition consists of group of processes that are used for the production of nanoparticles and for the deposition of thin layers of materials. These thin layers generally lie in the nanometre or micrometre range. In PVD technique, mainly three steps are involved: (1) the first step involves vaporization of the materials that are mainly from solid source; (2) in the second step, vaporized material is transported; and (3) the third step is the nucleation and growth for the generation of thin films and nanoparticles (Okuyama and Lenggoro 2003). Some of the frequently used PVD methods for synthesizing the nanomaterials (Hatakeyama et al. 2011; Veith et al. 2007; Bouchat et al. 2013; Asanithi et al. 2012; Ichida et al. 2014; Ghosh et al. 2007; Veith et al. 2005; Ramalingam et al. 2013; Zhang et al. 2004; Takahashi et al. 2004; Dmitrieva et al. 2006; Stellacci et al. 2002; Hsieh et al. 2010; Naessens et al. 2001; Ong et al. 2008; Andrea et al. 2009; Jing et al. 2014) are (i) sputtering, (ii) vacuum arc, (iii) pulsed laser deposition and (iv) electron beam evaporation.

#### 8.2.2.2 Chemical Method

For the synthesis of nanomaterials, several chemical methods are also used such as hydrothermal synthesis, chemical vapour synthesis, sol-gel method, chemical vapour deposition, microemulsion technique and polyol synthesis.

##### 8.2.2.2.1 Sol-Gel Method

The term sol gel consists of two components: ‘sol’ and ‘gel’. The sol is a colloidal suspension in which the dispersed phases are solid particles while the dispersion medium is liquid. However, the gel is a polymer containing liquid. Hence, in this process, ‘sols’ are formed in the liquid phase. During the first step of the sol-gel process, hydrolysis takes place wherein the presence of water bonds of the precursor molecules is disintegrated (*Sol-gel science: the physics and chemistry of sol-gel processing* 1990). After hydrolysis, condensation occurs allowing nanomaterials to form. Finally, water is removed for the generation of the final structure of the material (Behnajady et al. 2011). Synthesis of nanoparticles by the sol-gel method is widely dependent on different experimental conditions; therefore, the reflux temperature, reflux time, solvent percentage or calcination temperature should be optimized.

##### 8.2.2.2.2 Microemulsion Method

Microemulsions are homogenous, and transparent dispersion consists of mainly three components: polar phase, nonpolar phase and surfactant. Polar phase generally includes water, and the hydrocarbon liquid or oil is used as nonpolar phase. Surfactant molecules are used so that (i) separating layer may be created between the polar and the nonpolar phase and (ii) interfacial tension may be reduced between the microemulsion and the excess phase. This technique is mainly used for the preparation of inorganic nanomaterials that may be Au, Pt and Pd nanoparticles (metal nanoparticles); $BaCO_3$, $SrCO_3$ and $CaCO_3$ nanoparticles (metal salt nanoparticles); CdS, PbS, CuS, CdSe and $Cu_2S$ nanoparticles (semiconducting metal sulphite nanoparticles); and $ZrO_2$, $GeO_2$, $TiO_2$, $Fe_2O_3$ and $SiO_2$ nanoparticles (metal oxide nanoparticles) (Solanki and Murthy 2011; Zhang et al. 2006). Au nanoparticles were synthesized from Au(III) ions by its reduction in the presence of alkaline solution of 2,7-dihydroxynaphthalene (DNP).

##### 8.2.2.2.3 Hydrothermal Method

This method generally involves the fabrication of nanoparticles of some metal oxide, lithium iron phosphate and iron oxide. In this method, we have control over the different properties of the particles by varying the conditions of pressure and temperature (Hayashi and Hakuta 2010). This method is carried out in vessel called autoclave that is operated under high pressure, and the reaction is carried out in the aqueous solution. In hydrothermal method, by taking care of the reaction temperature, pressure, solution composition, solvent properties, etc., we have better control over the particle size as well as morphology (Abedini et al. 2013). Hydrothermal synthesis is generally carried out in two types of systems that are continuous hydrothermal and the batch hydrothermal processes. Du et al. synthesized Pt nanoparticles by hydrothermal method through one-pot synthesis (Du et al. 2014). Hydrothermal method is also widely used for the synthesis of iron oxide ($Fe_3O_4$, $Fe_2O_3$), copper oxide, silver oxide, zinc oxide and nickel oxide nanoparticles (Liu et al. 2015; Tadic et al. 2014; Sue et al. 2011).

#### 8.2.2.3 Biological Method

The biological method or biosynthesis creates low toxicity and is cost-effective and also environment friendly. Hence, it is considered a green synthesis method. In these methods, several bio-organisms such as bacteria, viruses, fungi, yeast, plant extracts, etc. are involved in the production of metal and metal oxide nanoparticles. This biosynthesis method can be categorized into three major parts.

##### 8.2.2.3.1 Biosynthesis Using Microorganisms

In this method, several microorganisms are used in the synthesis of nanoparticles. Target ions from their environment are captured by the microorganisms, and then, the metal ions are converted into their elemental form through several enzymes produced through cellular activities. Depending on the location of nanoparticle synthesis, it is further classified as extracellular and intracellular. In the intracellular method, metal ions are transported into the inside of the microbial cell for the formation of nanoparticles, whereas in extracellular synthesis method, metal ions are trapped on the surface of the cell followed by reduction in the presence of enzymes (Zhang et al. 2011). Bacteria can reduce the metal ions by utilizing several functional groups, proteins, enzymes and also some reducing sugars (Nanda and Saravanan 2009). Biosynthesis by utilizing fungi has several advantages over other microorganisms: (i) it can show high bioaccumulation, (ii) it is economically viable, and (iii) it also shows easy handling of biomass (Mukherjee et al. 2001).

##### 8.2.2.3.2 Nanomaterial Synthesis Using Biomolecules as Templates

Several biomolecules such as nucleic acid, viruses, membranes, etc. are used as templates in the synthesis of nanomaterials. DNA is considered one of the most useful templates in the synthesis process as it shows very strong affinity with the transition metal ions. Au nanoparticles are formed by incorporating gold, Au (III) metal ions to DNA. In this process, reduction of Au (III) occurs, and hence, the Au atoms and metal cluster developed which will result in the formation of nanoparticles on the chain of the DNA (Zinchenko et al. 2014). Similarly, Ag nanoparticles were synthesized by Kundu et al. by using DNA as a template. These Ag nanoparticle clusters that have developed on the surface of the DNA also show good catalytic activities in the reduction of some aromatic nitro compounds (Kundu 2013; Kundu et al. 2008; Kundu and Nithiyanantham 2014).

Biological membranes are also used as templates in the synthesis of nanoparticles because they are having ultrafine pores in their structure. In the synthesis of Au nanoparticles, rubber membrane prepared from the *Hevea brasiliensis* trees is used as template where reduction of Au (III) takes place at 80 °C (Cabrera et al. 2013).

#### 8.2.2.3.3 Nanomaterial Synthesis Using Plant Extracts

The generation of nanomaterials from the plant extracts is eco-friendly and a rapid process. Hence, this method is widely used for the synthesis of nanoparticles of noble metals, metal oxides and some bimetallic alloys (Iravani 2011). Gold nanotriangles were synthesized by using lemongrass leaf extract. For this preparation, Shankar et al. treated the lemongrass leaf extract with the aqueous solution of the AuCl ions (Shankar et al. 2014). In a similar way, the leaf extract of *Aloe vera*, *Tamarindus indica* and *Emblica officinalis* and leaf extract of some other plants are also used for the synthesis of the Au nanoparticles (Chandran et al. 2006; Ankamwar et al. 2005a; b). Some other nanoparticles such as Pd nanoparticles and Pt nanoparticles were also synthesized by using the extract taken out from different parts of diverse species of plants (Coccia et al. 2012; Sathishkumar et al. 2009). Shankar et al. also synthesized Ag nanoparticles from the leaf extract of *Azadirachta indica* and also from the fruit extract of *Emblica officinalis* (Ankamwar et al. 2005b; Shankar et al. 2004).

### 8.2.2.4 Hybrid Method

In this category of nanomaterial synthesis method, physical, chemical and biological are all involved. Hence, it is considered a hybrid method for the synthesis of the nanomaterial. It involves several methods such as chemical vapour deposition, chemical vapour synthesis or arresting of the particles in the glass or zeolites or polymers.

#### 8.2.2.4.1 Chemical Vapour Deposition and Chemical Vapour Synthesis

In this method, solid films are deposited from the vapour phase through chemical reactions taking place at high temperature. This method was earlier used for the production of thin films. However, nanoparticles can also be produced by this method if the following conditions are optimized:

(i) In the hot wall reactors, the temperature should be kept high.
(ii) The partial pressure of monomers should be kept high so that high supersaturation can be achieved.
(iii) Residence time should be kept slow.

This modified process is then called chemical vapour synthesis (CVS) or chemical vapour reaction (CVR) or chemical vapour precipitation (CVP) or chemical vapour condensation (CVC). During the synthesis process, either solid, liquid or gaseous precursors are converted to their vapour form (Swihart 2003). Chemical vapour synthesis method is generally utilized for the generation of nanoparticles by using several material such as ZnO, iron oxide, silicon oxide and copper oxides, Cr-doped zinc oxide, Al-doped zinc oxide, etc. (Hartner et al. 2009; Jin et al. 2007; Suffner et al. 2010; Lahde et al. 2011; Lee et al. 2009). One disadvantage of this method is that high temperature is required for this method, and hence, there are difficulties in providing such high temperature.

### 8.2.3 Synthesis of Nanoparticles

Nanoparticles show wide range of applications as they are used in number of optical, electronic, and magnetic devices and also they are used as catalysts, adsorbents, sensors and ferrofluids. Most of these applications of nanoparticles depend on their size as well as their shape, and hence, the synthesis of nanoparticles should be carried out in such a way so that well-defined shape and size of the nanoparticles may be obtained. One of the very common techniques used is the formation of metallic colloid dispersions by the reduction of metal complexes. Sizes of nanoparticles depend on the type of reducing agent used during its synthesis. If the reducing agent is strong, then the rate of reaction will be fast. Hence, the nanoparticles formed are of smaller size, but the nanoparticles formed will be of larger sized when we use a weak reducing agent because it induces slower rate of reaction.

Aggregation of nanoparticles can be prevented by the use of some polymeric stabilizers. These polymeric stabilizers form a single layer on the nanoparticle surface, and in this way aggregation can be prevented. These polymeric stabilizers are also called as capping material. But when we use polymeric stabilizers, the growth of nanoparticles gets inhibited because the monolayer of polymer has the tendency to significantly affect the process of growth by blocking the growth sites. Polymeric stabilizers also have the tendency to affect the shape of nanoparticles when they are used in different amounts.

### 8.2.4 Synthesis of Nanowires, Nanorods and Nanotubes

Several monodimensional nanomaterials like nanowires, nanorods and nanotubes can be synthesized by different techniques:

(a) Spontaneous growth – It involves various methods such as evaporation-condensation, vapour-liquid-solid growth and stress-induced recrystallization
(b) Template-based synthesis – The methods used here are electroplating, electrophoretic deposition, colloid dispersion, melt or solution filling and chemical reaction.
(c) Electrospinning
(d) Lithography

In spontaneous growth due to the preferential direction for crystal growth, there is formation of nanowires and nanorods in their single-crystal form.

Long ribbon-like nanostructures were synthesized by Pan et al. (2001) by evaporating the metal oxide powders at high temperatures, and for this they used mainly zinc, tin, indium, cadmium and gallium in their oxide form having semiconducting behavior (Pan et al. 2001).

One method for nanowire synthesis involves filling a template having cylindrical holes of size ranging in nanoscale. It is one of the most straightforward and common methods. It involves synthesis of nanowires into the cylindrical pores present at thin membrane by reducing the metal salt. This method has one advantage of producing nanowires and nanorods of controlled size when there is availability of template with homogeneous pores, and for this requirement, there are a number of permeable templates available that can accomplish such necessities (Martin 1994; Thurn-Albrecht et al. 2000).

By using various techniques, several nanochannel silicon membranes are prepared having pores in perfectly structured form, and such membranes of silicon are widely used in the synthesis of modulated-diameter gold nanorods. By using electrodeposition techniques, this method has been adapted for the creation of nanorods having different metal segments. They are widely used for tagging molecules in analytical chemistry and in the field of biology (Ozin and Arsenault 2006).

Some nanorods have functional molecules introduced to its some selective locations, and these functional molecules which anchored nanorods have wide applications in the today's research. One of the applications of this method involves functionalization of gold nanorod end with thiolated DNA. The complimentary DNA is Rhodamine labelled and is coupled to the nanorods through its end. By using a similar approach, gold nanorods side are also functionalized with DNA, i.e. gold nanorods with DNA side functionalized are also created, and these are directed for self-assembling on complimentary DNA-functionalized gold surface sites with soft lithographic pattern.

## 8.3 Enzyme Immobilization

Our main aim for immobilization of enzyme is to improve the stability of enzyme and its handling as well as storage properties. Enzymes are utilized for their several applications where indigenous form of them is not possible. Biosensors based on enzyme reactions are used for their certain therapies, but the enzymes used for biotransformation in industries are based on the use of some stable biocatalysis. These necessities can be fulfilled by immobilizing the enzymes (Sheldon 2007; Tran and Balkus 2011; Garcia-Galan et al. 2011; Hanefeld et al. 2009). There are certain techniques available for effective immobilization of enzymes, and these can be achieved by (i) binding the enzyme molecules with solid carrier (support), (ii) embedding the enzyme in an open matrix (encapsulation) or (iii) cross-linking through physical or chemical interactions (Singh and Gopal 2007). These different techniques are represented in Fig. 8.4.

(a) Binding to a support (carrier) involves several interactions that may be physical (e.g. hydrophobic and van der Waals interactions), ionic or covalent (Sheldon and van Pelt 2013; Cao 2005). However, among these several interactions,

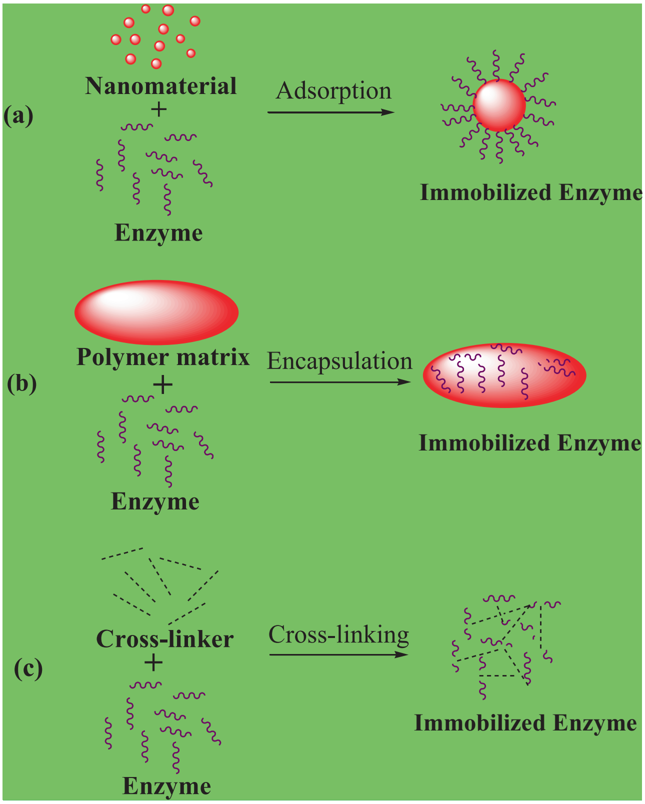

**Fig. 8.4** Scheme showing different techniques for enzyme immobilization

physical binding has no sufficient strength to hold the enzyme with the carrier during the rigorous industrial environment such as high concentration of reactant and product and high ionic strength. Ionic interaction is somewhat stronger; however, the covalent bond between the enzyme and the carrier usually does not allow the leaching of enzyme through its surface. However, the covalent bonding between the enzyme and the support has one limitation, that is the enzymes become irreversibly deactivated making the enzymes and the support useless. Some examples of support in immobilizing the enzymes are synthetic resins, biopolymers such as polysaccharides or inorganic solids such as mesoporous silicas or zeolites.

(b) Encapsulation of enzyme can be performed in matrix of organic polymer such as polyacrylamide or in matrix of inorganic polymer such as silica sol gel or in a membrane device such as hollow fibre or a microcapsule. Entrapment can be done after synthesizing the polymer matrix when enzymes are being there. One example that shows this behaviour is that when immobilization of enzymes is performed in mesoporous silica which is already fabricated, the enzyme would be located inside the mesopores but this cannot be supposed as encapsulation;

however, when enzyme is being there during the process of synthesis of a silica sol gel, the enzyme would be encapsulated.

(c) The carrierless macroparticles can be prepared by cross-linking the enzyme aggregates or crystals having the bifunctional reagent. Cross-linked enzyme crystals (CLECs) (Roy and Abraham 2004) and cross-linked enzyme aggregates (CLEAs) (Sheldon 2011) are some carrier-free immobilized enzymes. These approaches provide number of advantages such as high stability of enzyme, high enzyme activity or low costs of production, and hence it has the potential to replace other expensive carrier.

### 8.3.1 Active Nanomaterials in Enzyme Immobilization

The major advantage of nanomaterials in the immobilization of enzyme is that surface area-to-volume ratio is high that increases the effectiveness of the enzyme. Large numbers of nanoparticles are already being employed in immobilizing the enzymes that leads to the construction of some nanostructured systems such as nanofibres, nanotubes, nanomaterials, nano hollow fibre materials and some nanoporous material (Misson et al. 2014).

(a) *Sol-gel networks*

Enzymes can be immobilized by embedding them through this sol-gel process. During the process of enzyme immobilization, enzyme is added first to the sol materials and due to this structure of enzyme would be frozen. This enzyme becomes entrapped into the sol-gel network and is called xerogels that are fine powdered substance and stable from the mechanical point of view (Avnir et al. 2006).

(b) *Protein matrices*

During the creation of protein-based matrices, enzymes are connected by covalent binding, and there is a generation of cross-linking between the enzymes by some bifunctional agents. These materials actually do not have any solid carrier, but they are called nanostructured immobilized enzyme. This method shows a lot of advantages as it is simple and cost-effective and can be applicable for a number of enzymes. The major benefit of this method is that immobilized enzyme can be created without any intermediate materials and also without the need for cleaning. However, this method shows some disadvantages also, that is, the enzymes are located on the outer surface, and hence, it may come in contact to the substrate without any problem, but the transport of material by the enzymes located inside the matrix is difficult. Another problem is that binding of cross-linked agents shows some negative effect on the structure of enzyme by disturbing the activity of enzyme, making it inactive.

### (c) *Nanofibres*

Both inside and outside of the nanofibres created by electrostatic fibre production are used to immobilize the enzymes. After creating the nanofibres, the enzyme solution is allowed to mix with the polymer solution when the immobilization is carried out inside the nanofibres. The enzyme immobilization can show stability, security and some better quality for the enzyme functionality when immobilization is carried out inside the nanofibres in comparison to the immobilization of enzyme on the outer side of the nanofibres.

### (d) *Nanotubes*

Nanotubes are used in electronic, optic fibres and in different sensors due to their mechanical, thermal and structural properties. These are among the largely used enzyme carriers that are especially used in the development of biofuel cells. Both the single-walled and multi-walled carbon nanotubes are broadly used for immobilizing the enzymes. Single-walled nanotubes are used because they show high surface area and hence act as successful carrier material for the enzymes, while the multi-walled nanotubes are used because of their ease in dispersion property. The interactions or the type of bonding involved in immobilization are covalent or secondary bonding. Adsorption occurs through H-bonding or hydrophobic or π-π interactions, and the advantage of adsorption is that original behaviour of both the enzymes and the support is conserved. But it may dissolve easily during the applications due to which catalyst may become free; however, the covalent bonding gives more robust and durable association between the enzyme and nanotube (Feng and Ji 2011).

### (e) *Magnetic nanoparticles*

Successful immobilization of enzymes on magnetic nanoparticles is widely utilized for diagnostic purposes such as in measurement of blood sugar, biological transformations with stereoselectivity, etc. Nanosized magnetic particles show number of beneficial behaviour. Therefore, the cross-linked iron oxide particles, monocrystalline iron oxide nanoparticles and ultrasmall superparamagnetic iron oxide are used as an imaging agent in magnetic resonance imaging. Nowadays, magnetic nanoparticles are used for immobilizing the enzymes because they have the tendency to be easily separated out from the reaction mixture, they have the tendency to enhance the stability of bioelement and they can also increase the stability of enzymes. These magnetic nanoparticle-immobilized enzymes illustrate several applications in biotechnology or in analytical devices such as biosensors or in nanomedicine where nanoparticles are used for ease in diagnosis and disease treatments (Govan and Gunko 2014). Due to the advantage of magnetic nanoparticles in the ease of their separation, Liang et al. tried to immobilize the *Candida rugosa*

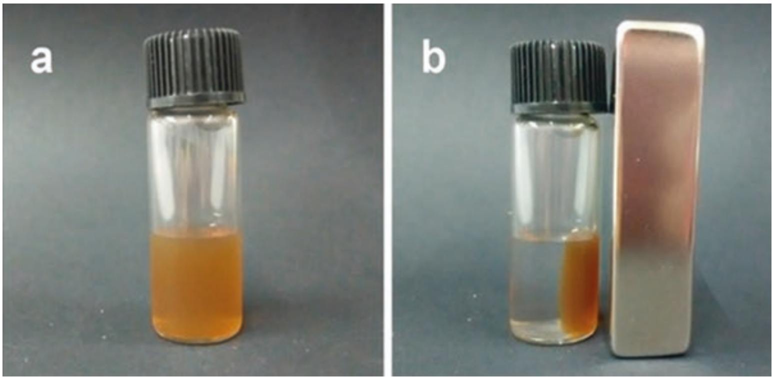

**Fig. 8.5** The behaviour of magnetic nanoparticle-immobilized CRL enzyme, i.e. CA-$Fe_3O_4$@Zn/AMP CPs. (**a**) Before separation and (**b**) after separation. (Li et al. 2017)

lipase (CRL) enzyme by using magnetic iron oxide nanoparticles modified with citric acid (CA-$Fe_3O_4$ NPs) in the presence of nucleotide-hybrid metal coordination polymers (NMCPs). Hence, the activity as well as stability of the CA-$Fe_3O_4$@Zn/AMP nanofibres-immobilized CRL enzyme gets increased (Li et al. 2017). Their separation behaviour is shown in Fig. 8.5.

### 8.3.2 Immobilized Enzymes in Biotechnology

Enzymes show one of the attracting roles, that is, they act as natural biocatalysts due to the ability of enzyme to hasten the rate of all biological reactions, but they themselves are not consumed during the reactions. They also do not affect the equilibrium between the reactants and the products. However, the immobilized enzymes show a number of advantages such as they show ability of easy reusability and lower degradation in comparison to free enzymes available in solution. In addition to these advantages, the rate of reaction can be controlled and also prevent contamination of the substrate with enzyme or protein. Stability of enzymes can also be improved by expanding their half-life through immobilizing the enzymes. Immobilized enzymes can work in a broader range of environments that have the ability to increase the stability of enzymes against temperature, pH, contaminants and impurities. Enzymes after their immobilization show improved biocatalytic activity and efficiency that make them highly attractive and therefore show high applicability in numerous varieties of growing biotechnologies (Gerday et al. 2000; Bradley and Wang 2015; Kell et al. 2015; Joshi and Satyanarayana 2015; Tavano 2013; Hyeon et al. 2013; Yu et al. 2015; Wendisch 2015).

### 8.3.3 Immobilized Enzymes in Biomedicine

Immobilized enzymes or proteins are widely used for the detection and treatment of several diseases, and these show their applications in the field of medicine. Several immobilized antibodies, receptors and enzymes are used in the form of biosensors for analysing different biologically active compounds in diagnosis. Other use of immobilized enzymes in the field of biomedicine includes synthetic cells and the manufacture of some systems that are used in drug delivery for dosing of proteins or enzymes and are well controlled. The development of some enzyme-based electrodes is another important application of immobilized enzymes in the field of biomedicine. Those enzymes that are highly specific and reactive towards its substrate are widely used in biosensor. Hence, the biosensor developed has high reliability, sensitivity and accuracy, showing ease in their handling, and is of low cost in comparison to the usual analytical methods.

Some important applications of bioreactors that are broadly used in the field of medicine for human are:

(a) In the poisoning of organophosphate, degradation of organophosphate can be done by the use phosphotriesterases (Chatterjee et al. 2014; Petrikovics et al. 1999).
(b) Alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase are used for the conversion of alcohol to its acetate form in the alcohol poisoning (Pei et al. 1995; Magnani et al. 1993).
(c) Repairing of damaged DNA in aging of skin and cancer by the use of DNA repair enzyme (liposome) (Yarosh et al. 1999; Yarosh et al. 1996).
(d) In hypercholesterolemia, hydrolysis of phospholipids can be done by the use of phospholipase $A_2$ (liposome) (Jørgensen et al. 1999).

## 8.4 Applications of Nanomaterials in Enzyme Immobilization

Nowadays, researchers are using two types of carriers (i.e. microcarriers and nanocarriers) for immobilizing the enzymes so that the immobilized enzymes can be utilized for several biotechnological, biochemical, bioanalytical or biomedical applications. These micro- and nanocarriers are magnetic nanoparticles and cross-linked enzyme aggregates (CLEAs), respectively. However, among these two carriers, the magnetic nanoparticles show a number of applications in biomedical, bioanalytical, biophysical and biomedical field. Magnetic nanoparticles are widely used in these fields because the nanosized structures have smaller size, excellent magnetic properties and large surface area-to-volume ratio (hence, they have higher surface energy). Therefore, they are used as carriers to which different active substances can easily bind. Several enzyme immobilization methods such as adsorption, chelating or metal binding, affinity binding or covalent binding help in the production of some efficient and stable magnetic nanoparticles bounded with enzyme. The development and synthesis of such materials have immense contribution in the present-day research in the form of imaging agents, sensors, drug delivery targets/vehicles and diagnostic tools. The application of nanomaterial in this field has enhanced the scope of studies.

### 8.4.1 Gold Nanoparticles as Enzyme Immobilization Templates

Gold nanoparticles are widely used in enzyme immobilization. When the aqueous gold nanoparticles are assembled on the surface of polyurethane (PU) spheres, it leads to the formation of [gold nanoparticle shell]-[polyurethane core] structure. The conjugation of gold nanoparticles occurs easily on the microspheres of polymer because nitrogen in the polymer interacts with the nanoparticles. One essential step in the core-shell structure protocols based on the polymer requires performing some additional modification on the surface of the polymer microspheres. Finally, the nanogold-PU material is allowed to make conjugation with enzyme such as pepsin which leads to the creation of a novel category of biocatalyst. In this way, the enzyme-conjugated material shows some enhanced biocatalytic activity and considerably improved stability to withstand broader pH and temperature range in comparison to the free enzyme in solution. One another advantage of this pepsin bioconjugated gold nanoparticle-labelled polyurethane microsphere is that it can be separated out from the reaction medium without any difficulty and it shows higher reusability of the bioconjugate up to six reaction cycles. In DNA immobilization and detection methodologies, the use of such gold nanoparticle shell-PU core structures is currently being envisaged (Phadtare et al. 2003).

## 8.5 Conclusion

Enzymes show environmental friendliness, high selectivity, high specificity, low cost and mild reaction conditions, and hence, they have the potential to replace several chemical catalysts. Enzymes are used as important biocatalysts, and hence they show wide applications such as in biosensors, pharmaceuticals, chemicals and foods. Due to low operational stability and short lifetime, enzymes have only limited applications. However, these limitations can be overcome by immobilizing the enzymes. Several materials have been tried for immobilizing the enzymes such as carboxyl-functionalized grapheme oxide, polyurethane foam support, SBA-15 and chitosan. However, the nanostructured carriers are attracting much attention of the researchers and are considered as ideal material for immobilizing the enzymes due to their specific surface area, high dispersibility and lower mass transfer resistance. Many nanomaterials have been used for immobilizing the enzymes so that improvement should be achieved in the enzyme activity and stability. However, the recovery of nanomaterials is very difficult, and hence, they cannot be recycled. Therefore, the magnetic nanosupport would be the most excellent alternative so that recovery of nanostructured immobilized enzymes may become easy by using magnet as the separation medium. Therefore , the magnetic nanoparticles such as iron oxide as well as ferric oxide have been tried successfully for immobilizing the enzymes. In this way, the immobilization support has the tendency to increase the enzyme stability, activity, specificity, lifetime, productivity and structural rigidity.

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# Nanomaterial-Immobilized Biocatalysts for Biofuel Production from Lignocellulose Biomass

## 9

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## Abstract

Enzymes are employed in several fields of basic and applied research as biocatalysts in green chemistry, biosensor, nanobioelectronics, biofuel, and pharmaceutical, agricultural, and biotechnological industries. In present scenario, the diminution of fossil fuels gained the attention of researchers for substitute and sustainable renewable energy resources for biofuel production to combat worldwide energy consumption. The enzyme immobilization as biocatalysts for biofuel applications from lignocellulosic biomasses is found to produce highest percentage of bioethanol. The enzyme immobilization is a fundamental tool to reduce the cost and harness their benefits. The stabilization of enzymes using immobilization helps in efficient recovery from the reaction conditions after biocatalysis and hence makes laborious separation steps easy and permits repetitive use of enzymes. Besides this, it offers several other advantages such as stabilization against harsh reaction conditions, thermodynamic and kinetic stability, surface- and volume-confined enzyme environments, ability to design multi-step reaction, and reduced formation of undesired products which makes easy separation of soluble end products than free enzymes. The different methods of enzyme immobilization either involve adsorption or covalent bonding or encapsulation or a combination of different methods. Several types of nanoparticles and nanocomposites are being used for the stabilization of enzymes which retain the enzyme activity even after immobilization. This book chapter will cover the developments in coupled strategies and the deeper knowledge in stabilization of enzymes with special emphasis on the possibilities of nanomaterial coupled immobilization for operational stabilities in biofuel application.

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_9

**Keywords**
Enzymes · Stabilization · Enhanced activity · Cost · Applications · Nanomaterials

## 9.1 Introduction

Enzymes perform their activity selectively under different physiological conditions. Enzymes are explored in the areas of food, feed, and pharmaceutical, energy production such as biodiesel and bioethanol, and developing green chemical processes. However, quite often these proteins do not fulfill necessary prerequisite for an effective industrial biocatalyst (Iyer and Ananthnarayan 2008; Sheldon 2007a, b). The applications of enzymes are technically feasible only if the enzymes are stable under extreme conditions such as temperature and pH and occurrence of salts, surfactants, and alkalis. Overall, the enzyme immobilization with any strategy aims to produce a better and stable biocatalyst using chemical alteration (Rodrigues et al. 2011) or exploration of enzymes from the thermophiles (Cowan and Fernandez-Lafuente 2011) or genetic manipulation (Hernandez and Fernandez-Lafuente 2016; Rodrigues et al. 2013). Therefore, there is a need to stabilize the enzyme against adverse environmental conditions such as thermal or alkaline deactivation (Iyer and Ananthnarayan 2008). On a broad note, the applications of enzymes for a vast range of industrial aspects would not be economically viable using conventional chemical methods. Under nonliving conditions, these biomolecules need to be recovered, stored, and recycled for developing sustainable processes. The recent substantial developments in microbiology and molecular biology on the exportation of enzymes from extremophiles such as thermophilic organisms for improving enzyme properties due to directed evolution (Cowan et al. 2005; Rubin-Pitel and Zhao 2006; Schmeisser et al. 2007; Turner 2009) and biochemical engineering (Avnir et al. 2006; Cabana et al. 2009; Figallo et al. 2007; Richter et al. 2008; Sheelu et al. 2008) or immobilization supports and protocols (Avnir et al. 2006; Rusmini et al. 2007); Betancor and Luckarift 2008; Siqueira Jr et al. 2010; Zhang et al. 2005) have enabled to fulfill the enzyme deficiencies as industrial biocatalysts. These tools permit increased efficiency of enzymes as biocatalysts in chemical and industrial sectors (Karel Hernandez 1980).

Several technologies such as cross-linking enzymes, immobilization using microwave, use of mesoporous materials, and nanoparticle-based immobilization are currently used to upsurge the enzyme activity and to minimize the cost for industrial application (Ansari and Husain 2012). The immobilization of enzymes allows the repeated use of enzymes which cut down the cost and allow easy recovery of soluble end product and perform multiple reaction with ease and simplifies the overall design and performance. To address the limitations associated with enzyme applications, efforts to immobilize enzymes by different methods enable cutting down the cost of enzyme as well as purification of the soluble end product (Tischer and Wedekind 1999; Lalonde and Margolin 2002). Since the initial report on immobilization of *Aspergillus oryzae* aminoacylase, for racemic amino acid resolution by Chibata in 1967, several efforts have been made in the past few decades (Brena and Batista-Viera 2006; End and Schoning 2004; Sheldon 2007a, b; Wong et al. 2009). In England, two other non-immobilized enzymes were commercialized on large scale. The first immobilization of penicillin acylase was used for preparing 6-aminopenicillanic acid from penicillin G, whereas glucose isomerase was immobilized as a second enzyme in the USA to convert glucose into fructose. Thereafter, extensive studies on enzyme technology led to an increased number of industrial methods based on immobilized enzymes (Katchalski-Katzir 1993). The term "immobilized enzyme" was coined in 1971 with the suggestions of Ephraim Katchalski-Katzir at Enzyme Engineering Conference at Henniker, New Hampshire, USA, for representing enzymes which are restricted physically or localized for repeated and continuous use with retention of enzyme activities (Katchalski-Katzir 1993). Even in recent times, efforts to stabilize the enzyme of different nature are gaining attentions for converting them into powerful and efficient factories. For example, stabilization of monomeric and oligomeric enzymes via covalent linkage and immobilization of enzyme subunits prevent subunit dissociation.

At present, the immobilized enzymes are the center of research, due to their multiple benefits over soluble enzymes such as stabilization against harsh reaction conditions, thermodynamic and kinetic stability, selectivity, surface- and volume-confined enzyme environments, ability to design multi-step reaction, and reduced formation of undesired products, where soluble end products are easy to collect and reduce the buildup of inhibitory or reactive intermediates, compared to free enzyme (Fernandez-Lafuente 2009; Iyer and Ananthnarayan 2008; Karel Hernandez 1980; Mateo et al. 2007a, b; Polizzi et al. 2007; Tischer and Kasche 1999). In some cases, unexpected significant decrease or enhancement in enzyme activity has been reported, compared with soluble preparation of enzyme. For example, the immobilization of subtilisin was 27 times less active than the soluble enzyme (Schmitke et al. 1996). On the other side, immobilization of lipase as lipoprotein in ester synthesis showed 40-fold enhancement in enzyme efficiency, compared to enzyme powders (Ottolina et al. 1992; Tischer and Kasche 1999). At present, the major emphasis in enzyme immobilization aims at the development of new supporting materials which includes use of mesoporous materials with uniform pore size (2–50 nm) with enormous surface area for the efficient biotransformation and improved stability (Drechsler et al. 2004; Yiu and Wright 2005; Palocci et al. 2007). Moreover, immobilization of enzymes onto macro/micro surfaces enables their usage for other applications including biosensors, developing environmental remediation materials, and other applied fields. The recent developments of site-selective protein immobilization and the use invention of synthetic biology are emerging as a powerful and effective site-specific covalent coupling method. Additionally, several other methods of anchoring or immobilizing such as the fabrication of functional protein microarrays have emerged (Bilitewski 2006; Drager et al. 2007; English et al. 2006; Kingsmore 2006; Wong et al. 2009; Zheng et al. 2005). In parallel, efforts to develop continuous flow systems such as the PASSflow system to streamline production and immobilization of recombinant His6-tagged proteins might be explored as efficient biocatalyst (Drager et al. 2007; Wong et al. 2009).

With increased attention on enzyme immobilization, their massive usage is inevitable in future applications. The routine use of enzymes as soluble homogeneous solution makes the recovery of enzymes difficult; hence their recycling and renewal is impossible. Therefore, the enzyme immobilization could be the probable solution since it can significantly increase the stability of enzymes. Nanobiotechnology has gained attention from enzymologists and has potential to provide biocompatible environment for the enzyme immobilization with high stability and reuse (Ahmad and Sardar 2015; Bosio et al. 2016; Rostro-Alanis et al. 2016). However, the immobilization of enzymes such as amylolytic on the surface via adsorption or covalent linkage or by entrapment is difficult. In addition, the immobilization of enzymes such as amylolytic on the surface via adsorption or covalent linkage or by entrapment is difficult. The large surface area of nanomaterials, high rate of immobilization, high stability (Husain et al. 2011; Husain 2017), and comparatively low steric hindrances offer unique properties for enzyme immobilization. Moreover, nanomaterials with magnetic properties are easy to reuse (Husain 2017).

Biodiesel offers an attractive alternate and renewal source of energy, compared to conventional fossil fuel, due to low level of sulfur and carbon monoxide (Sharp et al. 2000; Srivastava and Prasad 2000). For biofuel production from lignocellulosic biomass, the major associated bottleneck is its structural composition, owing to the complexity of cellulose and hemicellulose to lignin. The production of biofuel is mostly carried by transesterification using corrosive chemicals (Moser 2011). The environmental issues and downstream processing led to a shift in focus for alternative eco-friendly production methods (Bisen et al. 2010; Hama et al. 2007; Kim et al. 2006c; Verma et al. 2013). It demands economically viable and appropriate pretreatment technology (Naresh et al. 2018). To achieve this, several collective conventional pretreatment processes such as acid-alkali, uncatalyzed or acid-catalyzed steam explosion, and ultrasound-assisted ionic liquids have been explored. The limitation of conventional pretreatment processes is they are energy-intensive. Moreover, a series of steps for biomass conversion and diverse nature of lignocellulosic biomass, followed by the formation of inhibitors for fermentation, impede the process. Several attempts have been made for overcoming the technical hurdles by using alternative biological methods of pretreatment (Naresh Kumar et al. 2018). The biological methods based on enzymes have several advantages such as reduction in the formation of fermentation inhibitors, environmentally friendly, and low-cost production. Still, the low hydrolysis rate, cost, and need of longer pretreatment time need to be addressed. Therefore, focusing on coupling of multi-enzyme systems for uninterrupted conversion of plant biomass to final end product is gaining increased attention. For example, coupling of cellulase, laccase, and α-glucosidase through immobilization for efficient biocatalytic pretreatment has been tested to achieve the goal (Betancor and Luckarift 2008).

This book chapter will cover the recent developments in coupling strategies and deep knowledge in stabilization of enzymes with special emphasis on nanomaterial coupled immobilization for operational stabilities in biofuel application.

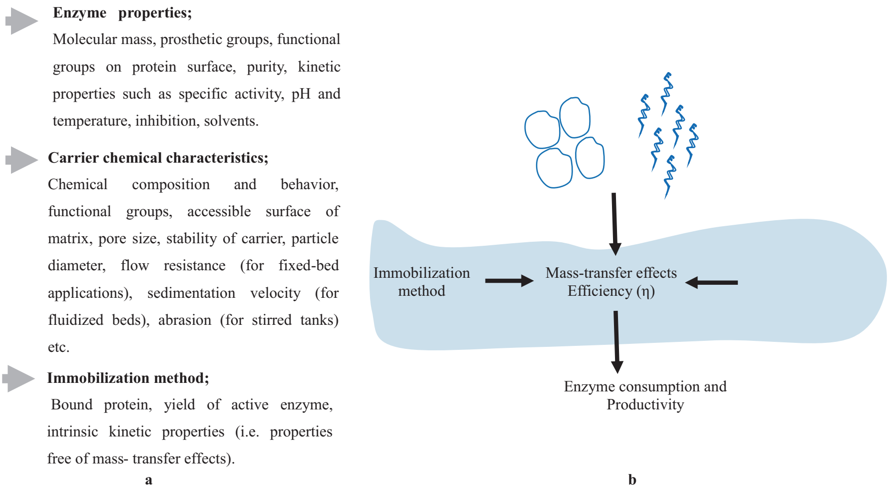
## 9.2 Enzyme Immobilization

Enzyme immobilization discovered in 1916 (Nelson and Griffin 1916) was used for the immobilization of invertase enzyme onto a firm carrier such as charcoal or $Al(OH)_3$. Initially, the enzyme immobilization has very low enzyme loading capacity which led to the more refined and better methods of enzyme immobilization. In late 1990s, a number of enzyme immobilization methods were discovered. Still, due to the commercial importance, attentions for enzyme immobilization have increased (Ahmad and Sardar 2015; Brady and Jordaan 2009; Cantone et al. 2013). The immobilization of enzymes was initially done by the use of polymer-based matrices and linkage onto carrier materials. Considerable efforts have been made for cross-linking of enzymes either using protein-based methods or using inactive materials (Silman and Katchalski 1966). The history of confining enzymes is dated back to the 1950s. Use of prefabricated carrier materials for binding is a method of choice, whereas cross-linking enzyme crystals also appears as an interesting alternative (Tischer and Kasche 1999). The method for immobilization is categorized based on the kind of chemical reaction used for binding (Buchholz and Kasche 1997; Kennedy and Melo 1990). Due to the low hydrodynamic and mechanical stability of materials obtained by enzyme cross-linking, researchers opted other methods. The cross-linking-based immobilization is typically support-free and includes formation of cross-linkages usually by the addition of cross-linking reagents such as glutaraldehyde to achieve three-dimensional arrangement (Murty et al. 2002; Öztürk 2001).

Currently, immobilization of enzymes can be done by binding to carrier or encapsulation or cross-linking. The linkage to carrier can be of physical nature through hydrophobic, covalent, van der Waals, and ionic forces. However, physical linkage is weak that it cannot hold the enzyme to the supporting carrier during applications. Here, the carrier can be of synthetic origin such as resin/biopolymer, or inorganic in nature such as (mesoporous) silica/zeolite. The immobilization of enzymes to different materials such as nano-size supports including spheres, tubes, and fibers (Kim et al. 2006a, b; Martin and Kohli 2003; Yim et al. 2003), mesoporous materials (Lei et al. 2006), silica-based nanotubes (Mitchell et al. 2002), inside of phospholipid bilayers (Hamachi et al. 1994), corridors of R-zirconium phosphate (Kumar and McLendon 1997), in Langmuir–Blodgett flicks (Troitsky et al. 2003), on polystyrene particle (Caruso and Mohwald 1999), on self-assembled monolayers (Hung et al. 2006), inside a polymer matrix (Kim et al. 2005a, b), gold nanoparticles accumulated on polymers and zeolite (Bhat et al. 2003; Phadtare et al. 2003) and thermal evaporated fatty lipid layers (Sastry et al. 2002) have been industrialized with their own distinctive advantages and limitations. Encapsulation of enzymes via inclusion in a polymer system such as an organic polymer or a silica sol-gel or a hollow fiber can be done. Here, the physical restraints are weak and cannot avoid enzyme leakage entirely. Therefore, often extra covalent attachment is done. The support binding and entrapment is not always clear. The binding to a prefabricated support regardless of enzyme immobilization on the external or internal surface is referred to as carrier binding. On the other hand, encapsulation involves the synthesis of polymer network in the presence of enzyme. For example, enzyme immobilization in a prefabricated mesoporous silica is carrier binding, whereas during the preparation of silica sol-gel, the enzyme is entrapped.

Conceptually, the immobilization of enzymes can be done on any other protein or on solid support (Hanefeld et al. 2009; Mateo et al. 2007a, b; Palazzo et al. 2014; Sheldon and van Pelt 2013; Vashist et al. 2014). Besides simple adsorption, the carrier is modified using an organic linker which contains combative functional groups. Then, the linkers are directly attached by covalent attachment. The coating can be made up of adsorbed or covalently linked self-assembled monolayer or bilayer of amphiphilic molecules including proteins such as bovine serum albumin and avidin (Vashist et al. 2014) or polymer (Fornera et al. 2012a, b). The organic layer coating may lead to enzyme inactivation upon direct interaction with the solid support. Sometimes, polymer–enzyme conjugates prepared previously in solution are adsorbed (Küchler et al. 2015a, b). Enzymes immobilized on a surface, in some cases, are more stable than their counterpart free enzymes (Küchler et al. 2016). A brief overview of different characteristic features of enzyme immobilization are mentioned in Fig. 9.1.

The enzyme crystals are one of the examples where the crystallized protein acts both as a carrier and catalyst. Here, multiple forces of non-covalent nature stabilize the crystals, compared to cross-linked assemblies of amorphous proteins. In addition, the chemical bonding using cross-linker further stabilizes the crystals in an aqueous solution (Persichetti et al. 1995). Immobilization using non-covalent-based adsorption is beneficial in non-aqueous solutions, where desorption may be ignored due to the little solubility of enzyme in the aqueous environments. This process is the choice for the preparation of lipase in water-immiscible solvent (Balcao et al. 1996; Malcata et al. 1997). The adsorbed enzymes (Antrim and Auterinen 1986) and proteins (Tischer 1992) are widely used in industrial applications. Immobilization



**Enzyme properties;**
Molecular mass, prosthetic groups, functional groups on protein surface, purity, kinetic properties such as specific activity, pH and temperature, inhibition, solvents.

**Carrier chemical characteristics;**
Chemical composition and behavior, functional groups, accessible surface of matrix, pore size, stability of carrier, particle diameter, flow resistance (for fixed-bed applications), sedimentation velocity (for fluidized beds), abrasion (for stirred tanks) etc.

**Immobilization method;**
Bound protein, yield of active enzyme, intrinsic kinetic properties (i.e. properties free of mass- transfer effects).

**Fig. 9.1** Overview of enzyme immobilization and characteristic features of enzyme immobilization (**a** and **b**). (Adopted from Tischer and Kasche) of enzymes can be attained by the diffusion of dried and water-soluble preparation of enzyme in water-immiscible organic solvents. Lipases have been immobilized using this method. In non-aqueous conditions, this type of preparation catalyzes hydrolytic reactions in the opposite way by carefully controlling the water content (Bell et al. 1995).

Immobilization by inclusion includes retaining of the enzyme within a membrane such as polymeric networks, hollow fibers, or microcapsules. For simple inclusion, derivatization of the enzyme is not required, and harmful effects of binding forces are not required to be considered. However, protein–carrier interactions lack additional stabilization. Use of polymeric networks is one of the most effective methods when used with other additional binding methods like the linkage of vinyl groups to the enzyme surface, and its incorporation into a rising polymer cable is another method. Such modified enzyme preparations can be combined into “biocatalytic plastics” which are active in aqueous as well as organic media (Wang et al. 1997). There are certain dissimilarities between binding on carriers, cross-linking, and crystallization. The last method primes particles of high enzyme concentration, frequently with an equal distribution. However, immobilization onto preassembled supporting material allows binding onto surfaces with comparative low enzyme concentration but with special binding on the outer covering of the carrier materials (Tischer and Kasche 1999).

## 9.3 Basic of Enzyme Immobilization

The enzyme immobilization using standard protocol is referred to as “random immobilization.” However, in general, the enzyme immobilization is never a complete random process and often leads to protein immobilized in a determined and very similar orientation. The selective enzyme immobilization on the surface using a particular immobilization method can lead to immobilized enzyme molecules with a specific unreachable surface area (Fuentes et al. 2006). Therefore, the challenge associated with different immobilization protocols, changes in enzyme properties, and even inversion of enzyme’s specific action (Fernández-Lorente et al. 2007; Mateo et al. 2007a, b; Palomo et al. 2002, 2007; Palomo 2009; Pišvejcová et al. 2006; Terreni et al. 2001) would be difficult to explain. For example, the trypsin-based lysis of penicillin G acylase to Eupergit (Mateo et al. 2000) or glyoxyl agarose (Mateo et al. 2005; Mateo et al. 2006), reveals dissimilarity in the orientation of protein in two methods (Karel Hernandez 1980; Temporini et al. 2010). In fact, a random immobilization of enzyme on a carrier is quite difficult to attain. Researchers have advocated the use of various immobilization methods in a coupled way to achieve the truly random immobilization (Fuentes et al. 2006; Karel Hernandez 1980). The adsorption can be done using H-bridges or hydrophobic or π–π interactions. The covalent binding is more robust and long-lasting. Initially, carrier interacts covalently with protein at one point via major amino group in the protein such as Lys amino acid. However, the non-ionized amino groups are considered better nucleophiles in the reaction (Diána 2015).

## 9.4 Methods of Immobilization

The immobilization of enzymes can be performed by physical or chemical methods. Physical procedures involve the weak interaction amid carrier and enzyme, whereas the chemical approaches involve covalent bond between the both. The selection of enzyme immobilization method is of vital importance to avoid the damage of any enzyme activity by maintaining the binding site of enzymes. In recent time, the applications of site-selective enzyme immobilization have gained significant attention. The prior knowledge of the enzyme active site and how it can be protected using protective groups which can be detached later on without any loss of enzyme activity are really helpful. Use of adsorbents, covalent linkage, entrapment, and cross-linking are the most common methods of enzyme immobilzation (Ahmad and Sardar 2015; Brady and Jordaan 2009). Even though the basic procedures of enzyme immobilization are classified into four types as mentioned above; several variants, based on combination of these basic procedures, have been industrialized for enzyme immobilization. Each of these method owns their merits and demerits for enzyme immobilization.

Further, based on interactions between enzymes and supporting carrier, different methods of enzyme immobilization are categorized into irreversible and reversible types (Brena and Batista-Viera 2006). In irreversible immobilization, enzyme immobilized onto supporting material cannot be detached by modulating the properties of both, whereas in reversible immobilization, it is possible to detach the enzyme from the supporting material using congenial environment. The covalent bonding, cross-linking, and entrapment are one of the most common methods, for irreversible enzyme immobilization, whereas physical adsorption and non-covalent binding through affinity and chelation binding are reversible type of immobilization methods (Zhao et al. 2015).

## 9.5 Adsorption of Enzymes

One of the oldest and simple methods of immobilization, the enzyme is adsorbed onto an insoluble support. This method is widely used and offers high capability of enzyme loading. Here, enzyme is mixed with suitable carrier adsorbent under suitable pH and ionic conditions. After that, unbound enzyme is removed by washing, whereas the immobilized enzyme is available for use. The interaction between adsorbent and enzymes are van der Waals forces, hydrogen, and ionic and hydrophobic types. These forces are very weak but operate in large scale. The adsorption usually includes multipoint adsorption between proteins and binding locations on the immobilization surface which may lead to alteration in the protein microenvironment (Johnson et al. 1996). The nature of adsorbent determines the amount of enzyme leakage. To avoid any chemical alterations and side effects to the enzyme activity, the surface property of support is also vital. The easy desorption of enzyme is a major disadvantage of this method (Rao et al. 1998) (Fig. 9.2a).

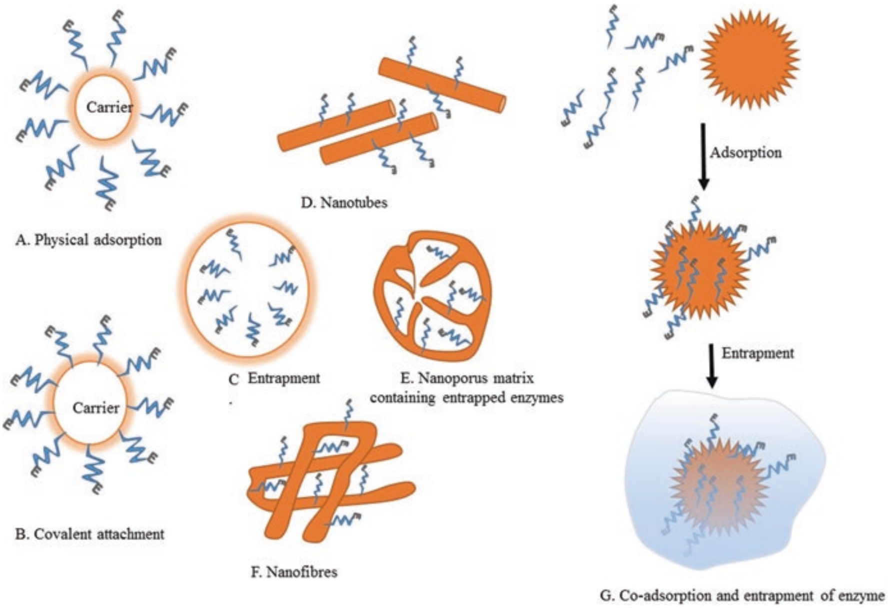


**Fig. 9.2** Overview of enzyme immobilization. Basic structures: (**a**) physical adsorption. (**b**) Covalent attachment. (**c**) Entrapment. (**d**–**f**) Nanosupports for enzyme immobilization. (Adopted from Wang 2006). (**g**) Immobilization of enzymes using co-adsorption method)

## 9.6 Covalent Binding of Enzymes

For covalent immobilization, the enzyme is conjugated to support matrix via the formation of covalent bonds. Similar to the adsorption of enzymes, the immobilization must be carried in an environment which does not lose enzymatic activity. The covalent linkage of protein to support involves exploration of amino acids on side chain like arginine, histidine, and aspartic acid which are also determined by the reactivity of imidazole, phenolic hydroxyl, and indolyl (D'Souza 1999). Use of peptide-adapted carriers for enzyme linkage leads to high specific activity as well as stability (Fu et al. 2011). The interaction between the supporting material and enzyme can be obtained either by direct bonding or by intercalated linkers known as spacers. The spacer molecule provides better flexibility to the coupled enzyme; therefore the enzyme activity has enhanced as compared to directly coupled enzyme. The activation of functional groups on support matrix is found to facilitate the covalent linkage. Chemicals such as glutaraldehyde, cyanogen bromide agarose, and cyanogen bromide-activated sepharose holding carbohydrate moiety are found to enhance the thermal stability of covalently bound enzymes (Hartmann and Kostrov 2013; Singh et al. 2013) (Fig. 9.2b).

## 9.7 Entrapping of Enzymes

Entrapment involves the restriction of enzyme movement in a porous gel or fibers; still it is available as free molecules in reaction volume. It is a convenient protocol where processes involve low molecular mass substrates and end products. Still, the lower diffusion rate or mass transfer effect limits the usage of enzymes entrapped for substrates with high molecular weight. The process of entrapping can be purely physical separation using cage or covalent binding. Thermal reverse polymerization is deployed for entrapping natural polymers like agar, gelatin, and agarose, whereas ionotropic gelation is preferred for alginate and carrageenan (Datta et al. 2013). Besides this, polymers like polyvinyl alcohol hydrogel (Grosová et al. 2008) and polyacrylamide (Deshpande et al. 1987) are also explored for entrapping enzymes (Fig. 9.2c).

## 9.8 Cross-Linking of Enzymes

A relatively simple process of cross-linking involves covalent bonding for enzyme immobilization. It also leads to frequent conformational changes which results in loss of activity; therefore, it is not a preferred immobilization method. Still, it is explored in enzyme immobilization methods particularly for minimizing the leakage of enzymes occurring by adsorption. Glutaraldehyde is one of the most common bifunctional agents extensively used, owing to its little cost, higher efficiency, and stability. The aldehyde groups of glutaraldehyde at the both ends link unrestricted amino groups of enzymes. Here, enzyme is generally immobilized in the presence of a protein like gelatin, collagen, and albumin. The undesirable activity losses probably occur due to the participation of catalytic groups for the immobilization. Another problem associated with cross-linking is lack of control, and therefore, it is challenging to get large-scale enzyme aggregation simultaneously with high activity. Moreover, the gelatinous behavior of the immobilized enzyme is another hurdle which limits the use of immobilized enzymes for several applications. In recent times, enzyme precipitation from aqueous solution in the form of physical aggregates either using salts or water-miscible organic solvents or non-ionic polymers is being explored for developing new immobilized enzymes known as cross-linked enzyme aggregates (CLEAs) (Sheldon 2007a). The physical aggregates are bound, organized by non-covalent interaction. Subsequently, the physical aggregation makes them permanently insoluble without disturbing their native structure and the catalytic activity. CLEAs are very effective, exhibit satisfactory stability as well as performance, and can be formed using economical and effective methods. The methodology can be deployed across the enzymes, even including for cofactor-dependent oxidoreductases (Sheldon 2007b).

## 9.9 Nature of Supporting Material for Enzyme Immobilization

The carriers of different nature have been explored over a period of time for enzyme immobilization (Datta et al. 2013; White and Kennedy 1980) (Fig. 9.2d–f). The nature of carrier plays decisive role in determining the performance of the enzyme immobilization. An ideally perfect support should be inert, have resistance to physical compression, microbial attack, biocompatible and available at low cost (Brena and Batista-Viera 2006). Polymers including alginate, cellulose, chitin, chitosan, starch, collagen, sepharose, pectin, and other natural polymers are common supporting materials (Datta et al. 2013). Additionally, synthetic polymer and inorganic materials are found to have good mechanical stability and can be modified easily (Bryjak and Trochimczuk 2006; Yong et al. 2008). The inorganic materials including alumina, silica, zeolites, and mesoporous silica (Datta et al. 2013; Hartmann and Kostrov 2013; Hudson et al. 2008) and silica-based materials are one of the most suitable and explored matrices for enzyme immobilization for commercial applications (Hartmann and Kostrov 2013; Ho et al. 2004) and research purpose (Vianello et al. 2000).

## 9.10 Nanomaterial-Based Enzyme Immobilization

Diverse types of carrier-based techniques for enzyme immobilization have been used in recent times to enhance the enzyme efficiency and stability and for lowering the cost of enzyme in industrial applications. These include use of aggregation, microwave-assisted immobilization, and mesoporous and nanoparticle-based materials. Enzymes/proteins are encapsulated within polymer silica (Frenkel-Mullerad and Avnir 2005) and gold nanoparticles. With the emergence of nanotechnology, the magnetic nanoparticles have gained a considerable attention. The unique presence of specific physiochemical properties of nanoparticles led to their advent in large number of biotechnological applications with better characteristics. The high surface-to-volume ratio of nanoparticles results in a high density of the immobilized entity. These enzyme preparations are active under broad pH and temperature range than free enzymes. The nanoparticles have well-structured core–shell and are uniform in size and shape, easy to prepare without any surfactants and toxic chemicals, and have particle size as per utility limits. Additionally, it is likely to co-immobilize the multi-enzymes on nanoparticles (Ansari and Husain 2012). The application of nano-immobilized enzymes is considered an exciting and emerging area which provides robust nanostructured materials as enzyme carriers for tailoring enzyme scaffolds (Xin et al. 2010). Recent advancements in nanobiology provide diverse range of nanomaterials and the approaches for immobilizing enzymes on the nanosupports.

The higher surface-to-volume ratio offers dense enzyme loading and hence a high activity of immobilized enzyme compared with conventional materials (Gupta et al. 2011; Hwang and Gu 2013; Verma et al. 2013). For example, protein of up to

204 mg/gm dry support for nanoparticles has been achieved compared to the 40 mg/gm of dry support for macroparticle. The high enzyme density leads to maximum enzyme activity units of nanoparticle-immobilized enzymes as compared to per gram of dry supporting macroparticle-immobilized enzymes (Klein et al. 2012). The nanoparticle-based enzyme immobilization clearly revealed that the carrier area-to-volume ratios rather than the carrier area are a better indicator of assessing supporting material's potential. The estimation of enzyme molecules immobilized on the materials can be assessed based on the physical property of the supporting material and enzymes. For example, presuming the spherical shape and the concentration of $Fe_3O_4$ which is 5.18 g/cm$^3$ and molecular mass of esterase which is 33,000 dalton, it is possible to estimate the number of esterase molecules conjugated to $Fe_3O_4$ nanoparticles (Shaw et al. 2006).

The enzyme activity of nanomaterial-bound enzymes is high due to low mass transfer resistance (Kim et al. 2008, 2006c). The stable mono-dispersion behavior of nanomaterial-immobilized enzymes is largely due to significant "Brownian motion" in aqueous suspension (Verma et al. 2014; Wang 2006). The improved diffusion and mobility of nanoparticles can impact the activity of mobilized enzymes (Jia et al. 2003). Therefore, promoting nanoscale structures for enzyme immobilization aims at maximizing the functional surface area and reduces diffusion limitations (Xie and Ma 2009). Other advantages such as thermal stability and augmented surface area can accelerate their potential applications. In recent times, different kinds of nanoparticles have been deployed for the fabrication of different nanomaterials such as nanotubes, nanorings, nanowires, etc. (Ali and Winterer 2010; Ni et al. 2007). Moreover, conjugation of various polymer microspheres and metal nanoparticles with proteins and enzymes has been done successfully (Schuler and Caruso 2000; Willner et al. 2006). The nanoparticle surface alteration using enzymes and biomembrane provides a distinctive biofunctionality to them (Yamashita 2001). Nanoparticles offer several other unique properties for the conjugation and functionalization which imparts advantages for diverse range of applications including clean energy generation and biodiesel production, biological/chemical sensing, drug delivery, and disease diagnostics (Ding et al. 2015). An overview of different nanoparticle-based enzyme immobilization methods is given below:

### 9.10.1 Enzyme Immobilization Using Magnetic Nanoparticles

The magnetic nanoparticles offer unique property such as superparamagnetism, large and modifiable surface, low toxicity and compatibility compared to bulk material. In this context, the iron oxide based magnetic nanoparticles are an attractive choice for enzyme immobilization. Since, iron oxide based magnetic nanoparticles are attracted to a magnetic force, this limitaion can be overocome once the force is removed. Nanoscaled carrier helps in achieving the upper limits in effective enzyme loading by harmonizing the key features that regulate the efficiency of biocatalysts such as high surface area-to-volume ratios and mass transfer resistance. The unique behavior of nanomaterial-coupled enzymes distinguishes it from conventional immobilized systems and hence shows potential applications in biocatalyst engineering. Still, nanoparticles often form clumps, and therefore, temporary dispersion is required. Moreover, their separation either by centrifugation or membranes is difficult due to their size. Therefore, use of superparamagnetic particles can solve these problems. Such type of particles can easily disperse in solution, and the suspended superparamagnetic particles in the reaction can be removed using an external magnet (Johnson et al. 2011; Liese and Hilterhaus 2013; Yang et al. 2008; Kopp et al. 2014). Magnetite nanoparticles ($Fe_3O_4$) are one of the most extensively used superparamagnetic supports (Gupta et al. 2011).

The usage of magnetic materials for enzyme immobilization was established on the basis of magnetic particles sequestered from magnetotactic bacteria (Matsunaga and Kamiya 1987). Thereafter, magnetic (maghemite) nanoparticles were explored for the immobilization of *Candida rugosa* lipase (Dyal et al. 2003). Recent developments are focused on the use of magnetic nanoparticle synthesis (Yiu and Keane 2012). The physiochemical attributes of magnetic nanoparticles enable them for several applications. In the last several years, magnetic nanoparticles have been explored as a potential material for enzyme immobilization (Yang et al. 2008). The parting of an immobilized enzyme using magnet is easy where either the immobilized enzyme is recollected using magnetic field or substrate solution removed or vice versa (Dyal et al. 2003; Gardimalla et al. 2005).

The advantages of nanomaterials in the form of shape, size, and functionalization ensure their suitability for enzyme immobilization as nanobiocatalysts. The nanoparticle-immobilized enzymes may be mobilized, directed, and then isolated by magnetic field. The magnetic nanoparticles can be used for enzyme immobilization provided the nanoparticles have adequate geometry and surface property. The magnetic immobilization of the enzymes is done either by the incorporation of magnetic particles during support polymer synthesis (Robinson et al. 1973) or coating of magnetic particles using supporting material such as agarose or dextran (Rusetski and Ruuge 1990). The magnetic particles/supports such as microspheres of different biomaterials or polymers with magnetic elements have been explored with great success (Dyal et al. 2003; Koneracka et al. 1999; Kouassi et al. 2005; Saiyed et al. 2003). The occurrence of amphoteric hydroxyl (OH) groups can directly facilitate the binding of proteins onto magnetic particles (Mehta et al. 1997). The magnetic particles have been utilized for the immobilization of several proteins including bovine serum albumin (BSA), lipase, and streptokinase (Fernandes et al. 2006; Koneracka et al. 2002), trypsin and chymotrypsin (Jordan et al. 2006), glucose oxidase and cholesterol oxidase using CDI as coupling agents (Koneracka et al. 2002; Kouassi et al. 2005; Kuroiwa et al. 2008; Mehta et al. 1997; Pimentel et al. 2007). Conjugation of proteins onto unmasked magnetic particles offers numerous inherent benefits (Saiyed et al. 2007). Magnetite ($Fe_3O_4$) is one of the most used magnetic materials, whereas matrices including nickel-impregnated silica paramagnetic particles (NSP) are also used for immobilization (Prakasham et al. 2007). The enzymes immobilized on NSP are found more stable and active on extreme pH and temperature limits which may be due to change in enzyme configuration under an immobilized condition (Prakasham et al. 2007).

Similarly, *Bacillus subtilis* keratinase has been immobilized onto polyethylene glycol-supported $Fe_3O_4$ superparamagnetic nanomaterial (Konwarh et al. 2009), haloalkane dehalogenase of *Xanthobacter autotrophicus* strain GJ10 immobilized on silica-coated or silica-uncoated $Fe_3O_4$ superparamagnetic particles (Johnson et al. 2008), lipase from *Candida rugose* immobilized on $Fe_2O_3$ magnetic nanoparticles, and lipase from *C. rugosa* immobilized on novel nanosupport, and phosphorous-containing polyurethanes are found more stable (Guncheva et al. 2011). The pretreatment of the supporting material with polar or non-polar solvents resulted in an undesirable impact on *C. rugose* lipase immobilization, whereas non-ionic surfactants enhanced the enzyme immobilization to 30–40% (Guncheva et al. 2011).

### 9.10.2 Novel Nanoparticles for Enzyme Immobilization

Gold nanoparticles are one of the outstanding biocompatible materials for enzyme immobilization. The interaction of gold nanoparticles between amino and cysteine group of proteins is equally robust to that of thiol derivative usually used. The proteins can be immobilized on gold nanomaterial directly without using any alteration (Crespilho et al. 2006; Lia et al. 2010; Xu et al. 2007). On the other side, the gold nanoparticles can be functionalized for protein immobilization using thiolated molecules and carboxylic groups (Jun et al. 2001; Vertegel et al. 2004). The zeolite-gold nanoparticle "core–shell" structures have been used for immobilizing fungal proteases (Phadtare et al. 2004). The gold nanoparticles are assembled on the zeolite surfaces through the amino groups present in 3-amino-propyltrimethoxysilane. The protein linked to "core–shell" assemblies can be easily separated from by centrifugation and then reused. The polyurethane microsphere–gold nanoparticle preparations are found to enhance enzyme activity in extreme pH range, shelf-life, as well as thermal stability (Kumar et al. 2003).

### 9.10.3 Enzyme Immobilization Using Nonmagnetic Nanoparticles

The enzyme immobilization on actuated supports allows the simplification and repeated use of enzymes and their biochemical properties (Gaberc-Porekar and Menart 2001; Iyer and Ananthnarayan 2008; (Mateo et al. 2007a, b). The numerous nanomaterials including nanoparticles, nanotubes, nanofibers, and nanoporous matrices have been extensively used for biotechnological applications (Wang 2006). Nonmagnetic materials such as cellulase immobilized on nanomaterials of precise polymethyl methacrylate (PMMA) core and cellulase shell have been used (Ho et al. 2008; Kin et al. 2008). Similar to other enzyme preparation, the immobilized cellulase showed high thermal stability and residual activity than free enzyme solution. Therefore, this type of methods can be explored for the immobilization of thermally stable enzyme for commercial applications (Ho et al. 2008). These types of nanoparticles contain thick and consistently dispersed enzyme shells. Such types of preparation can produce solid content up to 18% (w/w) concentration (Ho et al. 2008). The other nonmagnetic materials such as epoxy groups are useful for covalent bonding with amino, thiol, and hydroxyl groups present on the surface of enzyme. However, the interaction amid epoxy clusters and soluble enzyme is a slow process. Here, using initial intermolecular physical or chemical interaction, new functional groups are added to the enzyme surface. The subsequent intramolecular covalent linkage between the epoxy groups of the supporting material and the immobilized enzyme is performed. In this process, the initial immobilization regions are strengthened using multipoint covalent linkage (Mateo et al. 2007a, b).

The nonmagnetic nanofibers are created by electrostatic fiber production, and then enzymes can be immobilized into inner or outer surface. For immobilization on inner side, the enzymes are embedded in situ either between the polymer chains or inside the nanofiber. The enzyme immobilization can be on the surface of nanotubes using covalent and secondary binding. The nature of polymer material and buffered media of enzymes influence the enzyme immobilization. The biocompatible water materials such as soluble polymers or gelatins are considered as the most effective and homogeneous medium. The single-walled nanotube offers highly specific surface area, whereas the multi-walled nanotubes are preferred, due to their easy dispersion property. The immobilization on inner side of nanofibers also provides higher stability and enzyme activity than enzyme immobilized on outer surface of fiber materials. The use of other nanomaterial such as carbon nanotubes of both single-walled and multi-walled nature is explored for the enzyme immobilization (Asuri et al. 2006; Chen et al. 2003; Karajanagi et al. 2004; Shim et al. 2002). Presently, multi-walled nanotubes are the preferred material for nanobiocatalysis. The reports on both loss and increase in enzyme activity after multi-walled nanotube immobilization are presented in the literature (Asuri et al. 2006; Shah et al. 2007). The enzyme immobilization using carbon nanotubes is one of the most widely used carrier supports for developing biofuel cells. Other than $SiO_2$ and iron oxides, nanomaterials such as polynanofibers and superparamagnetic particle-based nanocrystals are also described in the literature for enzyme immobilization (Herricks et al. 2005; Jia et al. 2003) (Fig. 9.3).

Another novel and synthetic method involving single enzyme molecule based immobilization on porous nanostructure known as “single enzyme nanoparticles (SENs)” is also established (Kim and Grate 2003). Here in this method, the armored nanoparticles and a porous and amalgamated organic/inorganic network surround every enzyme molecule. The thin and porous nature of SENs prevents mass transfer effect on the substrate. The nanohybrid enzyme structure is a potential method to stabilize enzymes and hence can be used for diverse applications (Kim and Grate 2003). The fabrication of SENs involves a multistep process including the surface modification of enzyme and polymer growth on the enzyme surface followed by polymerization by condensation which attach polymer into a network surrounding the enzyme (Yang et al. 2008).

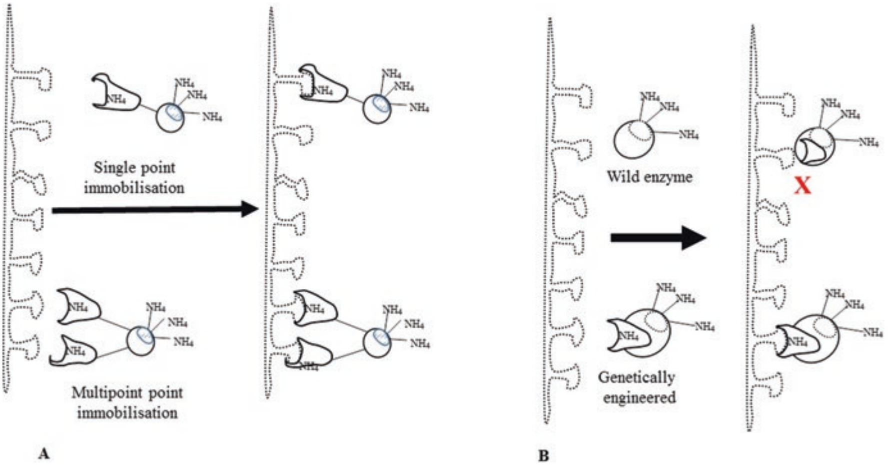


**Fig. 9.3** (**a**) Single- and multipoint enzyme immobilization. (**b**) Engineering enzyme for improved tethering on nanoparticle surface

## 9.11 Methods of Nanomaterial-Based Enzyme Immobilization

The amount of active enzyme present on the carriers is broadly influenced by bound enzyme which is typically 0.1–10% of the total weight of the supporting carrier material (0.1–500 units per mg protein of enzyme activity). There is a need to optimize the reaction volume and amount of enzyme and carrier materials. However, carriers can impart novel properties to enzymes. In crystal-based preparations, the carrier itself is an enzyme protein which determines the crystal weight and has the potential to provide high enzyme density. It can be of special importance for enzymes with low activity. The surface functionalization of nanomaterial offers effective enzyme immobilization. The use of interfacing proteins, agreements the possibilities to discover novel and robust conjugates for different uses (Chronopoulou et al. 2011). In another novel immobilization method based on prefabricated nanophase amphiphilic network, the enzyme is positioned in hydrophilic domains consisting of poly(2-hydroxyethyl acrylate) (PHEA). Here, the hydrophobic polydimethylsiloxane (PDMS) phase-diffusing substrate can contact the enzyme via the bulky interface and allows its isolation and abnormal swelling behavior. In the presence of water, the hydrophilic domain swells, and the polymer conjugated with the enzyme after immersion in an aqueous solution. After drying, the phase shrinks in size and contains trapped enzyme. The immersion of polymer network in organic solvent leads to the hydrophobic PDMS domain swelling and then permits entry of dissolved substrate via the interface (Sheldon 2007a, b).

Enzymes can be adsorbed onto carbon nanomaterial such as nanotubes via covalent or non-covalent methods (Lee et al. 2010). One of the major issues with the adsorption involves leaching of enzyme from nanoporous support. However, it can be regulated using covalent processes and cross-linkers such as glutaraldehyde (Gao et al. 2010). The use of site-specific affinity tag such as his-tagged and single-walled carbon nanotubes, altered with Na,Na-bis(carboxymethyl)-L-lysine hydrate, can overcome the limitations of diffusion and enzyme leaching and achieve proper orientation of active site (Wang and Jiang 2011). Cellulase attached to gold-fixed silica nanoparticles using affinity method is used for the hydrolysis of cellulose to glucose in a single step (Cho et al. 2012). The functionalization of core–shell nanoparticles with amino groups and PEG chains is also explored for the covalent linkage of β-glucosidase (Georgelin et al. 2010). Lipase of *Burkholderia cepacia* is immobilized onto the silica nanowires which is prepared by capping different numbers of alkyl groups. The immobilized lipase exhibited superior performance ascribed to improve mass transfer efficiency of reactants of silica nanowires and the activation of enzyme by capped-octadecyl groups in the aqueous and non-aqueous environmental conditions (Jin et al. 2018). Moreover, nanowires-immobilized lipase can be explored for developing porous biocatalytic membrane for the easy removal of enzyme from end products (Jin et al. 2018). The use of glutaraldehyde as a cross-linker for covalent immobilization to nanoparticle surface is the most commonly explored method (Wang et al. 2009). It offers high enzyme loading of up to 25 milligram of lipase/g particles as well as good activity retention up to 70% (Xie and Ma 2010). Lipase has been immobilized to the carbon nanotubes using carbodiimide-based activation (Ji et al. 2010). The immobilized enzyme also reserved the selectivity of the native enzyme as well as high stability. Use of amidination process for the activation of nitrile groups of the polyacrylonitrile nanofibers is also a preferred method (Li et al. 2011). The activated nanofiber showed high protein loading up to 43 milligram of lipase per gram of fiber and retained enzyme activity of up to 80% than free enzyme. The enzyme immobilization on nanomaterials is executed using free amine present on the enzyme surface with carboxylic acid, created by nanotube oxidation using chemicals such as nitric acid and sulfuric acid. The cross-linkage is also enabled using other chemicals such as EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (Saifuddin et al. 2013). The mesoporous silica such as MCF, SBA-15, MCM-41, and MCM-48 has been examined for lipase immobilization (Kim et al. 2007).

## 9.12 Analytical Tools for Investigating Enzyme–Nanomaterial Interaction

Understanding the interactions of nanocarrier and enzymes at the organizational level is a priority for enhancing the applications of the nanomaterials (Cruz et al. 2010; Pavlidis et al. 2012a, b). The biophysical changes after post immobilization can be observed using scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), circular dichroism (CD), UV-vis spectroscopy, Raman spectroscopy, microcalorimetry, time-of-flight secondary-ion mass spectrometry (TOF-SIMS), XPS (X-ray photoelectron) spectroscopy, and Fourier transform infrared spectroscopy (FTIR) that are used to elucidate the structure of enzymes adsorbed on/in the nanomaterials. The conformational stability of immobilized enzymes using these tools led to the finding of novel properties of nanomaterial-immobilized enzymes responsible for enzyme stability and activity (Andrade et al. 2010; Lee et al. 2009). The maintenance of native and active structure of enzyme is determined by the distinctive conformational stability, the nature of the immobilizing carrier, and the intermolecular interaction between the enzyme and carrier (Cruz et al. 2010; Shang et al. 2007).

The possible stabilizing outcome of nanostructured materials is enzyme-specific (Ganesan et al. 2009). The conventional tools for determining enzyme structure which depend on the light scattering phenomena are mostly unproductive for nanomaterial-based protein immobilization studies, due to the background noise of the carrier and possible aggregation. Therefore, the morphology and size of nanosupports prior to immobilization and after the immobilization of enzyme are examined by SEM, TEM, and AFM, respectively. For example, the XRD analysis revealed that there is no change in the crystal phase of magnetic nanoparticles after immobilization to the support (Xie and Ma 2010). FTIR-based comparative analysis of the free and immobilized enzyme revealed the change in the peak pattern for both types of enzyme preparations. CD spectroscopy is a method of preference for defining the impact of carrier addition onto the secondary structure of enzyme (Ganesan et al. 2009). CD spectrum analysis of lipase immobilized to carbon nanotubes revealed that it retained 62% of the α-helix structure of the native lipase (Ji et al. 2010). The sophisticated analytical techniques explain in situ characterization of structural and operational stability of immobilized enzymes on supports (Ganesan et al. 2009).

## 9.13 Applications of Nanoparticles in Enzymatic Hydrolysis of Lignocellulose in Biofuel/Bioenergy

The continuous depletion of fossil fuels, a growing concern for its contribution to greenhouse gas emissions, global warming, and other political crisis, demands new alternate and sustainable energy resources. Therefore, alternative and renewable energy sources such as lignocellulosic biomass which is mostly unused materials and rich in carbohydrates are a potential substrate for alternative energy source. Energy crops such as perennial grasses, *Miscanthus*, and poplar are one of the main sources of lignocellulosic biomass (Kirupa Sankar et al. 2018). Lipases (EC 3.1.1.3) have been employed for the biodiesel production (Wang et al. 2011a) and cellulases (EC 3.2.1.4) for the saccharification of plant biomass for ethanol production (Abraham et al. 2014; Lee et al. 2010; Margeot et al. 2009; Matano et al. 2013; Puri et al. 2012). Cellulase representing a complex of endoglucanase, exoglucanase, and β-glucosidase works in synergistic way to produce glucose as end product which is afterward fermented into bioethanol (Chandel et al. 2012; Garvey et al. 2013; Lynd et al. 2002). Since, the enzymes immobilized onto nanomaterials are stable and active in extreme environmental conditions (Dordick et al. 2012) therefore these immoblised enzymes can be explored for variety of applications. These molecular machines are extensively used for catalyzing multiple reactions for alternate energy production from renewal energy sources (Johnson et al. 2011; Kim et al. 2008). A list of immobilized enzymes deployed for biofuel production is mentioned in Table 9.1.

Although several processes for immobilization of lipases are available, the major hurdle for the technology transfer is the extraordinary cost of enzyme and limits of repeated use, e.g., the market price of Novozyme 435 is $1000/kg (Stoytcheva et al. 2011). Therefore, to reduce the cost, the techniques to immobilize the enzyme must be available at low prices and high constancy to avoid enzyme leakage or efficiency loss. Among different commercial immobilized lipases, Novozyme 435 is widely explored for biodiesel production. China, for the first time in 2006, started biodiesel production with a capacity of 20,000 tons per year using "Lipozymes TL" and "Novozyms 435." Thereafter, another biodiesel production plant in 2007 using mebrance immoblised lipase of Candida sp. 99–125 with an annual manufacturing capacity of 10,000 tons was started *Candida* sp. 99–125 has been immobilized on membranes was started. The biodiesel production using immobilized lipase of *Candida* sp. was found to be economical compared to single-time used Novozyme 435 (Zhao et al. 2015).

The lipase and cellulase immobilization has been done on silica, magnetic, gold, cellulose, $TiO_2$, and other nanomaterials (Cho et al. 2012; Huang et al. 2011; Pavlidis et al. 2012a, b). The use of nanomaterials as carrier materials for enzyme immobilization in biotechnology has been successfully employed for biofuel production (Verma et al. 2014). For biodiesel application, lipases have been immobilized on magnetic $Fe_3O_4$ nanoparticles at a reactor scale (Wang et al. 2009). Similarly, porcine pancreas lipase, *Candida rugosa* lipase, and *Pseudomonas cepacia* lipase have been immobilized onto the amino-activated $Fe_3O_4$ nanoparticles. Porcine pancreas lipase with natural resistance to methanol has been used for transesterification enzymatically (Wang et al. 2009). Here, high amount of biodiesel production from soybean oil was recorded, and immobilized enzyme retained 100% of its activity for three cycles (Kaieda et al. 2001). The high amount of protein loading (85% of solution) rendered high enzyme activity to the immobilized enzyme 95% compared to 82% of free enzyme which ultimately led to high biodiesel production. In another study, high biodiesel production was reported for amino-functionalized magnetic nanoparticles covalently immobilized to *Thermomyces lanuginosus* lipase (Xie and Ma 2009, 2010) and nanomaterial's activated glutaraldehyde cross-linked lipase (Liu et al. 2012). *Burkholderia* sp. lipase immobilized onto hydrophobic magnetic particles without losing any enzyme activity has been successfully used six times for transesterification, and its activity was comparable with Novozyme 435. Lipase obtained from *Pseudomonas cepacia*, immobilized via adsorption onto the nanoporous gold support, achieved biodiesel production of up to 90%, whereas the transformation of soybean oil by free lipase was 74% after 24 h (Wang et al. 2011a, b). In addition, the performance of the enzymes was improved under extreme reaction conditions such as high temperature and pH conditions.

As discussed earlier, lipase immobilized on nanoparticles is most commonly done by covalent or non-covalent methods (Wang et al. 2009) and is found to have

**Table 9.1** List of immobilized enzymes currently employed in biofuel production

| S. no. | Enzymes | Immobilization method | Material | Properties | Reference |
|---|---|---|---|---|---|
| 1. | *Burkholderia cepacia* lipase | Ultrasonic emulsification | Soybean oil | High biodiesel yield of 68.6% in 180 min | Murillo et al. (2019) |
| 2. | Lipase | PAN-PEI-SA-$CaCl_2$ | | High enzyme loading (36.90 mg/g), repeated use (20 cycles), and 2.5-fold increase in enzyme activity (54.47 U/g) compared to Novozyme® 435 were recorded | Li et al. (2019) |
| 3. | Endo-1,4-β-D-glucanase, cellobiohydrolase, β-glucosidase, endo-1,4-β-xylanase, and β-xylosidase | Magnetic nanoparticles, SE-CLEAs, and M-SE-CLEAs | Lignocellulose-based biomass | Single-step saccharification of lignocellulose using nanoparticle-immobilized enzyme produced reducing sugar up to 250 mg/g of biomass.] | |
| 4. | Cellulase and β-glucosidase | | Biomass of *Typha angustifolia*, *Arundo donax*, *Saccharum arundinaceum*, and *Ipomoea carnea* | The co-immobilized trienzyme biocatalyst are potential one-pot pretreatment for the production of bioethanol from lignocellulosic biomass | Kirupa Sankar et al. (2018) |
| 5. | Porcine pancreatic lipase | Zirconium-pillared clay using adsorption and cross-linking | Canola oil | Biodiesel production | Colín-Luna et al. (2018) |
| 6. | Hydrolases | | *Spirulina platensis* biomass | Bioethanol production from microalgal biomass | Rempel et al. (2019) |
| 7. | Cellulolytic enzymes | Superparamagnetic nanoparticles | | The bio-nanocomposites on the membrane prevent direct membrane-foulant interaction for long period, stabilized the enzyme, and hence led to continuous productivity | Gebreyohannes et al. (2018) |

| | | | | | |
|---|---|---|---|---|---|
| 8. | Chimera construct of xylanase and lichenase | SpyTag/SpyCatcher | | A novel strategy, easy to scale up and, potential to meet the demands of biofuel industry | Lin et al. (2018) |
| 9. | Lipase immobilization | Silica xerogel with protic ionic liquid and bifunctional agents | Olive oil, sunflower, soybean, and colza oils | Application in biodiesel production | Carvalho et al. (2018) |
| 10. | *Pseudomonas* sp. lipase | Activated biochar | | Immobilized lipase yield (92.23%) of fatty acid methyl esters, and it can be reused for 3 cycles | Khosla et al. (2017) |
| 11. | *Trichoderma reesei* cellulase | Chitosan-coated magnetic nanoparticles | | The immobilized enzyme showed better thermal and storage stability, retained ~80 % of its activity 15 times, and can be easily separated with external magnetic field compared to free enzyme | Sánchez-Ramírez et al. (2017) |
| 12. | *Candida antarctica* lipase | Core–shell magnetic nanoparticles | Waste cooking oil | The immobilized enzyme produced biodiesel by transesterification of waste cooking oil, higher thermal stability, and methanol tolerance compared to the free enzyme | Mehrasbi et al. (2017) |
| 13. | Amylases | | Saccharification of microalgal biomass | Immobilized enzyme is a promising alternative toward development of the bioethanol production | Rodrigues et al. (2017) |
| 14. | *Candida* sp. 99–125 lipase | Entrapment of lipase in alginate and support of diatomite | | Higher biodiesel yield (92%); repeated use (11 times) was recorded at 40 °C in 24 h; maintained about 70% of its activity after 11 repeated batch reactions | Zhao et al. (2017) |
| 15. | *Burkholderia cepacia* lipase | Nanoporous carbon derived from metal–organic framework | Soybean oil | A promising green and sustainable catalytic system for industrial application | Liu et al. (2017) |

(continued)

**Table 9.1** (continued)

| S. no. | Enzymes | Immobilization method | Material | Properties | Reference |
|---|---|---|---|---|---|
| 16. | *Streptomyces* sp. strain W007 lipase | | Waste cooking oils | High biodiesel yield (~95%); mobilized lipase retained ~70% of its initial activity after four batch cycles | Wang et al. (2017) |
| 17. | Cellulase | Magnetic nanobiocomposite | *Sesbania aculeata* biomass hydrolysate | Maximum bioethanol production of 5.31 g/l under optimal conditions | Baskar et al. (2016) |
| 18. | Lipase | Superparamagnetic $Fe_3O_4$ hollow submicrospheres | Waste cooking oil | FAME yield exceeded ~93% over a range of temperatures from 10 to 40 °C | Liu et al. (2016) |
| 19. | *Candida parapsilosis* lipase | Synthetic resins (Accurel MP 1000 and Lewatit VP OC 1600) | Jatropha oil | The production of biodiesel by transesterification of jatropha oil with methanol in a lipid/aqueous system | Rodrigues et al. (2016) |
| 20. | *Candida* sp. 99–125 lipase | | Waste cooking oil | The immobilized lipase conversion ratio exceeds 70% even after 10 subsequent reactions, in particular which is better than commercial Novozyme 435 and TLIM | Zhao et al. (2014) |
| 21. | Lipases | Silica nanoparticles | | The immobilized enzyme can be reused and retained 95% of initial activities after 7 and 15 cycles of the reaction | Babaki et al. (2016) |

decent preservation of enzyme activity, extraordinary loading efficiency, as well as higher enzyme activity (Yu et al. 2005; Gao et al. 2010; Georgelin et al. 2010; Ji et al. 2010; Lee et al. 2010; Li et al. 2011; Saifuddin et al. 2013; Wang and Jiang 2011; Xie and Ma 2010). Immobilized lipases have high activity and stability, and the maximum transformation rate up to 75% was documented at 12 h. In comparison, after nine cycles, the transformation rate of only 18.5% was observed in the batch process (Wang et al. 2009). This decrease is probably due to low deactivation rate of the immobilized lipase. Another four-PBR system of immobilized lipase offered higher conversion rate of up to 88% for 192 h and last 75% after 240 h of reaction (Hama et al. 2007; Thanh et al. 2011; Wang et al. 2011a).

Cellulase complex immobilized to gold-doped silica nanoparticles using affinity technique is also used for single-step catalysis of cellulose to glucose (Cho et al. 2012). The nanohybrid-immobilized cellulase loaded at 20 FPU per gm of pretreated bagasse is found to produce reducing sugar of 38.87 gm per liter which resulted in 0.38 gram per gram of ethanol after fermentation. Moreover, the immobilized nanohybrid was recycled up to five times. The co-immobilized laccase, cellulase, and α-glucosidase for the bioethanol production from *Typha angustifolia*, *Arundo donax*, *Ipomoea carnea*, and *Saccharum arundinaceum* lignocellulose biomass are more stable, compared to free enzyme solution. The saccharification of *S. arundinaceum* biomass produced reducing sugar of 205 ± 3.73 milligram per gram of biomass. The high amount of bioethanol up to 63.43 ± 9.35% was obtained with *I. carnea* (Kirupa Sankar et al. 2018).

## 9.14 Site-Directed Mutagenesis to Enhance the Biochemical Properties of Immobilized Enzymes

The prior information of the protein tertiary structure and basics of enzyme immobilizations can play vital role in developing an efficient system. The usage of site-directed mutagenesis can help to reserve the enzyme role as well as improve immobilization process. An escalation in the quantity of the ionized amino acids on proteins allows the immobilization of relevant enzymes on suitable supports. For example, the introduction of eight Glu residues using site-directed mutagenesis led to decrease in the isoelectric point of the protein from 6.4 to 4.3 (Montes et al. 2007). Therefore, a wild-type enzyme which is difficult to adsorb on anionic exchangers can be adsorbed on DEAE or polyethylenimine-coated supports. Other alternative to site-directed mutagenesis has been explored for enzyme immobilization. For example, the method to increase the formate dehydrogenase residual activity involves entrapping *Candida boidinii* lipase in polyacrylamide gels (Ansorge-Schumacher et al. 2006). Here, the chemical alterations of Lys or the modifications of the hydrophobicity of the medium, due to acrylamide and tetramethylethylenediamine exposure, may lead to inactivation of enzyme (Bordini et al. 2000; Chiari et al. 1992; Dobryszycki et al. 1999). The formate dehydrogenase variants obtained after site-directed mutagenesis reserved high residual activity after entrapment in polyacrylamide gels compared to native enzyme type. Other parameters such as the polymerization method also affect the enzyme variations. Both the immobilization and directed evolution strategies can be explored in coupled fashion for achieving optimized catalyst activity (Brandt et al. 2006; Rai et al. 2016). In general, Cys residues are explored for direct immobilization of the protein on a support containing disulfide groups or gold. Additionally, use of His tagged protein, carrier altered with immobilized metal and other support materials such as glyoxyl or epoxy disulfide or with higher potential and multipoint covalent connection are also explored (Karel Hernandez 1980).

## 9.15 Conclusion and Future Prospective

The continuously growing industries demand technologies which are more effective, selective, and sustainable than the previous technologies. The commonly used chemical-based methods for the saccharification of plant biomass for biofuel production are promising due to environmental concerns. The biocatalysts for industrial applications are on the edge of evolution, and catalytic processes can be performed under organic or aqueous environments. The substrates can be selectively and effectively modified with enzymes (Schmid et al. 2001). The recent developments in biotechnology and nanobiology for nanobiocatalysts are emerging as attractive choice for the manipulation of nanoscale surroundings of the enzymes by generating nanoscale materials as carrier macroscale reactors. The immobilization on carrier provides specific biochemical and kinetic properties to the enzyme, ease of separation, and even shelf-life from the reaction that can minimize the costs and increase their competitiveness for technical applications (Tischer and Kasche 1999). The benefits of immobilized enzyme can easily compensate the cost of biodiesel production. The option of designing and developing green and efficient operating systems for biodiesel production from waste has been achieved using novel magnetic nanoparticle (Ngo et al. 2013). The properties of immobilized enzymes are administered by the enzyme and the carrier material properties. The enzyme immobilization methods use prefabricated carrier materials for enzyme stabilization through adsorption, conjugation, and entrapments as crystals or powders. The immobilization of enzyme on carrier provides specific biochemical and kinetic properties to the enzyme (Tischer and Kasche 1999).

The immobilization is a valuable tool to cut down the enzyme cost and offers several technological advantages including stability against harsh reaction conditions. It also, permits the repetitive enzyme use which significantly reduces the cost of immobilized enzymes. Moreover, the enzyme can be simply detached from the reaction media. However, the immobilization of enzymes onto planar surfaces may limit their activity due to factors such as alteration in the configuration of native protein, steric interference, and slow diffusion rate. To eliminate the undesirable effects of enzyme immobilization, nanoparticles (NPs) as enzyme carriers are attractive alternative (Ding et al. 2015). Still, the challenges associated with exploring enzymes for biofuel production are enormous including high enzyme costs, inactivation of enzymes by solvents, and difficulties of large-scale production

(Watanabe et al. 2000). The nanomaterial-based efficient methods of enzyme immobilization can cut down the cost to a significant extent. Besides the advantages and limitations of nanomaterials, health and environmental concerns are of paramount importance prior to their usage in biofuel production (Arico et al. 2005; Cui et al. 2005; Krumov et al. 2009). The homogeneous size of nanomaterials and stabilization from aggregation might be addressed by coating with a polymer shell (Rozenberga and Tenne 2008).

The enzymes immobilized by nanostructured materials can provide promising solutions for industrial biotransformation. Use of nanomaterials for fabricating nanobiocatalysts for several enzymes including hydrolase, glucose oxidase, and alcohol dehydrogenase has been achieved with great accomplishments (Kim et al. 2008; Petros and Desimone 2010; Wang et al. 2009; Ginet et al. 2011). The nanomaterial enzymes have lower inactivation and hence can maintain higher activity even after repeated uses (Husain 2017). In recent times, use of nanomaterials for numerous applications has been explored to immobilize enzymes. The development of PBR systems for frequent and efficient use of immobilized lipase (Wang et al. 2011a) and a better understanding of enzyme interactions with nanomaterial surface at the support or in solvent interface can remarkably increase both the assembly (Pavlidis et al. 2012a, b) and efficient nanomaterial-based enzyme immobilization.

In addition, mass transfer effects can reduce the enzyme activity. The resulting enzymes are not only affected by the method adopted for immobilization but also by a decrease in the availability of enzymes or substrate molecules. The mass transfer effects can result in substrate or pH gradient which can decrease the reaction rate and product yield. This also reduces access to substrate and leads to deliberate restriction in the movement of enzyme which affect the access of enzyme for substrate and partition effects of substrate concentrations and decrease the enzyme efficiency as compared to free enzymes. How to avoid and minimize the mass transfer effect and other limitations responsible for reduced enzyme activity during enzyme immobilization needs to be addressed which reduces the enzyme efficiency. Altogether, these factors can offer an accurate measure of enzyme productivity or enzyme consumption (Tischer and Kasche 1999). Therefore, owing to the biochemical properties of enzymes and the varying conditions of reaction technology, there is a need to explore the wealth of methods for developing novel processes of immobilization. Efforts to address the regulated movement and diffusion which primes to reduction in enzyme activity required to be explored using mathematical and other methods. The associated concerns of enzyme immobilization include reduction in enzyme activity or catalytic structures, distortions in the enzyme structure and enzyme inactivation, due to unfolding of the native enzyme structure followed by kinetically irreversible changes through aggregation or covalent changes in the enzyme.

The expansion of methods to stabilize the enzyme structure is an interesting challenge. Compared to enzyme immobilized on micrometric supports, nanobiocatalysts have potential to achieve higher enzyme loading on its surface. Current advances of nanobiocatalytic methods have improved the performance using various nanomaterials, but challenges to cope with mass transfer efficiency and enzyme activity need to be optimized for low cost and efficient processes. The immobilization of enzymes onto nanostructured surfaces has led to numerous benefits compared to the use of free enzymes or immobilization on larger materials. So far nanomaterial-supported enzyme preparations have been explored for biofuel production. The possibility to co-immobilize the multi-enzymes on nanomaterials certainly can enable the efficient use of different enzymes in targeting biomass for biofuel (Alper and Stephanopoulous 2009). However, studies are necessary to know the practical bottlenecks including efficient synthesis techniques for nanomaterials and evaluation of safety guidelines. Regardless of the development in the preparation of nanomaterial–protein conjugates, limited studies have generated thorough evidences on the structure and function of proteins, attached to nanomaterials (Asuri et al. 2007).

Therefore, the biotechnological perspective of enzyme immobilized on nanomaterials is of supreme importance. Keeping in view, the structural and functional properties of nanomaterials for enzyme immobilization and stabilization method, care must be taken for possible modifications in catalytic efficiency. Further, the optimization of stabilizing parameters will prime to a better and efficient processes and hence may upsurge the economic potential in existing enzymatic processes and for other novel areas where enzymes have not been used till now because of their uncertainty.

**Acknowledgment** Authors are thankful to SEED division, DST, GOI for the award of project under Scheme for Young Scientists and Technologists (SEED-TIASN-023-2018).

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# Carbon Nanotubes Synthesized by Green/Eco-friendly Technique Potential for Bioenergy Applications

# 10

Kelvii Wei Guo

## Abstract

The multi-walled carbon nanotubes (MWCNTs) were synthesized by homemade equipment with the waste alcohol. The synthesized MWCNTs were characterized by scanning electron microscopy (SEM), transmission electron microscope (TEM), and Raman spectroscopy. Moreover, in confirming the synthesized green/eco-friendly technique, MWCNTs were grown on some of the micropunches to show the attractive potential for bioenergy application compellingly and convincingly. Results show that due to the usage of the waste alcohol, not only is this technique definitely green/eco-friendly to the environment, but also MWCNTs as a protective layer on the micropunch can lengthen the service life of the micropunch by up to 35% without directly affecting the size of the punched holes compared with that of without MWCNTs. Moreover, the punched microholes are definitely quite distinct from that of traditional punching. Besides the fracture band is absent, the areas of rollover and burr are also extremely narrow, which implies the attractive punched microhole can be used directly without any posttreatments.

## Keywords

Multi-walled carbon nanotubes (MWCNTs) · Green/eco-friendly technique · Bioenergy · Biomass power plant · Waste alcohol · Nanotechnology · Microhole · Micropunch · Service life

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_10

## 10.1 Introduction

Carbon nanotubes (CNTs) have attracted intense attention since their discovery (Iijima 1991, 1993). The past two decades have witnessed their spread in a range of applications including building blocks in modern electronics, field emission sources, photodetectors, nonvolatile memory, ultrasensitive chemical and biosensors, and transparent conductive membranes.

It is well known that CNTs are intrinsically composed of pure carbon atoms that arrange and interact with each other by the strong $sp^2$ carbon-carbon chemical bonds and form the unique geometric structure of a carbon network; this gives CNTs fascinating and attractive properties, such as electronic, mechanical, and thermal properties. These properties have convinced scientists that CNTs and related nanostructures may play crucial but irreplaceable roles in many different kinds of applications by either replacing the traditional materials or exploring novel functions.

To date, CNTs' production capacity exceeds several 1000 tons per year (De Volder et al. 2013). Advances in CNTs' controlled growth, purification, modification, and processing are showing great advantages in many aspects of everyday life. CNTs have been integrated into various commercial products, ranging from rechargeable batteries, mobile screens, conductive additives, sporting goods, and water filters (Sehrawat et al. 2016; Li et al. 2017; Yadav and Singh 2016; Moreno et al. 2017; Jäckel et al. 2016; Ho et al. 2018; Puch and Hopmann 2014; Zhai et al. 2016; Luzardo et al. 2017; Paramo et al. 2018).

Because of its unique structure, excellent electrical conductivity, mechanical property, high tensile and flexural strengths, high elastic modulus, and high aspect ratio, CNTs have been regarded as an attractive contender in tribological applications. Interesting results were reported with significant reduction in friction and wear rates in nanotube polymers (Pöllänen et al. 2011; Green et al. 2009), ceramics (Zhan et al. 2003; Hvizdoš et al. 2012), and metal composites (Guiderdoni et al. 2013; Bakshi et al. 2011). Theoretically speaking, friction coefficient between the walls of MWCNTs can be extremely low (Damnjanović et al. 2002; Dickrell et al. 2005; Dickrell et al. 2006).

Advances in telecommunication, transportation, and medical fields lead to challenges in microfabrication, miniaturization, and multifunctional technologies in manufacturing. Feature sizes are in the range of a few microns up to hundreds of microns. The ability to fabricate microholes in large quantities has potential applications in microscale batteries and fuel cells (Baik and Seo 2018; Fischer 2018; Price et al. 2018; Shin et al. 2018; Christwardana et al. 2018; Kim and Park 2018; Ali et al. 2018; Takahashi et al. 2018; Martinez et al. 2017a; Hashemi et al. 2017; Torelli et al. 2017; Dubois et al. 2017; Shetzline et al. 2017; Pratyusha et al. 2017; Palecki et al. 2017; Cherevko et al. 2016; Belarb et al. 2016; Fukuzumi 2016; Calabriso et al. 2015; Theodorakakos et al. 2014a; Virkar 2012), fluidic microchemical reactors requiring microscale pumps (Baghel and Mali 2018; Haghighi et al. 2018; Kant et al. 2017; Calvo-López et al. 2017; Guler et al. 2017; Hatsuzawa et al. 2017; Madhavi and Hiremath 2016; Derkus 2016; Liu et al. 2016; Hilber and Jakoby

2015), valves and mixing devices (Asim et al. 2019; Pradeep et al. 2018; di Capaci et al. 2018; Zangl et al. 2018; Hsu et al. 2018; Mohan et al. 2018; Ravi et al. 2018; Silaskar and Shinde 2018; Ferraro et al. 2018; Cortes et al. 2017; de Vries et al. 2017; Hayes et al. 2017; Makaryants 2017; Okhotnikov et al. 2017; Sayindla et al. 2017; Tverskoy et al. 2017; Tesař 2017; Willich and White 2017; Banyai et al. 2016; Farzaneh-Gord and Khoshnazar 2016; Gehlot and Tripathi 2016; Lisowski and Filo 2016; Malama et al. 2016; Ramesh et al. 2016; Edvardsen et al. 2015; Kudźma and Stosiak 2015; Muniak 2015; Singh and Tharakan 2015; Amirante et al. 2014; Rogers et al. 2014), microfluidic systems (Dvořák et al. 2018; Hoysall and Garimella 2018; Massarwi et al. 2018; Samih et al. 2018; Trotta et al. 2018; Tsur and Shamir 2018; Abed et al. 2017; Dittmeyer et al. 2017; Martinez et al. 2017b; Munk et al. 2017; Kowsari et al. 2016; Kuriakose et al. 2016; Sarafraz et al. 2016; Chavoshi and Luo 2015; Kenig et al. 2013), biomedical and medical implants (Affes et al. 2018; Bellemère 2018; Block 2018; Choi et al. 2018; Rupp et al. 2018; Taniguchi et al. 2018; Cristofari et al. 2017; Rittel et al. 2017; Baino and Potestio 2016; Ferraris and Spriano 2016; Hakimi et al. 2016; Nyström and Malmsten 2016; Pina et al. 2016; Ramos and Mesnard 2016; Sharma et al. 2014), diagnostic and remediation devices (Tharmavaram et al. 2018; Gomis-Berenguer et al. 2017; Rosace et al. 2017; Tanimu et al. 2017; Ferreira et al. 2016; Monošík and Angnes 2015; Nguyen 2013; Schütze et al. 2013), cooling holes in jet turbine blades (Fraas et al. 2019; Kim and Kim 2018; Lee et al. 2018; McInturff et al. 2018; Enagi et al. 2017; Al-Zurfi and Turan 2016; Mazaheri et al. 2016; Park et al. 2016; Ali and Janajreh 2015; Satta and Tanda 2015; Sciubba 2015; Chung et al. 2014; Krewinkel 2013), fiber optics (Pakarzadeh et al. 2019; Bhattacharya 2018; Kumar et al. 2018; Gaona-Reyes and Bermudez 2017; Tu et al. 2014), micro-nozzles for higher energy efficiency (Do et al. 2017; Krishnamoorthi and Malayalamurthi 2018; Ranjan et al. 2017; Saadati and Roohi 2015; Theodorakakos et al. 2014b; Torre et al. 2011) and less pollutant emissions to the environment (Boncel et al. 2011; Gutierrez et al. 2012; Tofighy and Mohammadi 2011; Upadhyayula and Gadhamshetty 2010; Tiusanen et al. 2012), micro-molds and deep X-ray lithography masks, optical lenses, and micro-components in daily life products such as compact disc players, air bags, and inkjet printers. Currently, with the ever-increasing demand for smaller, higher-quality, and lower-priced products from almost all fields of industry, household equipment, and entertainment electronics, the development of manufacturing methods which are tailor-made for the micro-system technique with higher precision, lower cost, and larger quantities and more eco-friendly to the environment is extremely urgent.

The trend for micromanufacturing is more focused on miniaturizing or downscaling both conventional and nonconventional methods to produce microproducts. Manufacturing processes can be categorized according to the type of energy used in the process itself, such as mechanical, chemical, electrochemical, electrical, and laser processes. The working principles behind each process include consideration of mechanical forces, thermal effects, ablation, dissolution, solidification, recomposition, polymerization/lamination, and sintering. According to the way in which components/products are to be made, general manufacturing processes can also be classified into subtractive, additive, forming, joining, and hybrid processes. The classification is equally applicable to micromanufacturing. For subtractive processes, there are micromechanical cutting (milling, turning, grinding, polishing, etc.), micro-EDM, micro-ECM, laser beam machining, electron beam machining, and photochemical machining. For additive processes, the relevant techniques are surface coating (CVD, PVD), direct writing (inkjet, laser-guided), microcasting, microinjection molding, sintering, photoelectron forming, chemical deposition, polymer deposition, and stereolithography. For deforming processes, there are microforming (stamping, extrusion, forging, bending, deep drawing, incremental forming, superplastic forming, hydroforming, punching), hot embossing, and micro-/nano-imprinting. For joining processes, the related techniques are micromechanical assembly, resistance, laser, vacuum soldering, bonding/welding, and gluing. For hybrid processes, there are microlaser ECM, LIGA, and LIGA combined with laser machining, micro-EDM and laser assembly, shape deposition and laser machining, laser-assisted microforming, micro-assembly injection molding, combined micromachining, and casting (Mamalis 2005; Janssen et al. 2008; Uhlmann et al. 2016; Korkmaz et al. 2017; Cole et al. 2017; Biasetti et al. 2017; Singh et al. 2018; Saxena et al. 2018).

Punching is the process of forcing a punch through the material and into a die to create a hole in the workpiece. It is often an economic way of creating shaped holes in mass production. Micropunching was demonstrated in the punching of circular and noncircular holes as small as 5 microns in size. Being a mechanical process that does not involve the use of chemicals, punching is environment-friendly. Therefore, micropunching can be an economic and environment-friendly way of fabricating shaped microholes in mass production.

It is well known that punching is the process of forcing a punch through the material and into a die to create a hole in the work piece. Tool wear is an important issue in micropunching. Even with punches made of hard and tough materials like tungsten carbide and cobalt (WC/Co), the quality of the punched holes declines rapidly under repeated punching (Dornfeld et al. 2006; Mark 2006; Bhattacharyya 2015; Luttge 2016; Knobloch and Kaminorz 2004; Mohamed 2006; Edwards 2006; Oliver et al. 2004; Johnstone and Parameswaran 2004; Brousseau et al. 2010).

The higher quality of the micropunches is a key to the successful application of micropunching. When the punching number exceeds 1000, the quality of the micropunch decreases obviously due to the serious wear of the micropunch. During the micropunching, the friction between the micropunch and the substrate deteriorates the micropunch. As a result, the quality of the punched holes decreases simultaneously. In addition, the price of WC/Co micropunches is costly (Guo and Tam 2011).

Because of the unique property of CNTs, the friction between the micropunch and the substrate can be decreased by forming a CNT coating on the surface of the micropunches to increase the wear property of the micropunches and improve the tool life in the end. Therefore, aiming to form an effective CNT coating on WC/Co micropunches, the multi-walled carbon nanotubes (MWCNTs) were synthesized by homemade equipment with the waste alcohol, which is green/eco-friendly to the environment. Meanwhile, the effect of synthesized MWCNT coating on the tool life of WC/Co micropunches has been fully investigated by confocal laser, SEM, digital balance, etc. Results show that the effect of MWCNTs synthesized by the waste alcohol is significantly efficient. The wear of micropunch coated with MWCNTs decreases obviously, even in the severe wear period. Moreover, the wear loss is definitely less than that of micropunch without MWCNT coating. This promising result is due to the formation of a transfer film at the contact region by rubbing the MWCNT forest. MWCNTs adhere to the surface of the micropunch avoiding direct contact with the substrate and providing lubricant properties to the interface by virtue of their graphitic nature.

## 10.2 Experimental Materials and Procedures

### 10.2.1 Experimental Materials

Micropunch made by Ultrahardness tools company in Japan with 75% volume fraction WC particle and 25% volume fraction Co particle of 50 μm mean size, 150 μm in diameter, is shown in Fig. 10.1. Specimens for punching experiments were pure titanium sheets 200 μm in thickness.

Figure 10.2 shows the surface texture of micropunch and its elements' (carbon, tungsten, cobalt) distribution. Ploughs induced by the ultraprecise machining can be obviously detected, and some debris are distributed on the surface of the micropunch. Moreover, the distribution of tungsten element is denser than that of both carbon element and cobalt element, which indicates that the hardness of the surface of the micropunch is hard and suitable for the micropunching process.

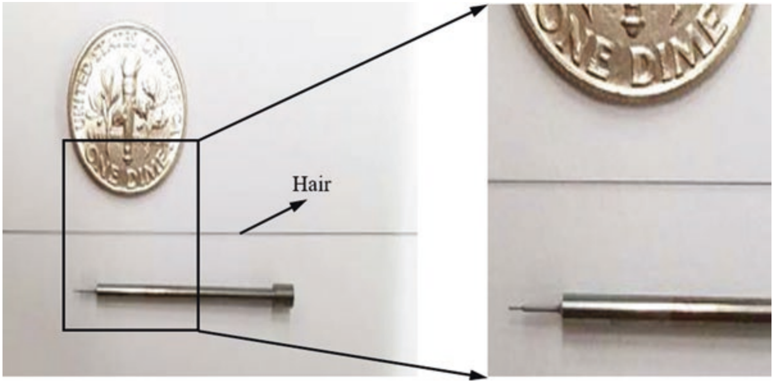


**Fig. 10.1** Profile of Ø150 μm micropunch

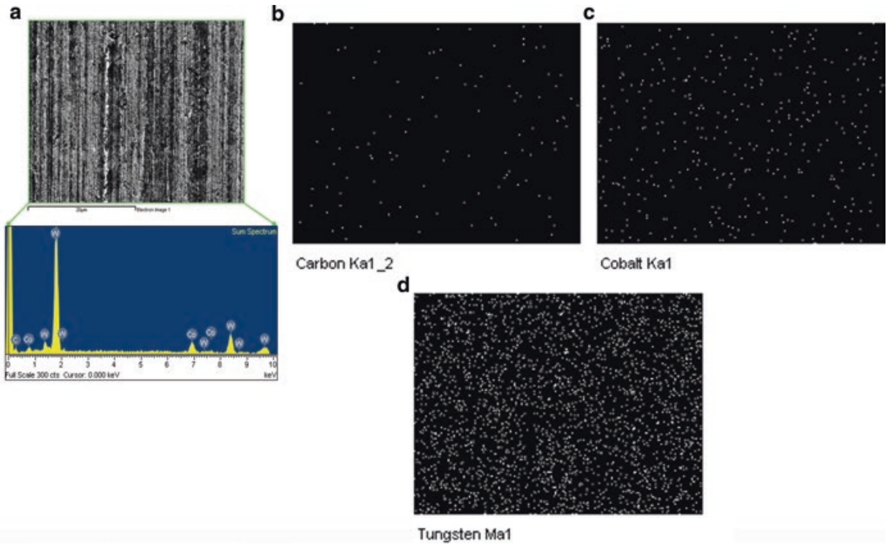


**Fig. 10.2** Surface texture of WC/Co micropunch and elements' distribution. (**a**) Surface texture and mapping of elements. (**b**) Carbon distribution. (**c**) Cobalt distribution. (**d**) Tungsten distribution

## 10.2.2 Experimental Procedures

### 10.2.2.1 CNT Growth

Micropunches were cleaned by acetone and pure ethyl alcohol for contaminant removal prior to CNT growth/deposition.

In this research, Fe was taken as the catalyst for CNT forming. In the initial, the catalyst was coated on the required area of the micropunch by electron cyclotron resonance (ECR). The relevant processing parameters are listed in Table 10.1. It is well known that the distribution of cobalt element in the micropunch is also a catalyst for CNT forming and CNTs will be consequently synthesized during the CNT growth. Therefore, in order to protect the other areas from synthesizing CNTs, aluminum foil is adopted as the cover for the remaining areas of the micropunches (Dupuis 2005).

For Fe coating process, the micropunches were set in a vacuum chamber for CNT forming. Figure 10.3 shows the relevant schematic diagram of the homemade CNT forming equipment. The micropunch was set on the heating area for CNT growth. The dimension of the vacuum chamber was Ø100 mm × 150 mm.

The waste alcohol, which was directly taken from the alcohol after the specimens' cleaning, met with the GB-HW06 standard, was put into a ceramic container under the heater to provide carbon source for chemical vapor deposition (CVD). Conditions for CNT growth were summarized in Table 10.2.

**Table 10.1** ECR processing parameters for Fe deposition

| Substrate | WC/Co micropunch |
|---|---|
| Catalyst | Fe |
| Irradiation time | 60 s |
| Accelerated voltage | 2500 V |
| Ion current density | 12.0 mA/cm$^2$ |
| Gas | Ar |
| Gas flow rate | 0.6 SCCM |
| Vacuum | $1.5 \times 10^{-4}$ pa |

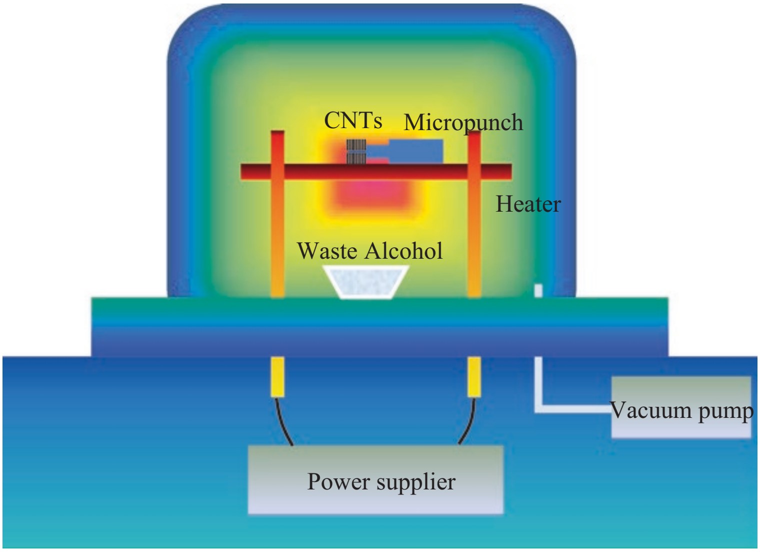


**Fig. 10.3** Setup for CNT growth

**Table 10.2** Parameters for CNT growth

| Heating rate | 3~4 A/s |
|---|---|
| Cooling rate | 5 A/s |
| Reaction direct current | 38~40 A |
| Reaction time | 10 s |
| Vacuum | $1 \times 10^{-2}$ pa |

#### 10.2.2.2 Micropunching

The acetone and pure ethyl alcohol were taken as cleaner to make the substrate-pure titanium sheet clean the contaminants. After that, the substrate is carefully put into the microdie. When all is set properly, the micropunching is in process using the microprocessing machine MP50 (made in Japan), where the federate of micropunching was 2 mm together with 20 pulses in 1 min.

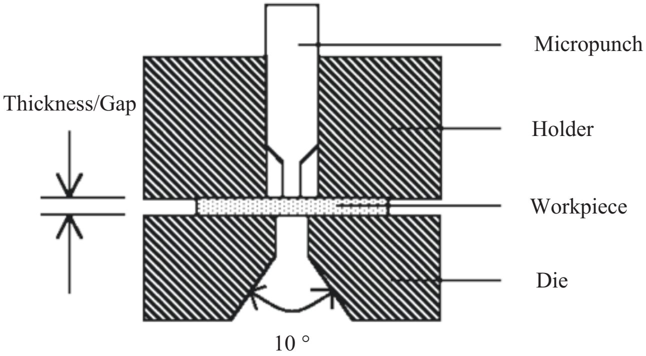


**Fig. 10.4** Schematic diagram of micropunching

Along with the micropunching process, the in-house setup was used to make the accurate vertical alignment. The accurate vertical alignment is extremely important to the micropunching. Without it, the micropunch is easily broken and seriously worn during the punching process. Consequently, the punched microholes lack high quality, resulting in the failure of the microhole application afterward. In this research, the lateral error is less than 1.5 μm in the range of 20 mm vertical stroke. A *z*-stepper is taken as the stroke and load with the 20 N in maximum by a push rod. In the meantime, the bush was fabricated by the same material as the micropunch (tungsten carbide/cobalt) with a 200 μm through-hole in 10° taper. Both faces of the bushes were carefully polished to ensure flatness and parallelism. The relevant schematic diagram of micropunching is shown in Fig. 10.4.

The characteristics of microholes punched by WC/Co micropunch in various periods were investigated by LEXT confocal laser-OLS3000, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), optical surface profiler (OSP)-Veeco/Wyko NT9300, etc.

## 10.3 Results and Discussion

### 10.3.1 Micropunch with MWCNT Coating

An image of a CNT-coated micropunch is shown in Fig. 10.5, and its higher magnification image is expressed in Fig. 10.6. It illustrates that the synthesized CNTs coat on the surface of the micropunch successfully and the height of CNTs is about 15 μm. Meanwhile, CNTs vertically align with high density. Moreover, CNTs appear wiggly because they were tightly packed during growth. TEM image of CNTs is shown in Fig. 10.7. It indicates that CNTs are multi-walled nanotubes (MWCNTs) with a diameter of about 3–5 nm.

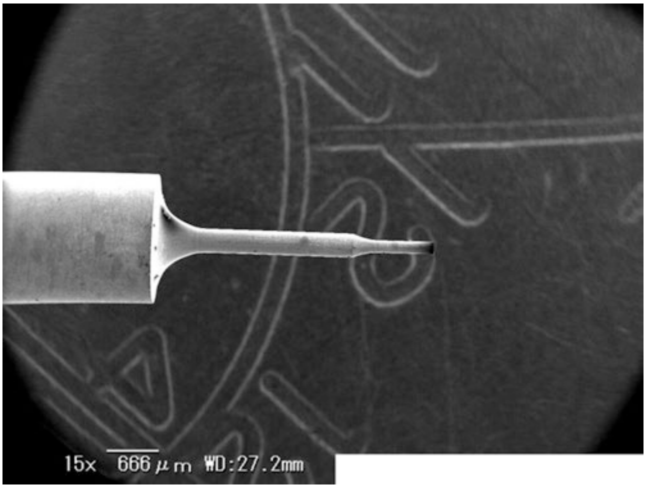


**Fig. 10.5** A micropunch with MWCNT coating

### 10.3.2 Raman Spectroscopy Analysis

Raman spectroscopy analysis is taken for testing the property of the synthesized vertically aligned MWCNTs. The parameters of Raman spectroscopy analysis are listed in Table 10.3. It is well known that the *G*-band, the *D*-band, and the RBM are included in the typical Raman shifts ($cm^{-1}$) and the corresponding peak of the *G*-band is at about 1590 $cm^{-1}$ and the *D*-band 1350 $cm^{-1}$. The characteristic of the synthesized MWCNTs is evaluated according to the ratio between the *G*-band and the *D*-band.

Figure 10.8 expresses the result of Raman spectroscopy analysis of the synthesized vertically aligned MWCNTs. It shows the ratio of IG/ID is about 0.75. Meanwhile, the peak of *D*-band is higher than that of *G*-band. It reveals that the synthesized vertically aligned MWCNTs are highly disordered. As the media in the tribology, the disordered MWCNTs will be more inclined to show their attractive lubricant characteristics more easily. Therefore, the micropunch with such MWCNTs must be more effective for the practical applications than that of the micropunch without MWCNT coating.

### 10.3.3 Wear Loss of Micropunches

During the micropunching, the micropunches vary during the various periods due to the wear of the micropunch along with the weight changes of the micropunches. The variation of the micropunches was measured by the intermittent weight calibration by digital balance during punching.

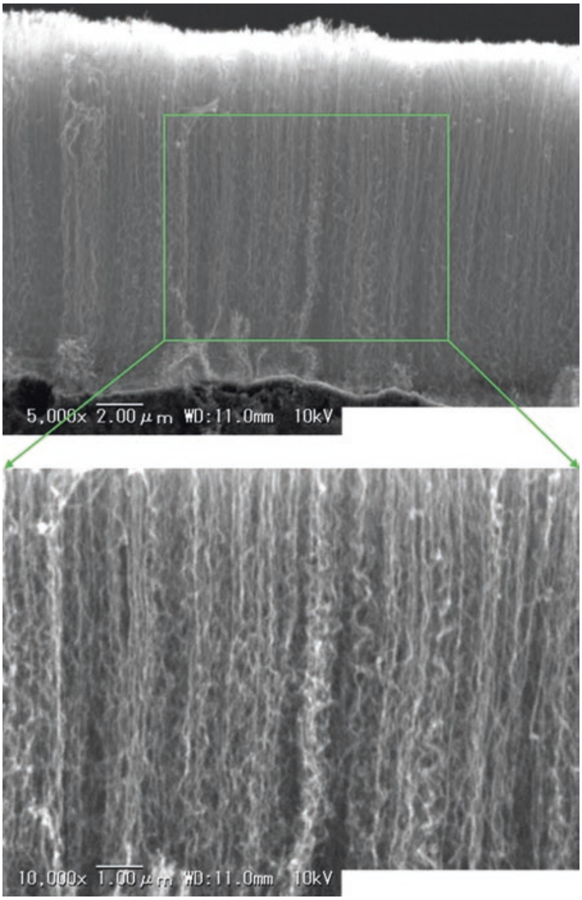


**Fig. 10.6** MWCNTs coated on the surface of micropunch

Results clearly show that there are three stages during the micropunching process; they are initial stage, quasi-stable stage, and severe wear stage. The initial stage is also known as the run-in stage. In this stage, for the micropunch without CNT coating (cycle no. <500), the wear loss of the micropunch is definitely obvious and relatively high. With the effect of friction between the micropunch and the substrate, the micropunch wears and the surface is smoothened. At the same time, the crests on the surface of the micropunch are lowered. When the punching number is in the range of 500–1200, the wear characteristic of the micropunch is quasi-stable. In this stage, the wear loss is very little, and the variation of the surface texture of the micropunch is also hardly detected. However, when the punching number exceeds 1200, due to the severe cobalt loss, which is the bonding material in the micropunch, tungsten carbide is more easily peeled off from the micropunch seriously resulting in a distinctive weight loss. Moreover, the micropunch is more inclined to break in this stage.

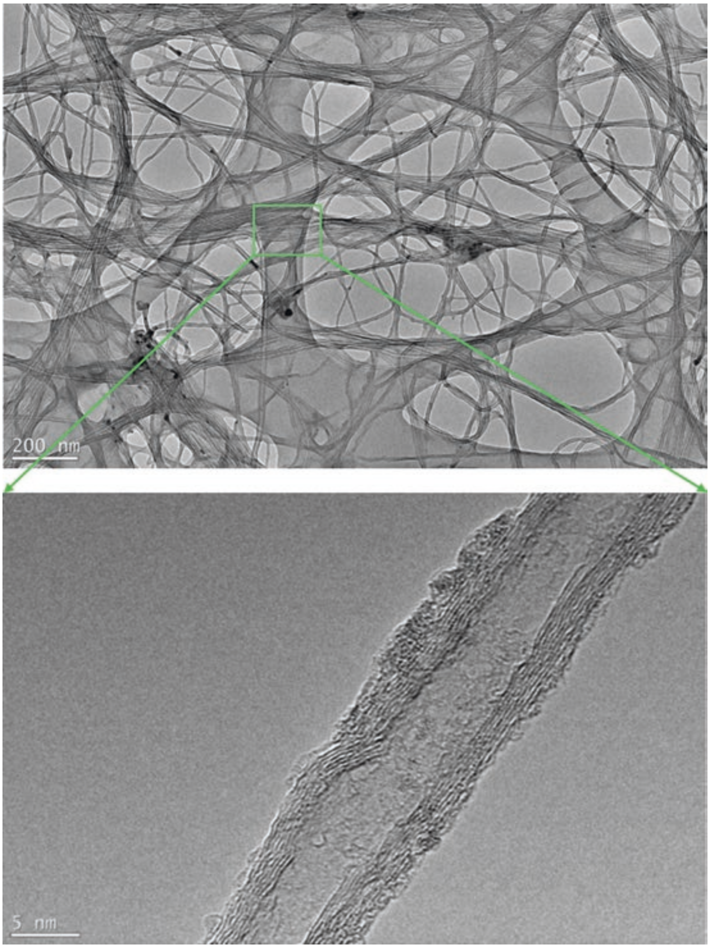


**Fig. 10.7** TEM image of synthesized MWCNTs

**Table 10.3** Conditions for Raman spectroscopy analysis

| | |
|---|---|
| Laser wavelength | 532 nm |
| Laser power | 30 mW × 0.5% |
| Irradiation spot | 5 μm |
| Integration times | 30 |

It also expresses that the wear characteristics of the micropunches with CNT coating are nearly the same as that without CNT coating. The attractive result of the micropunches with CNT coating is that the quasi-stable stage forwards and begins at 450. At the same time, the quasi-stable stage delays and ends at 1400. Moreover, the wear loss of non-coated/coated micropunches in the quasi-stable

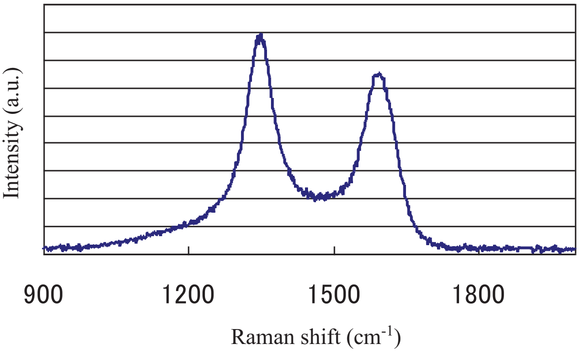


**Fig. 10.8** Raman spectra of synthesized MWCNTs

period is definitely attractive, especially for punching number from 500 to 1200 for non-coated micropunches and from 450 to 1400 for MWCNT-coated micropunches. In this period, the effect of MWCNTs on the wear loss of micropunch sounds obvious. In addition, the comparison between the micropunch with and without CNT coating is affirmatively distinctive. For the severe wear stage, the wear loss of the micropunches (both with CNT coating and without CNT coating) is certainly obvious. When the punching number exceeds 1200 for the micropunches without CNT coating and the punching number exceeds 1400 for the micropunches with CNT coating, the weight loss of the micropunches at both conditions is all serious, and the beneficial effect of the synthesized MWCNTs on the wear loss disappears, especially with the punching number increasing further.

It should be noted that with the favorable effect of MWCNTs synthesized on the surface of the micropunches, the start of the quasi-stable period is pushed forward, and the end of the quasi-stable period is postponed. It elucidates that the effective quasi-stable period of the micropunches with CNT coating is longer than that of without CNT coating on the surface of micropunches. For MWCNT-coated micropunches, it is 1400–450 = 950. By comparison, for non-coated micropunches, it is 1200–500 = 700. Furthermore, the total wear loss of MWCNT-coated micropunches is less than that of non-coated micropunches. It demonstrates that the life of micropunch is improved or prolonged evidently.

### 10.3.4 Profile of Microhole Punched by WC/Co Micropunch

The diameter of the punched microhole by the micropunch was measured by LEXT confocal laser-OLS3000.

It illustrates that in the different period, with the weight variation of the micropunch, the diameter of microhole changes correspondingly. In the initial period, the diameter of microhole decreases obviously with the increment of punching number. While the punching number is from 500 to 1200, the diameter of microhole relatively keeps stable due to the unvariation of the micropunch which is indicated by the near-zero weight loss of the micropunch in the quasi-stable period. With the punching number increasing further, especially when the punching number exceeds 1525, the diameter of microhole decreases remarkably because of the serious loss of cobalt. Consequently, the serious wear of micropunch mainly relies on tungsten carbide particles which are easily peeled off without cobalt as bonding material in the severe period of the micropunching. Meanwhile, due to the temperature increment with punching number increasing, tungsten carbide particles are more easily peeled off, resulting in more intensive wear loss. Moreover, if the pulses per minute increases, the wear loss of the micropunch will be more drastically.

Moreover, it also illustrates that for both cases (non-MWCNT coated and MWCNT coated on the surface of the micropunches), in the different wear conditions, the diameter of the punched microhole changes correspondingly. In the initial condition, the diameter variation of the two kinds of micropunches (with and without CNT coating) essentially overlaps, which is the same as the trend of the weight change. There is a distinctive diameter decrease of the punched microholes when the weight loss of the micropunch is fast in the initial stage. At this period, the beneficial effect of MWCNTs on the size of the punched microhole is not clear. At a result, the evolution of the size of the punched microholes relies mainly on the weight loss of the micropunches. The attractive results of the diameter variation of the punched microholes at both conditions (with and without CNT coating) in the quasi-stable stage are very small along with the very little weight loss of the micropunches. For the micropunches without CNT coating synthesized on the surface, the diameter of the micropunches decreases slightly faster consistent with the slightly faster weight reduction of the corresponding punch. The diameter of the punched microhole for the micropunches with CNT coating on the surfaces reduces from 174.2 μm to 172.9 μm and from 173.1 μm to 170.9 μm for the micropunches without CNT coating on the surfaces. The holes achieved by the CNT coated on the surface of the micropunches are slightly bigger than that of without CNT coated on the surface of the micropunches which reveals the definitely beneficial effect of CNT coating compellingly and convincingly.

The diameter of the punched microholes reduces rapidly in the severe wear stage. After 1600 shots, the diameter of the punched microholes reduces to 163.8 μm for punching with CNT coating on the surface of the micropunches. For the micropunches without CNT coating on the surface, the diameter of the punched microholes reduces to 161.0 μm. These scenarios indicate about 6% reduction from the nominal diameter in the quasi-stable stage. More seriously, near the end the reduction rate is about 6.5 μm per 100 shots for both micropunches. For the surface of the micropunches coated with MWCNTs, due to the lack of attached MWCNTs on the surface of the micropunches, the relevant wear characteristic is same as that of non-MWCNTs coated the surface of the micropunches. Consequently, the serious weight loss of the micropunches is the obvious characteristic in the severe wear stage. In the meantime, the relevant diameter of the punched microholes reduces remarkably.

Meanwhile, the variation of the diameter of the punched microholes meets very well with the wear loss of the micropunches.

### 10.3.5 Surface Texture of MWCNT-Coated Micropunch

Figure 10.6 shows the initial surface texture of MWCNTs coated micropunch are distributed as MWCNTs forest, MWCNTs densely populate, mutually tangle and distribute uniformly on the surface of the micropunch.

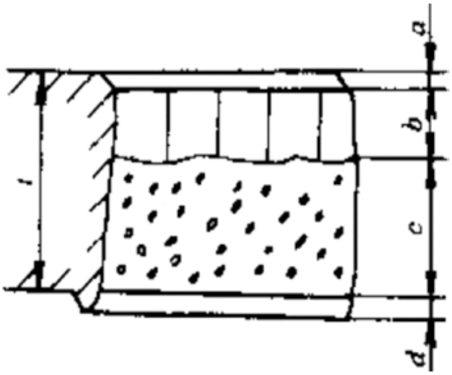
It can be seen that MWCNTs are distributed non-uniformly on the surface of the micropunch, and under the rubbing effect of interaction between the micropunch and the substrate, a bundle or bulk of MWCNTs attaches on the micropunch surface. The microtextures of the micropunch surface in the quasi-stable period show that the uniformly distributed MWCNTs attached on the surface of the micropunch form a favorable transfer film as a lubricant layer between the micropunch and the substrate in the micropunching procedure. Moreover, owing to this transfer film formation at the interaction area under the effect of CNT forest stripping, MWCNTs or the formed debris adhere to the surface of the micropunch (or the mating surfaces) averting the direct contact between the micropunch and the substrate in the micropunching process and are taken as the lubricant to the interface by virtue of their graphitic nature. Results definitely provide a promising prospect for the future prolonging the life of micropunches in the following practical applications.

When the punching number increases, it illustrates that MWCNTs distribute sparsely and disappear at the last stage. It reveals that the beneficial effect of MWCNTs is distributed initially on the surface of the micropunch lost. As a result, the severe wear of the micropunch appears.

## 10.4 Features of Punched Microhole

Actually, material shear and fracture in the micropunching with foil (thickness less than 200 μm) are different from those of punching in traditional punching. Figure 10.9 shows the distinguishing features of traditional punching, which includes four sections: (1) rollover, (2) shear/burnish zone, (3) fractured zone, and (4) burr. Most attractively, the features of microhole punched by micropunching are definitely quite distinct from that of traditional punching. The fracture band is absent in the punching of a 150 μm hole in titanium with the thickness of 200 μm as shown in Fig. 10.10. In the relevant fractrography of inner micropunched microhole, it indicates that the areas of rollover and burr are extremely narrow besides the absence of the fracture band. The most part of the inner microhole is the shear/burnish band. Figure 10.11 expresses its three-dimensional topography observed by optical surface profiler (OSP)-Veeco/Wyko NT9300, which agrees well with the results shown in Fig. 10.10.

**Fig. 10.9** Traditional punching: (*a*) rollover, (*b*) shear/burnish zone, (*c*) fractured zone, and (*d*) burr



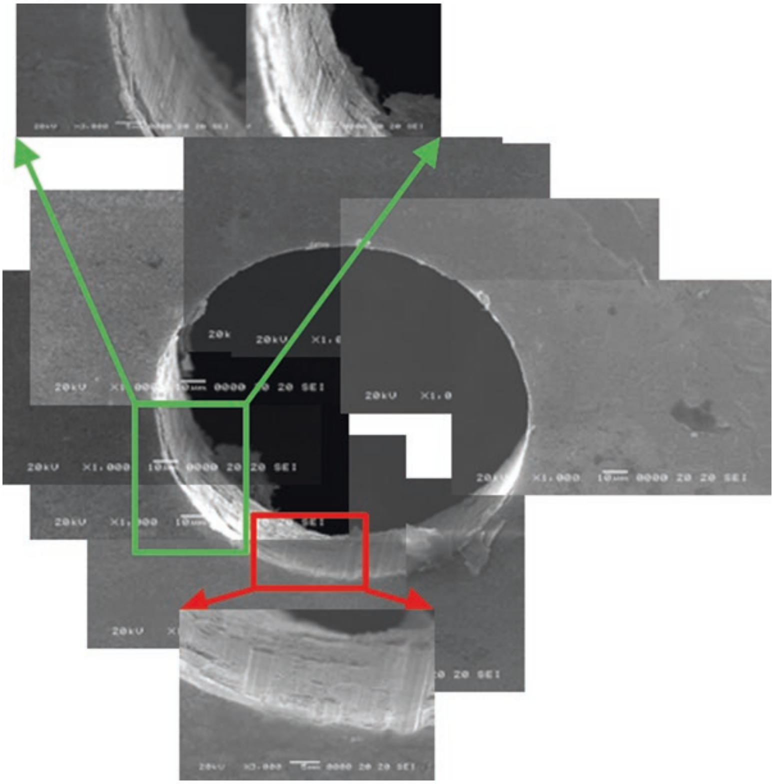


**Fig. 10.10** Fractrography of the inner microhole formed by micropunching

It indicates that how the punch and foil interact during punching at microscale must be quite different from that at millimeter-scale punching. Therefore, future investigation shall be pinpointed to look into better understanding of the related mechanism of punch-foil interaction in micropunching, which can

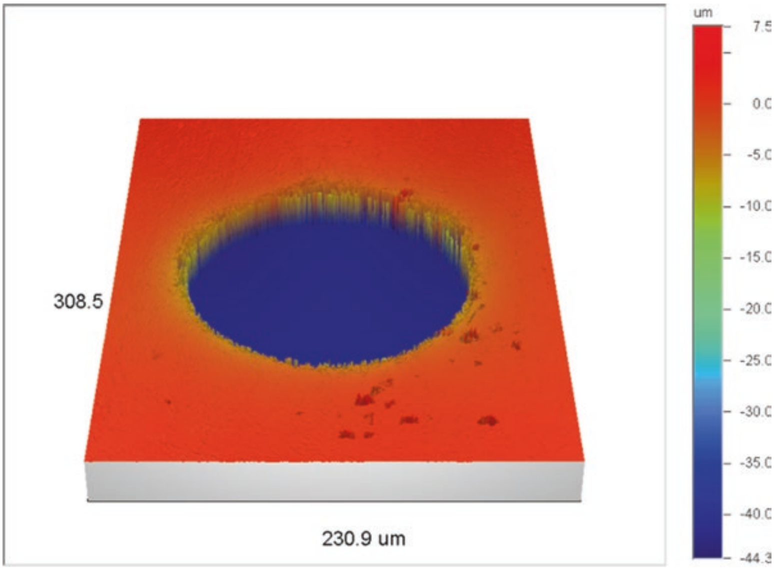


**Fig. 10.11** 3D topography of the punched microhole

ultimately lead to the improved micropunches and the quality of the punched microholes simultaneously.

## 10.5 Microhole Potential for Bioenergy

Bioenergy known as the promising renewable energy is generated from materials derived from biological sources. Among bioenergy, as a kind of renewable energy source, biomass is attained from the biological materials of the living including both animals and plants. Up to now, the energy such as heat or electricity is generated by burning biomass to produce the steam or convert to other useful things such as ethanol, methane, etc. At present, there are a lot of biomass power plants all over the world with the increasing share of usage of electricity. The relevant power plant performance is evaluated through energetic performance criteria: electrical power and thermal efficiency. As an effective method in the power plants, microholes can beneficially enhance fluid flow and heat exchange, especially for the turbines. Moreover, materials with microholes of special physicochemical properties can be applied in energy conversion and storage devices to distinctively improve the related efficiency, especially for the electrochemical areas, along with the minimal environmental impact.

## 10.6 Conclusion

A dense layer of packed and tangled MWCNTs was grown on the working regions of WC/Co micropunches by the waste alcohol eco-friendly to the environment, and the height of the MWCNTs is about 15 μm with the diameter of about 3–5 nm. In the micropunching process, the weight loss of MWCNT-coated micropunches could be divided into the initial run-in stage, quasi-stable stage, and severe wear stage, just like the case of micropunches without MWCNT coating. MWCNT coating helps to lubricate the micropunch and specimen interface. Moreover, MWCNT coating could extend the length of the quasi-stable stage by about 250 shots (or 35%). In addition, the attractive features of punched microhole are definitely quite distinct from that of traditional punching. Besides the fracture band is absent in the punching of a 150 μm hole in titanium with the thickness of 200 μm, the areas of rollover and burr are also extremely narrow. Meanwhile, the diameter of the front side of the punched microhole is almost the same as that of the back side even in the severe period, which implies the punched microhole can be directly used in biomass power plants without any posttreatments.

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# Synthesis of Iron Oxide Nanomaterials for Biofuel Applications

# 11

Misbah Ghazanfar and Muhammad Irfan

**Abstract**

The world is paying attention on exploring and establishing alternative modes of energy production instead of utilizing fossil fuels, because of the truth that conventional fuels are being squandered and the liberated chemicals of these fuels have become detrimental to the environment. In this context, production of biofuels can solve the issue, and nanotechnology can play a significant role for biofuel industry. Iron oxide nanomaterials are helpful and have great potential for enhanced production of biofuels. Many investigations have been conducted on the synthesis and use of iron oxide nanomaterials. There are different methods to synthesize iron oxide nanomaterials, and they are efficiently used in biofuel synthesis.

**Keywords**

Nanotechnology · Iron oxide · Biofuel · Nanomaterials · Nanoparticles · Bioethanol · Biodiesel · Renewable · Production

## 11.1 Introduction

Almost every country today is facing the issue of current energy requirements and future supplies (Waqas et al. 2018). Recently, more than around 75% of this energy is formed from nonrenewable sources. We are disregarding the outcomes of overuse of natural resources on our environment and the necessities of our coming generations (Nizami et al. 2017). Environmental concerns and the rapid exploitation of the fossil fuels lead to exploration of a substitute fuel (Balat and Balat 2010). Raising need for fossil fuel from the last several years consequences in the decline

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M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_11

of natural resource availability. Moreover, fossil fuel has a major issue like the discharge of carbon monoxide, which causes global warming. So, there is a greater need for alternate fuel (Ghazanfar et al. 2018).

Environmental, economic, and energy safety worries developing from imprudent dependence on petroleum products are compelling almost all the countries on the earth to find alternative fuel in the form of biodiesel and bioethanol (Farrell et al. 2006). To make progress toward renewable and sustainable energy systems, there have been rigorous development and research work done in the field of biofuels. It is considered that biofuel industry has a significant potential part in dealing the issues of climate change and atmospheric pollution, along with the renewable energy production as it is quickly developing (Rai and Da Silva 2017).

The establishment of renewable and substitute fuels is a very critical research area in the last several years. Biomass is earning extreme attraction as a renewable energy source, because of the world fuel need and distresses on the climatic influence of production of carbon dioxide. Biomass has innumerable advantages as compared to fossil fuels, e.g., low emissions of oxides of sulfur and nitrogen, carbon dioxide, locally produced, harmless, as well as renewable (Zhang et al. 2011). Advance living standards demand elevated mobility rate, and, as a result, transport is one of the biggest and speedy establishing energy-demanding areas. Increase in competitive agribusiness automatization also leads to a high energy need. However, due to anxiety on the negative impact of fossil fuels on the environment, the use of biofuels appears as an encouraging alternative that is moderately becoming technically and economically feasible. The development of lifestyle forces the chase for continual energy so that world energy demands across the world can be fulfilled (Demirbas 2010), while global problems such as global warming and environmental pollution are also caused by the utilization of fossil fuels because they burnt as the chief means of energy (Kiran et al. 2014). This situation leads to the discovery of eco-favorable, sustainable, and renewable energy by industrial, government, and energy areas (Shafiee and Topal 2009).

Liquid biofuels were preferred over others among renewable energies, as they constitute approximately 40% of the global energy consumption (Tan et al. 2008) because the utilization of liquid biofuels also causes a decline in liberation of greenhouse gas, making opportunities for job, supply security and regional development (Demirbas and Balat 2006).

Solar, wind, geothermal, and biomass are different options which constitute renewable energy. Due to the frequent availability of plant residues that can be employed to produce gaseous and liquid fuels or electricity, the attention in the latter has been raised. Utilization of this green waste can be in the form of direct biomass burning to produce electricity and vapor or after few alterations that permit a better employment of this energy supply (Antunes et al. 2017).

Researchers are making efforts to enhance overall constituents of the biofuel industry such as pretreatment of feedstock/biomass, process conditions and parameters, designs of reactor, yields and quality of product, and the inclusive optimization of process, in addition to the capital investment, approval by public, and availability for market for different biofuels (Hussein 2015).

Nonetheless, the strategies for the handling of biomass linger restricted by economical and technological barricades. Therefore, novel and innovative strategies and perspectives are essential to attain viable operations as well as bigger and quicker alterations of world energy pattern (Antunes et al. 2017). Nanotechnology is a generative field of investigation and research among the new developments, which includes auspicious potential in several application zones of industrial and social benefits.

Nanotechnology along other connecting technologies (information technology, cognitive sciences, and biotechnology) is capable to enhance human living standard having implementations in various fields such as life sciences, electronics, pharmaceutical and material development, etc. (Demetzos 2016; Wolf and Medikonda 2012). Due to their dynamic physicochemical characteristics, nanomaterials have the prospective for their commercial utilization. In the future, it is predicted that the commercial significance of nanoparticles will elevate distinctly and probably it would rise from 2000 tons as in 2004 to around 58000 tons in 2011–2020 (Shi and Ma 2010, 2011). Various fields including industries belonged to biofuel production and environmental remediation; there are vast applications of nanoparticles (Srivastava et al. 2014b, a). Efficacy of green fuels like bioethanol/biohydrogen can be improved by employing nanoparticles; thus nanomaterials can be supportive in the improvement of the production of biofuels (Srivastava et al. 2015a). Studies also revealed that the production of cellulases, their strength, and catalytic ability can also be enhanced employing nanomaterials. Furthermore, the presence of nanoparticles also enhances the pretreatment of lignocellulosic biomass (Srivastava et al. 2015a). Iron oxide nanomaterials have several crystalline phases, relying on their stoichiometry, such as goethite [$FeO(OH)$], ferrihydrite [$Fe_5HO_8(4H_2O)$], magnetite ($Fe_3O_4$), wüstite ($FeO$), hematite (a-$Fe_2O_3$), maghemite (g-$Fe_2O_3$), etc. $Fe_3O_4$ and g-$Fe_2O_3$ are the most investigated iron oxide nanomaterials (Ubale and Belkhedkar 2015), which are used extensively in different applications (Cavas et al. 2013; Haddad et al. 2015; Jia et al. 2005) as well as in pretreatment and hydrolysis of biomass to produce sugars (Srivastava et al. 2017a). Figure 11.1 indicated various applications of nanomaterials in the process of production of biofuels. According to the truth as mentioned prior, the purpose of this chapter is to describe the synthesis of iron oxide and its applications in enhancing biofuel production. In present chapter, we tried to project an overview of the biofuels and synthesis of iron oxide nanomaterials and their implementations in biofuel production.

## 11.2 Worlds' Fuel Demand and Biofuels

Presently, world energy requirements are executed primarily by hydro, natural gas, coal, nuclear, oil, and inexhaustible means (Brazilian Energy Balance 2015). From 2002 to 2030, the requirement of biofuels are expected to rise up to 57%. In 2005, the average cost of patrol was \$2.56 per gallon, which was \$0.67 greater than the average cost of patrol in the earlier year. Until now the average cost of petrol in the United States increased up to \$4.10 per gallon (Kumar et al. 2009). Investigations

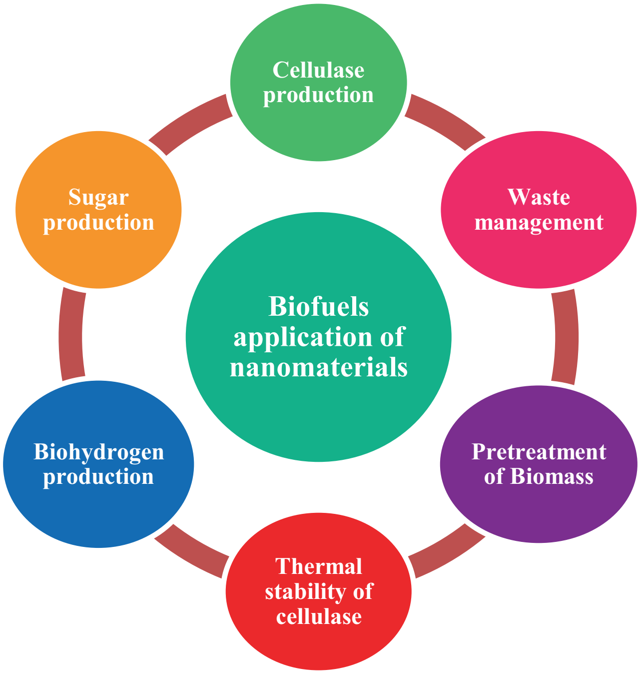


**Fig. 11.1** Different applications of nanomaterials in production of biofuels

for substitute energy means, particularly that obtained from inexhaustible matter, e.g., biomass, accelerated as the global energy need and oil reserves shrink significantly (Saxena et al. 2009).

Total world energy chief production has been raised from the last decades, and currently the major producers are (MTOE) Saudi Arabia (630), Russia (1334), Australia (357), China (2555), Brazil (26), Indonesia (457), the United States (1989), Iran (308), Canada (452), and India (571) (Yearbook Enerdata 2015a).

Reviewing various energy sources, energy supply of the world is fulfilled principally by oil, chased by naturally occurring gas and coal. Figure 11.2 illustrates the profile of world energy supply in 1971 and 2013 which distinctly depicted that the planet greatly relies on traditional fuels.

Whereas, from 40 years, renewable energy contributes for only about 10% of total in world energy supply (Brazilian Energy Balance 2015). Regardless of this scenario, green energy is being used in several countries. For instance, electricity production from inexhaustible energy is the foremost source of energy production in Portugal (62.6%), Brazil (73.4%), Chile (42.8%), New Zealand (79%), Sweden (58.5%), Norway (98%), Venezuela (62.8%), Italy (42.1%), Colombia (70%), and Canada (62.5%) (Yearbook Enerdata 2015b). Furthermore, share of solar and wind in electricity production is powerfully dispersed in Romania (9.6), Belgium (10.0),

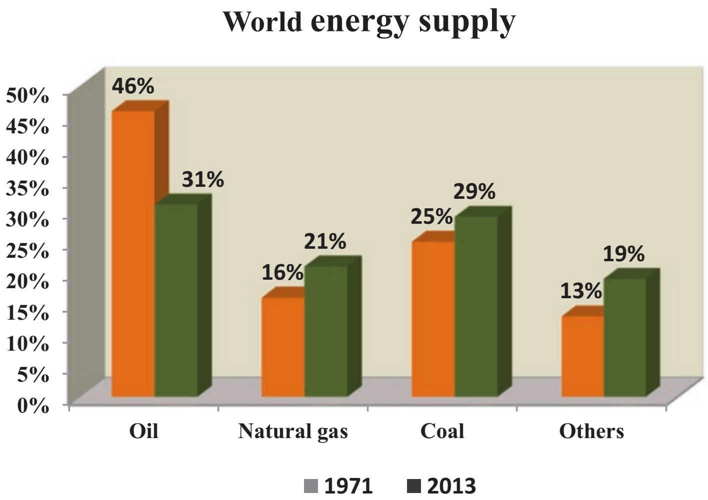
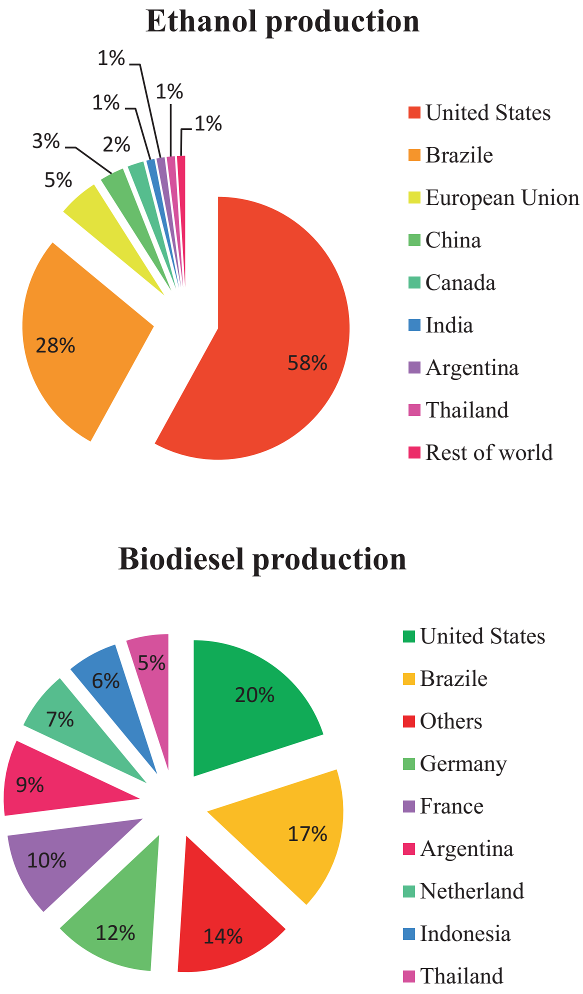


**Fig. 11.2** Profile of world energy supply in 1971 and in 2013. (Source: Based on International energy agency from the Key World Energy Statistics © OECD/IEA 2015, www.iea.org/statistics. Licence: www.iea.org/t&c; As redesign by present authors)

Portugal (24.5), Spain (23.9), the Netherlands (6.6), the United Kingdom (10.3), Germany (15.2), Sweden (7.6), Italy (16.7), and New Zealand (21.7) (Yearbook Enerdata 2015c).

Recently biofuels have secured much attention and are being produced with enhanced production in different countries. For instance, the United States applying corn as a crude matter and Brazil applying sugarcane juice as a source of carbon are the bigger producers of bioethanol in the world by producing around 14,000 and 7000 million of gallons per year, respectively. The overall bioethanol production in the world is approximately 25 million gallons annually (Renewable Fuels Association 2015). In 2006, Goldemberg (2006) suggested that carbon liberation can be decreased up to 66 million tons annually if bioethanol formed from juice of sugarcane can be substituted 10% of total utilized patrol around the globe. Another principal biofuel is biodiesel and is commonly synthesized through a transesterification reaction which is catalyzed chemically, between oils and fats from plants or animals and low chain length alcohols (mainly methanol), and the synthesis of this compound is improved too. For instance, currently European Union is producing around 3.35 billion liters of biodiesel, and in 2025, this production can be elevated up to nearly 4.14 billion gallons (Statista, The statistic portal 2016a). The largest producers of biodiesel in the world in 2014 were the United States (4.7 billion liters) chased by Germany (3.4 billion liters), Brazil (3.4 billion liters), Indonesia (3.1 billion liters), and Argentina (2.9 billion liters) (Statista, The statistic portal 2016b). In Fig. 11.3 we can observe an outline of the major producers of renewable fuel in the world in 2015.

**Fig. 11.3** Main producers of renewable fuel in world in 2015. (Source: Renewable Fuels Association 2015; Statista, The statistic portal 2016b)



Lignocellulosic biomass is the most abundant renewable source of carbon on earth as it is frequently found in plant residues, hence accounted as potential material for biofuel production. It is estimated that this green waste is generated from 10–50 billion tons annually and about millions of tons of predominant biomass can be feasibly reutilize (Zhao et al. 2012). For self-assisted formation of energy, biomass is burnt in boilers in different industrial processes. Cellulose and hemicellulose are found in green biomass and constitute its two-thirds polymeric carbohydrates (Antunes et al. 2014) and therefore can be employed in bioprocessing for production of biofuels and other valuable products.

So the green technology along with an advance approach like nanotechnology has immense prospective to contribute various commercial zones with huge need of synthesis within the necessitated conception of feasible development methods.

## 11.3 Biomass to Biofuels

Although field of synthesis of biofuels receives huge importance for the substitute of fossil fuels, it reinforces to lessen the atmospheric pollution, yet away from the viable implementations due the large formation price. Therefore, uninterrupted attempts are being made for the cheap formation of biofuels all around the globe to balance its economy. So, if we use lignocellulosic biomass as substrate, it can be cost-effective. This substrate is a biological waste; cost-effective, huge source of carbon, inexhaustible in character, and easily found on the earth in large amount. These lignocellulosic biowastes can be easily utilized as the substrate for the production of biofuels (Srivastava et al. 2015a, b). Over the last many years, many scientists have conducted researches on thermochemical pathways, fermentation, and biomethanation for the transformation of biomass to biofuels as origin of energy, which has recently become the area of interest. In general, the lignocellulosic biomass is a complicated mixture of organic compounds, e.g., carbohydrates (cellulose, hemicelluloses, and starch), proteins, lignin, and fats; however, the physiochemical attributes of biowaste differ in distinct pattern according to their origin. For example, the basic components of the biomass from forest or agricultural origins are lignin and carbohydrate which can differ with the variety of plant. The roots, seeds, and seed residue of plant or crop which is profuse in fats and starch are sources of biomass, whereas most of the biomass types are residues or byproducts of crops, human waste, cattle waste, municipal waste, demolition waste, construction waste, and forest remains. As it has been discussed above, the usage of biomass for liquid biofuels is an essential substitute to prevent hazardous influences of direct burning of biomass (as untreated or crude solid fuels) which can lead to air pollution, subsidiary pollution, and hazardous health effects (Verma et al. 2012).

Cellulosic biowaste can be converted into fermentable sugars by cellulase enzymes, and then the product can be additionally transformed into desirable biofuels. While cellulase enzyme performs an important role in the formation of biofuels, the cellulose enzyme production is the most costly step in the whole method of production of biofuels. So, huge expenses on cellulase enzyme formation increase the price of biofuels, and it contributes 40% of the total price (Bhalla et al. 2013; Srivastava et al. 2015a, b). Moreover, one of the major reasons to enhance the cost of formation is lack of effective preprocessing of lignocellulosic biomass (Sun and Cheng 2002). Other than the abovementioned facts, many factors like effectiveness of cellulase enzyme, its stability, and catalytic activity at different pH and medium components influence the entire process of formation of biofuels (Yeoman et al. 2010).

As a substitute origin of energy, the use of biomass fuels has become necessity of the present era to avoid the environmental pollution and the crises of fossil fuels (Barakat et al. 2012; Barnard et al. 2010). The transformation of these biomasses into fuels has enticed huge concentrations because they are inexhaustible in nature and have feasible origin of cellulose and there is broad presence of these lignocellulosic biomasses. Therefore, greater than 20,000 scientific research papers rely on the ISI Web of Knowledge database, and approximately 3000 international

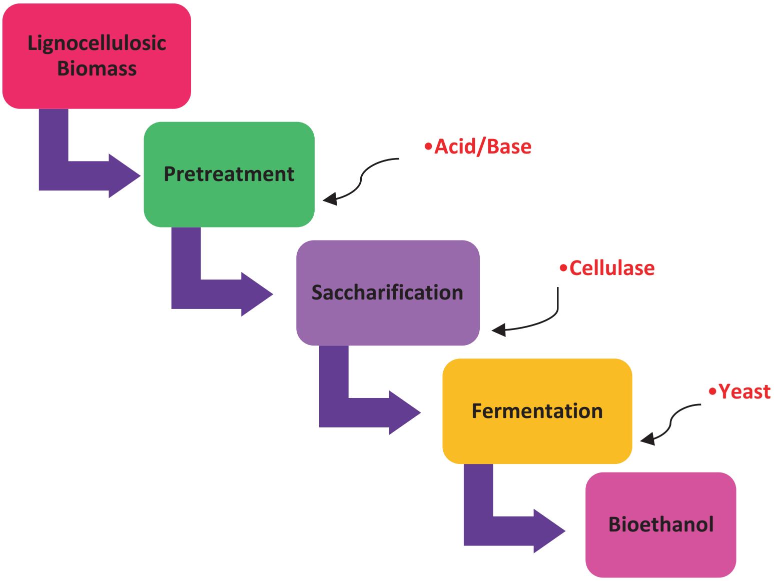


**Fig. 11.4** Overview of biofuel productions from lignocellulosic biomass

patents, according to the SpaceNet database, have been published in this field till date (Chaker Ncibi and Sillanpaa 2013). The immense amount of research publications and patent undoubtedly recommend conversion of biowaste into biofuels is among the most provoking research fields to further search its practical application. Figure 11.4 represents flowchart of process of formation of biofuels using the lignocellulosic biomass as the substrate. Cellulose-containing biomass undergoes suitable pretreatment process and then by the action of cellulolytic enzymes converted into fermentable sugar, and then fermentive microorganisms use these sugars and produce bioethanol/biohydrogen through the fermentation process.

In many countries in the world, the production of biodiesel from the transesterification of animal fats, vegetable oils, and discarded frying oils has increased principally, because transesterification is one of the most common procedures employed to decrease viscosity of oil. This is actually the manufacturing of a fuel, biodiesel, comprised of mono-alkyl esters of long-chain fatty acids. Biodiesel, because of its strategic, technical, and environmental advantages, is the most vastly believed substitute fuel for diesel engines (Enweremadu et al. 2011). Furthermore biodiesel is strategically powerful as traditional, petroleum-obtained diesel fuel and needs effectively no changes in the fuel dispensation fundaments. Decline of most dissipated liberation, greater flash point, inherent lubricity, and biodegradability are among the other benefits of biodiesel compared to petro-diesel (Haas et al. 2001), and the truth that it is of local emergence. To avoid pollution and retrench the biodiesel synthesis with the raising requirement of biodiesel, it is indispensable to minimize loads on the eatable oil and to produce biodiesel from discarded non-eatable fatty acids. Thermal transformation processes of biowaste involve direct burning, thermal breakdown, liquefaction, and gasification. Quick thermal breakdown of biomass is supposed to be a favorable thermochemical method in which biomass is transformed to pyrolytic oil, gas, and char (Zhang et al. 2011). With advantages of unburned hydrocarbons, small level of smoke, and carbon monoxide than fossil fuel-derived diesel, biodiesel is accepted as an inexhaustible and atmosphere-friendly fuel compassionate to petrodiesel. With biodiesel it is feasible to attain virtuous burning, consequently reduction in level of waste release by utilizing oxygenates as supplement antioxidants and nanomaterials as additives in green diesel. Currently, several investigators concentrated their focus on production of fuel strategy for gaining good implementation and liberation properties.

Bioethanol is the ethanol merely formed by fermentation of the sugar and starch ingredients of plants, renewable energy source. Agricultural residues of beetroot, sugarcane, rice, corn, potatoes, and currently employed grapes, banana, dates, and other wastes are being used for the production of bioethanol. The production of such biofuel is due to the declining quantity of fossil fuels, substitute energy origins required to be inexhaustible, feasible, powerful, inexpensive, appropriate, and secure (Chum and Overend 2001).

When conventional fuel became costly and environmental issues emerged, advance ethanol industry initiated in the 1970s. In 1975, the Brazilian government launched a pioneer program known as “Pro’alcool” (Pro-Alcohol) with two main objectives: to reduce the impact caused by oil price increases and, at the same time, mitigate the fall of sugar price in the international market. Between 1980 and 2002, over 5 billion dollars was invested on sugarcane agriculture and industry to expand alcohol fuel production. “Pro’alcool” is known worldwide for its positive effect on biofuel promotion. Nowadays, in Brazil, 20–25% of anhydrous ethanol is used as an additive in gasoline. Moreover, since 2003, flex-fuel vehicles, which can use alcohol, gasoline, or gasoline+alcohol, are on the market. Ethanol is the leading liquid biofuel used for transportation (Bolivar-Telleria et al. 2018). Among alternative fuels, bioethanol has an extensive history and vast application in transportation sector. Germany and France began to employ bioethanol as a substitute fuel in internal combustion engines (ICEs) in 1984. In 1925 Brazil started utilization of bioethanol. Until the early 1900s, Europe and the United States widely used bioethanol. Until the fuel crisis in the 1970s, the utilization of bioethanol was ignored after World War II because of its costly synthesis as compared to petroleum fuel. Since the 1980s, the attention toward bioethanol has been growing, and it has been believed as a good replacement in several countries. Global production of ethanol raised from 13.12 billion of gallons in 2007 to 25.68 of gallons in 2015 with a little reduction in 2012 and 2013 (Azhar et al. 2017). In 2015 with the synthesis of around 15 billion gallons, the United States is the largest ethanol producer in world. Eighty-five percent of the world’s ethanol is produced by the United States and Brazil. Bioethanol is also called ethyl alcohol and chemically $C_2H_5OH$ or EtOH. It can be utilized as mixed with patrol to form “gasohol” or can be used directly as pure ethanol (Staniszewski et al. 2007). In diesel-bioethanol mixture, bioethanol is used as a gasoline booster and octane improver and also contributes in reduction of the emission of harmful gasses (Pejin et al. 2009). Green ethanol offers various benefits over patrol, e.g., enhanced heats of vaporization, greater flame speeds, greater octane number (108), and wider flammability limits (Balat and Balat 2009). Contrary to gasoline, bioethanol is quickly biodegradable and little toxic and produces lesser atmospheric pollution (John et al. 2011). Production of bioethanol through the first, second, and third generation is carried out by using different types of feedstock. The first-generation production of bioethanol involves raw materials plentiful in sucrose such as sugar beet, sweet sorghum, sugarcane, and fruits and starch such as potato, corn, barley, cassava, wheat, rice, and sweet potato, while lignocellulosic biomass such as straw, wood, grasses, etc. are used for second-generation production of bioethanol. However algal biomass including macroalgae as well as microalgae is employed for production of third-generation bioethanol (Nigam and Singh 2011).

## 11.4 Nanomaterials and Biofuel Production

In 1959, the term "nanotechnology" was introduced by Feynman which refers to the synthesis and utilization of nanomaterials, e.g., materials which have at least one dimension smaller than 100 nm (Palaniappan 2017). Since then, many researchers and scientists around the world have explored the effects of nanomaterials in a wide variety of fields, ranging from power conversion equipments, integrated circuits, and food products (Wu et al. 2008). Compared to their large-scaled materials, the nanomaterials are said to have distinctive chemical, magnetic, mechanical, electronic, and optical characteristics (Biswas et al. 2012). Nanomaterials are said to perform a critical part in power generation fields because of their special structure, increased energy electrical storage capacity, comparatively great specific area, and relatively good efficacy of heating and lighting (Ansari and Husain 2012). Dimension-wise, they can be compared to biological macromolecules like enzymes or nucleic acids (Verma et al. 2013a). At the outset, it is first essential to understand the production mechanisms and basic characteristics of nanomaterials before using them to enhance biofuel production. Nanomaterials basically include a variety of substances ranging from nanocomposites, nanoparticles, nanotubes, nanosheets metal-based nanomaterials to carbon-based nanomaterials and nanocrystalline materials. There are various ways of nanomaterial production (Palaniappan 2017).

Nanoparticles are described as distinct bodies that have three dimensions of the order of 100 nanometers or less (Scenihr 2007); they own distinctive physical and chemical properties that are dramatically different from large materials (Osaka et al. 2006). By utilizing various kinds of the nanomaterials, nanotechnology has great power to increase the production of bioenergy. Nanoparticles have great chemical, electrical, and physical characteristics, which make them different from the bulk material. They are capable of enhancing the production of energy, hence serving as the solution to the issue of energy production. Nanotechnology can be employed to enhance different kinds of renewable energies such as solar, biogas, water, etc. (Hussein 2015).

Employment of nanoparticles is supposed as one of the major techniques these days to facilitate and improve biofuel formation process from waste biomass (Choedkiatsakul et al. 2011). The convenience to incorporate nanoparticles will particularly give more worth to green fuel formation method to become more sustainable by lowering the price and positive environmental impacts. Two-thirds of the lignocellulosic waste is covered by cellulose and hemicelluloses, apart from lignin which can be efficiently transformed into green fuels following the pretreatment (Alvira et al. 2010; Cai et al. 2011; Chang and Yao 2011; Hamelinck et al. 2005; Rawat et al. 2014; Srivastava et al. 2015a, b; Sun and Cheng 2002). Moreover, using the nanomaterials as the catalyst, the price of reagent used for the pretreatment of biomass may be reduced (Alvira et al. 2010; Srivastava et al. 2015a, b). The sugar production can be improved providing high yield, by using suitable pretreatment technology, which can further be used for biofuel production. That is why, by applying the suitable pretreatment process, more scientists are governing to enhance the biotransformation ability of lignocellulosic biomass. In one of the investigations by Wei et al. (2015), raised sugar formation was observed in the availability of iron oxide nanomaterials utilizing acidic pretreatment method of corn stover, employed as the substrate. This integrated acid nanocarrier-treated substrate as compared to without nanoparticles makes approximately 13 to 19% more xylose and glucose. Furthermore, results from investigations found that at 100 °C, the use of iron oxide nanoparticle for the pretreatment of substrate causes formation of increased sugar over the metallic iron. Likewise, it has been reported that $Fe_3O_4$–RGO–$SO_3H$ nanocomplex can enhance the sugar formation through enzymatic breakdown (Yang et al. 2015). These researches unlock a new pathway for the cheap formation of biofuels employing the nanomaterials, but still this field is at the very initial phase. So, there should be more feasible applications of biofuels because they are very effective alternative source of energy. Highly effective cellulase system should perform in severe conditions, for the remarkable enzymatic breakdown of discarded biomass. In many researches, utilization of several cofactors like metal ions is reported for upgrading the cellulase effectiveness and its commercial formation (Srivastava et al. 2014b, c). Recently, to enhance the enzyme stability, use of nanoparticles is growing as an advance avenue in the zone of biofuels (Dutta et al. 2014; Jordan et al. 2011; Singh et al. 2016; Srivastava et al. 2014a, b). There are very restricted but promising studies that have been outlined in this research field. For instance, Dutta et al. (2014) observed an enhanced cellulase formation by bacteria in the availability of hydroxyapatite nanocarriers. The enzyme employed in this investigation was extremely thermostable which at 80 °C kept its half-life. Also, Srivastava et al. (2015c) have described an enhancement in the formation of cellulase enzyme and its thermal stability in the availability of iron (II, III) oxide/alginate nanocomplex. Furthermore, an enhanced thermal stability is also noted in the availability of nickel cobaltite ($NiCo_2O_4$) nanoparticle (Srivastava et al. 2014a). In the availability of zinc oxide nanoparticle, increased pH stability of unprocessed cellulases has been reported by Srivastava et al. (Srivastava et al. 2016). Likewise, Verma et al. (2013b) have explored the stability of the β-glucosidase enzyme against temperature, which was enhanced in the existence of iron oxide nanoparticles, displayed its half-life at 70 °C. The aforementioned investigations distinctly demonstrate that the nanomaterials have capabilities to play a significant part to change the whole biotransformation method (Ansari and Husain 2012). At normal temperature, i.e., 45–50 °C, there are various disadvantages of performing enzymatic breakdown, which include incomplete hydrolysis, overall slow reaction, and twitchy to microbial spoilage. Hence, decreased yield of fermentable sugars is generally observed and hence needs greater enzyme stuffing (Lu et al. 2006; Singh et al. 2016; Wang et al. 2010). It is accounted that, to remove the above bottlenecks; the thermostable or thermophilic enzyme is an effective way (Viikari et al. 2007; Yeoman et al. 2010). In early studies, betterment in stability of cellulase by employing nanomaterials is reported (Dutta et al. 2014; Srivastava et al. 2015c). An enhanced stability of cellulase enzyme against temperature at 80 °C was obtained in the availability of calcium hydroxyapatite nanoparticles and afterward enhanced sugar formation (Dutta et al. 2014). Furthermore, 5.71-fold more D-xylose and 15-fold more other reducing sugars have been reported by these authors in availability of the hydroxyapatite nanoparticles. In the current conducted research of Srivastava et al. (2015c), greater stability against temperature and productivity of sugar with iron (II, III) oxide/alginate nanocomposite-treated cellulase have also been observed. Though capability of nanoparticles is investigated by various investigations, their precise mechanism in reaction is however ambiguous and requires even more investigations in this field. Implementations of nanoparticles are much investigated in the field of biohydrogen formation among several subsisting biofuels (Ivanova et al. 2009; Zhang and Shen 2007). It is reported that production of biohydrogen is much complex, and microorganism-assisted method relies on various physical and chemical parameters such as inorganic sustenance comprising metal ions, working condition, and nature of substrates (Wang and Wan 2008). Moreover microorganisms, which perform significant part in the formation of biohydrogen, can receive benefit of nanomaterials in anaerobic environment, because the shifting of electron is much feasible to acceptors in presence of nanomaterials (Beckers et al. 2013). Efficacy of biohydrogen formation was enhanced in the availability of gold nanoparticle in the research by Zhao et al. (2013). In addition to silver and gold, nanocarriers can also enhance the substrate transformation effectiveness in biohydrogen process. Enhanced biohydrogen was observed in the existence of hematite nanoparticles in the investigation by Han et al. (2011). Though struggles are being made to enhance the biohydrogen formation by acquiring various strategies and different types of nanomaterials, this field is yet in grappling stage for smaller- to larger-scale study. Applications of nanomaterials in the field of biofuel formation are summarized in Table 11.1.

**Table 11.1** Feasible applications of nanomaterials (Srivastava et al. 2017b)

| Biofuel areas | Feasible applications of nanomaterials |
|---|---|
| Pretreatment of cellulosic biomass | Enhance the liberation of hemicelluloses and lignin structure |
| Cellulase enzyme | Enhance the synthesis, pH, and thermal stability |
| Bioethanol production | Enhance the synthesis and resistance of microorganisms |
| Biohydrogen production | Enhance the synthesis and stability of nitrogenase and hydrogenase |

For future scenario, biofuels are supposed to be a better choice to substitute the fossil fuels and are probably an advanced and new research zone to offer a replacement in the form of inexhaustible energy. Currently, the United States and Brazil produce about 80% of the world's biofuels, and both countries are producing principally bioethanol. In future, worlds' requirement of fuel for transport is accounted to rise up to 55% by 2030 as compared to 2004, and according to this estimate, the demand of production of biofuels will increase. Internationally a number of efforts and studies are being done to upgrade the economical production of biofuels (Srivastava et al. 2017a).

The nanomaterials as supplements for biodiesel production have appeared as an advance favorable fuel supplement among the current fuel supplements to green diesel, for acquiring extreme betterment in the production and greatest possible decrease of harmful liberation (Prabu 2017). Dreizin (2000) noticed that the nanoparticle scattered test fuels exhibit good thermal and physical characteristics due to its greater surface to volume ratio and perform as an oxygen buffer regarding NO liberation. Kuo et al. (2003) investigated later that nanomaterials increase the heat shifting estimates because of its greater specific surface area. Idriss (Idriss 2004) conducted a research on the advancement of cerium oxide nanomaterials with ethanol and found that nanoparticles could be applied as a fuel-formed supplement in hydrocarbon liquid fuels. Farfaletti et al. (2005) later applied ceria nanomaterials as supplement in diesel admixture fuel and reveal critical decline of particulate material, carbon monoxide, and unburned hydrocarbon emission.

Singh et al. (2016) stated that in the current years, nanotechnology has attained greater concentration due to extensive limits of technological and engineering implementations. Due to their diverse physical and chemical characteristics, nanomaterials have the power for their industrial use. It is also believed that the commercial value of nanomaterials would elevate in future years, and probably it is will raised from 2000 tons in 2004 to around 58,000 tons in 2011 to 2020 (Shi and Ma 2011). Several sectors covering industries belonged to atmospheric amendatory and biofuel synthesis have vast use of nanoparticles. Production of biofuel can be enhanced by use of nanomaterials; for example, effectiveness of biofuels like bioethanol/biohydrogen can be getting better by applying nanoparticles. Use of nanomaterials can also enhance the production of cellulase enzyme, its hydrolysis efficacy, and stability. Furthermore, nanoparticles also play part in the improvement of pretreatment of lignocellulosic biomass (Srivastava et al. 2017a). The advantages of implementing nanotechnology to catalysis involve lifetime, resistance to poisoning, better activity, and other new efficiencies (Mahmood and Hussain 2010; Mahmood et al. 2010). These enhancements and advances cannot be gained consistently with catalysts synthesized by other processes. Cobalt-based catalysis is an illustration of extending the catalytic efficiencies of regular catalysts by nanoscale modifications. The structure, size, and other supplementary constituents, such as Mg, Si, and Ni, may influence the cobalt nanoparticle catalysts (Hussain et al. 2009; Kesavan et al. 2001; Son et al. 2002; Yang et al. 2001). Particularly being inexpensive, cobalt nanoparticles require gentle reaction environment for greater yields of products in less reaction times relative to the conventional catalysts (Mahmood and

Hussain 2010; Mahmood et al. 2010). In gasification, nickel catalyst is broadly employed (Davidian et al. 2007; Hussain et al. 2009; Svoboda et al. 2007). Guo et al. (1995) reported that the size and structure of nanoparticles influence the melting point and catalytic ability increases on merging nanoparticles of cobalt and nickel. X-ray diffraction, scanning electron microscope, and transmission electron microscope are used to study the characteristics of nanoparticles (Salavati-Niasari et al. 2008). For catalytic reduction of carbonyl and aromatic compounds, nickel nanoparticles can be employed (Kidwai et al. 2006; Mahmood and Hussain 2010; Mahmood et al. 2010). Kidwai et al. (2006) accounted that nickel nanoparticles can also be employed for the selective and effective catalysis of the reduction of aldehydes in the availability of additional functional groups, e.g., –CN, $–NO_2$, and double bonds at α and β places. Catalytically the nickel nanomaterials perform principal part in the aldehydic groups' reduction. In addition to nickel, cobalt too performs a critical character in alteration of the chemistry of fatty acid. It was distinctly observed that cobalt nanoparticles (Wu et al. 2004) extended the band of the carboxyl group of pure liquid and takes to the synthesis of two novel bands with property of the symmetric cis (COO-) and the asymmetric trans (COO-) stretch.

Increasing rates of purified products of crude oil and rates of crude oil itself are a clear indication of the continuing depletion of global nonrenewable fossil fuels, namely, coal, crude oil, and natural gas. One study has indicated that the world's fossil fuel reserves would be diminished by 2050 at this rate of consumption, which is roughly about 105 times nonfaster than that the nature can actually create (Satyanarayana et al. 2011). Apart from the rising prices, fossil fuels are also said to contribute considerably to environmental pollution and ecological devastation, causing depletion of ozone layer and global warming and destroying both the biosphere and the geosphere. The environmental concentration of the main greenhouse gas, carbon dioxide, has been steadily rising to reach a level of about 390 parts per million and is expected to reach 750 parts per million by the end of this century (Ahrens 2011). This carbon dioxide is capable of trapping the sun's infrared radiation and thereby causing a rise in the global temperature. These two factors have led the fuel industry to move toward sustainable sources of alternative energy or, in other terms, renewable energy sources. Sustainable sources of alternative energy can range from solar, wind, tidal, and geothermal energies to energies generated from biomass, termed as biofuels. Currently about 90% of the world's energy demands are met by fossil fuels, which is expected to reduce to around 50% by 2040 with the advent of the abovementioned renewable energy sources (Hussein 2015). Biofuels utilize domestically available renewable resources and are able to decompose biologically and nonpoisonous. They can be formed from numerous raw materials such as vegetable fats and biowastes. Anaerobic digestion of agricultural residues, animal manures, organic food wastes, sewage sludge, and various other energy crops yield yet another renewable source of energy known as biogas.

Nanoparticles are at the frontline of quick establishment in nanotechnology. Their unique size-dependent characteristics of nanoparticles form these materials requisite and predominant in several zones of human affairs (Salata 2004). Iron has the status of pillar in the present infrastructure because of being one of the most contemporaneous transition metals in the crust of Earth (Huber 2005). Although, as compared to other group elements like nickel, cobalt, platinum, gold, and iron oxides are ignored to some extent. There are nearly 16 known iron oxides, formed by chemical combination of iron and oxygen. In the form of rust, iron (III) oxide is available in nature (Cornell and Schwertmann 2003). Commonly, iron oxides are widespread and greatly used because they are cost-effective and perform a mandatory part in several geological and biological operations. Humans also use them widely, e.g., as hemoglobin and catalysts, iron ores in thermite, and long-lasting pigments (colored concretes, paints, and coatings) (Laurent et al. 2008). In nature hematite ($\alpha$-$Fe_2O_3$), maghemite (g-$Fe_2O_3$), and magnetite ($Fe_3O_4$) are the most usual kinds of iron oxides. In the area of scientific technology, these oxides play very important role (Teja and Koh 2009). Nanoparticles are made up of ferromagnetic matter, and their size range from less than 10–20 nanometer shows an idiosyncratic type of magnetism, particularly superparamagnetism. Alloys, oxides, elemental metals, and other chemical compounds are the ferromagnetic materials that can be magnetized by an exterior magnetic field. Normally, only the nanoparticle systems have the outstanding phenomenon (Ali et al. 2016).

Cellulases are the complex enzymes that comprise cellobiohydrolases, β-glucosidase, and endoglucanases and are the imperative constituent in the hydrolysis of lignocellulosic biomasses, produced by different species of protozoans, bacteria, and fungi. Exploiting the hydrolytic expertise of these enzymes permits for the effective transformation of inexhaustible naturally occurring supplies into fermentable sugars. Regrettably, the feasible implementations of cellulases are restrained by their hydrophilic nature when they undergo the complications of the reactions involved (Ho et al. 2008). For the manufacturing of a variety of functional materials or equipments, the immobilization of biomolecules is a significant tool onto insoluble aids (Tischer and Wedekind 1999). Feasible isolation from reaction mixture, improved stability, possible modulation of the enzymatic characteristics, effortless avoidance of microbial growth, etc. are among its most distinct advantages (Bornscheuer 2003). The employment of magnetic supports for immobilization is of specific interest. Magnetic nanocarriers accomplish two main actions in the way, they have a magnetic matter which grant the required magnetic characteristics to accumulate with the species to be isolated and they can have surface attributes which allow a discriminatory isolation (Jordan et al. 2011).

Nanotechnology has numerous implementations such as amendment in raw materials, establishing much effective catalysts, and many others in biofuels and bioenergy field. Enzymes, for example, are being immensely employed to catalyze synthesis of biodiesel from oils and fats or to convert biomass into biofuels such as biogas and ethanol (Michalska et al. 2015; Verma and Barrow 2015). Nanomaterials, in this scenario, could be employed to immobilize the enzyme or to substitute the enzymes; consequently more productive catalysis is gained or enhanced the recuperation of biocatalysts from media. Furthermore, this strategy involves modifications in which magnetic characteristics can be appended to systems that are immobilized (Rai et al. 2016; Verma and Barrow 2015).

Anaerobic breakdown of organic wastes such as animal, human, plant, and agricultural wastes can produce biogas. The liberation of energy from anaerobic operations relies on the carbon and nitrogen ratio, whereas these organic residues have frequent carbon and nitrogen sources (Feng et al. 2014). It is also reported that the production of energy can be increased by incorporation of small amount of metal ions which act as catalyst and enhancing the activity of methanogenic bacteria. As methanogenic bacteria need little quantity of iron, nickel, and cobalt for the anaerobic digestion, scientists reported that use of nanomaterials is advantageous instead of using bulky or atomic materials (Feng et al. 2010). Yang et al. (2015) reported that due to powerful paramagnetic characteristic and great coercivity, magnetic nanoparticles can be employed in the procedure of methanogenesis. In 2015, Abdelsalam et al. (2015) also observed that nickel and cobalt nanoparticles improved the production of methane gas. They also demonstrated that the action of iron oxide nanoparticles is greater than that of iron nanoparticles. Later Abdelsalam et al. (2016) investigated that the influence of various nanoparticles, e.g., iron, iron oxide, nickel, and cobalt maximum methane and biogas is synthesized by anaerobic digestion of cattle manure. It was also reported that when iron oxide nanocarriers were employed to the hydrocarbon matter in the anaerobic digester, improvement of the disintegration activity and high yield of biogas and methane production were noticed (Casals et al. 2014). Various food feedstocks like sucrose from sugarcane, starch from corn, plant oils, and animal fats are usually used for the synthesis of first generation of biofuels (Naik et al. 2010). Continuous exploitation of these raw materials broaches several worries, and therefore, new concept of second-generation biofuel is obtaining importance in the world scenario for biofuel production by employing non-food feedstock such as lignocellulosic materials, e.g., agricultural remains, wood wastes, and others (Eggert and Greaker 2014). However, second-generation biofuels have both pros and cons as the use of waste material is the advantageous prospect, while the high production cost and technological issues and infrastructure are the demerits of it. Therefore, in this situation, there is a need to establish effective strategies to deal these problems in treatment of mass and enhanced output in biofuels. Pursuing these approaches, implementation of nanotechnology can beat the aforesaid problems by offering the opportunity to amend the properties of feedstock matter for synthesis of biofuel. Because of extraordinary physiochemical characteristics, nanoparticles have deliberated uses in synthesis of biofuel. Many nanomaterials such as titanium dioxide, iron oxides, stannic oxide, zinc oxide, graphene, fullerene, and carbon presenting distinctive characteristics are being utilized for production of biofuel production. Furthermore, some characteristics such as quantum properties, greater surface to volume ratio, and ability to immobilize because of their little size, nanoparticles (magnetic) have broad applications in the area of biofuel production. In addition to these, the easy recovery of nanoparticles from reaction mixture by employing appropriate magnetic field makes them more significant (Ahmed and Douek 2013). Biodiesel fulfils the specific standards and hence can be employed as fuel in diesel engines; it is a mixture of esters, which are generally synthesized by transesterification of animal fats or vegetable oils with small-chain alcohols, e. g., ethanol or methanol. Biodiesel

**Table 11.2** Use of different nanocatalysts and feedstock used for biodiesel production

| Sr no. | Nanocatalyst | Feedstock | Yield (%) | References |
|---|---|---|---|---|
| 1 | Lithium-impregnated calcium oxide (Li-Cao) | Karanja oil and *Jatropha* oil | 99.0 | (Kaur and Ali 2011) |
| 2 | K2O/γ-Al2O3 | Rapeseed oil | 94.0 | (Han and Guan 2009) |
| 3 | MgO nanoparticles on TiO2 support | Soybean oil | 95.0 | (Mguni et al. 2012) |
| 4 | KF/γ-Al2O3/honeycomb ceramic (HC) monolithic catalyst | Palm oil | 96.0 | (Gao et al. 2015) |
| 5 | MgO | Sunflower oil and rapeseed oil | 98.0 | (Verziu et al. 2008) |
| 6 | Sulfamic and sulfonic acid-functionalized silica-coated crystalline Fe/Fe3O4 core/shell magnetic nanoparticles | Glyceryl trioleate | 95.0 | (Wang et al. 2015) |
| 7 | KF/CaO | Chinese tallow seed oil | 96.8 | (Wen et al. 2010) |
| 8 | Ca/Fe3O4@SiO2 | Sunflower Oil | 97.0 | (Feyzi and Norouzi 2016) |
| 9 | ZrO2 loaded with C4H4O6HK | Soybean oil | 98.03 | (Qiu et al. 2011) |
| 10 | Cs/Al/Fe3O4 | Sunflower oil | 94.8 | (Feyzi et al. 2013) |
| 11 | ZnO nanorods | Olive oil | 94.8 | (Molina 2013) |
| 12 | Iron/cadmium and iron/tin oxide nanoparticles | Soybean oil | 84.0 | (Alves et al. 2014) |
| 13 | CaO | *Jatropha* oil | 98.54 | (Reddy et al. 2015) |
| 14 | Hydrotalcite-derived particles with Mg/Al oxides | *Jatropha* oil | 95.2 | (Deng et al. 2011) |
| 15 | TiO2-ZnO | Palm oil | 92.2 | (Madhuvilakku and Piraman 2013) |

is much beneficial than conventional fuel as it is produced from renewable resources and has biodegradable and predominant emollient characteristics, without liberation of injurious chemicals (Feyzi and Norouzi 2016). Table 11.2 abridges various nanocatalysts and feedstock utilized for the biodiesel production and the yield belonged to each process as well.

As revealed that in this field, there are numerous different feasibilities of implementations of nanocatalysts. Several modified nanomaterials are being successfully employed in the biodiesel production. For instance, Wang et al. (2015) synthesized acid-modified magnetic nanoparticles and described their utilization as heterogeneous nanocatalysts for synthesis of biodiesel. In this investigation, acid-modified, i.e., $H_3NSO_4$ and $R - S(=O)_2 - OH$ silica-covered crystalline iron/iron (II, III) oxide shell/core, magnetic nanoparticles were produced and utilized for the synthesis of biodiesel from transesterification of glyceryl trioleate. Researchers observed that both the acid-modified nanocatalysts manifested the noticeable enzymatic activities. But the sulfamic acid-modified nanocatalysts presented greater activity as compared to the sulfonic acid-modified nanocatalysts. Tahvildari et al. (2015) conducted a research on the biodiesel production using cooking oils, employing calcium oxide and magnesium oxide nanoparticles produced by sol-gel and sol-gel self-combustion methods, respectively. Calcium oxide nanoparticles displayed principal improvement in the yield of biodiesel as compared to magnesium oxide nanoparticles.

A new approach known as magnetic nanomaterials is also getting importance as these are easy to recover and reuse and also favorable for economic application of the process. In this context, to produce biodiesel from soybean oil, Alves et al. (2014) suggested a quick and simple nanotechnological application. They employed a combination of iron/tin and iron/cadmium oxide nanoparticles having magnetic characteristics, formed by co-precipitation method as nanocatalysts for the production of biodiesel. Iron/tin oxide nanoparticles displayed the highest efficiency by forming approximately 84% biodiesel. These nanocatalysts proposed remarkable ability toward hydrolysis, transesterification, and esterification of soybean oil and their fatty acids. Likewise, Qiu et al. (2011) suggested the production by zirconium oxide nanocatalyst laden with potassium bitartrate, in size ranging from 10 to 40 nm. Moreover, authors explored the biodiesel production by utilizing the modified nanocatalysts for the transesterification of soybean oil and methanol in various molar ratios and other factors, e. g., concentration of nanocatalyst, time for reaction, and reaction temperature. The observations suggested that reaction mixture with oil and methanol in the ratio 1:16 having 6% nanocatalyst at 60 °C for 2 h ended in maximum production of biodiesel of around 98.03%. Use of nanomaterials for production of biodiesel is also reported in several investigations. For example, in 2010, Wen et al. (2010) produced potassium fluoride/calcium oxide nanocatalyst by saturation method with size ranging from 30 to 100 nm and further utilized it for biodiesel production from Chinese tallow seed. This study revealed about 96.8% yield of biodiesel depicting ability of such nanocatalyst for biodiesel industry. In another study, Deng et al. (2011) achieved about 95.2% production of biodiesel from pretreated *Jatropha* oil using nanocatalysts, hydrotalcite-obtained particles with magnesium/aluminum oxides created by using urea as precipitating agent through a co-precipitation method.

Feyzi et al. (2013) in a novel research found a new nanocatalyst, cesium/aluminum/iron (II, III) oxide, that was produced and assessed with respect to its catalytic efficiency for production of biodiesel. This research investigated the influence of various cesium/aluminum and cesium/iron molar ratio and calcination situations on the catalytic implementation. These nanocatalysts, in the molar ratio of 2.5:1 and 4:1, respectively, depicted prospective for biodiesel production, ending in 94.8% of process yield at 58 °C with continuous stirring for 120 min. Verziu et al. (2008) in their investigation about nanocatalysts demonstrated the establishment of magnesium oxide nanocatalyst in the form of nanosheet by aerogel method and revealed its application in the production of biodiesel from rapeseed and sunflower oil, substantiating process yield up to 98%. In a recent study, Feyzi and Norouzi (2016) developed a nanocatalyst $Ca/Fe_3O_4@SiO_2$ with powerful magnetic characteristics applying mixture of two different applications, namely, sol-gel and incipient wetness impregnation method for the formation of biodiesel. Developed nanocatalysts were suggested to be very efficient at optimum conditions and ends in maximum production of around 97%. The magnetic character of nanocatalyst favored its reuse for various times without principal is appearing in its catalytic action. In another recent study, Reddy et al. (2015) revealed the formation of new calcium oxide nanocatalyst using *Polymesoda erosa*, a seashell, through various steps like calcination, hydration, and dehydration techniques. They also found the feasibility of the formed nanocatalyst regarding production of biodiesel from the non-eatable raw oils, e.g., *Jatropha* oil. The highest production of 98.54% biodiesel was attained at conditions of oil to methanol ratio in 1:5.15 molar ratio, reaction time of 133.1 min at 0.02:1 (w/w) nanocatalyst ratio.

Bioethanol (ethyl alcohol) is usually formed by carbon means of grains, sugarcane juice, and others. However, the synthesis of this alcohol is also possible from the sugars obtained from the hydrolysis of vegetal residues and lignocellulosic biomass. The lignocellulosic biomass is generally made up of polymeric structures of carbohydrates, i.e., cellulose and hemicellulose, and lignin which is a complicated organic polymer comprised of chiefly phenolic compounds (Antunes et al. 2014). Different pretreatments of biomass are required to remove the stubborn barrier of lignin from the cellulose and hemicellulose, so that these complex polymeric frameworks can be converted into simple monomeric sugars. Generally the complex structures of cellulosic contents of biomass are hydrolyzed enzymatically into simple fermentable sugars, after an initial pretreatment. Using this procedure, simple sugars can be formed in moderate process parameters such as lower temperature, without requirement of pressure and the non-production of unwanted fermentation inhibitor compounds as compared to chemical process.

Around 18% of total expenses involved in the process of the production of bioethanol belong to the cellulases used which are responsible for the breakdown of lignocellulosic biomass. Therefore the development of advance approaches is required which could help in easy recovery and recycling of cellulases and could decrease production cost as well. Keeping this fact in mind, nanotechnology proposed immobilization of different enzymes like cellulases and hemicellulases, which are engaged in the production of bioethanol. For instance, enzyme immobilization on magnetic nanoparticles is a reliable procedure that delivers easy recovery of enzyme by assigning magnetic field that permits enzyme reuse and recovery for many runs (Rai et al. 2016). Enzymes involved in the production of bioethanol are reported to be immobilized on magnetic nanoparticles in many studies. Usually enzymes are immobilized on nanoparticles by physical adsorption or covalent binding. However covalent binding method is considered more satisfactory because this method decreases desorption of protein as a result of the formation of covalent bonds between nanoparticles and enzyme (Abraham et al. 2014). Nanomaterials need to be amended or loaded with chemically active polymer for stable immobilization of enzyme on them, to provide the functional group for binding of enzyme. Lee et al. (2010) investigated the entrapment method for the immobilization of β-glucosidase enzyme on polymer magnetic nanofibers for the production of ethanol from cellulosic matter. β-Glucosidase is literally the enzyme which catalyzes the transformation of cellobiose into glucose, and this glucose is converted into bioethanol by microorganisms. Actually, the stability of enzyme is achieved by entrapment of β-glucosidase on magnetic nanofibers, and this entrapment method also offers chances of repeated use, recycling and isolating them by implementing magnetic field. Likewise, Verma et al. (2013b) assessed immobilization of β-glucosidase isolated from fungus on magnetic nanoparticles, used as nanobiocatalyst for production of bioethanol. They reported 93% of enzyme-binding efficacy, demonstrating around 50% of its initial activity at 16th cycle. Jordan et al. (2011) also investigated the hydrolysis of microcrystalline cellulose by recycling of enzyme, employing carbodiimide as binding polymer for immobilization of enzyme on $Fe_3O_4$ nanoparticles. The enzyme could be restored easily and recycled for six times because of magnetic characteristics of nanoparticles. Goh et al. (2012) studied that enzyme used in the production of bioethanol was immobilized in single-walled carbon nanotubes, which was already subsumed, to give magnetic properties, by magnetic iron oxide nanoparticles. They concluded that by altering the concentration of iron oxide nanoparticles in the nanotubes, the performance of immobilized enzyme could be controlled. Thus, immobilized enzyme can be stored in acetate buffer at 4 °C for its longer storage. For immobilization of enzymes, various nanomaterials have been investigated. For instance, Xie and Wang (2012) conducted a research on the lipase immobilization on magnetic chitosan microspheres produced by chemical co-precipitation method. In this study, as linking molecules, glutaraldehyde was used for the covalent binding between magnetic chitosan microspheres and lipase enzyme. Furthermore, aiming for the use of production of bioethanol, through adsorption methods, enzyme immobilization on titanium oxide nanoparticles was also done successfully and employed for the breakdown of lignocellulosic biomasses (Ahmad and Sardar 2014). Cherian et al. (2015) in some other study immobilized cellulase enzyme isolated from *Aspergillus fumigatus* on nanoparticles of manganese dioxide by covalent binding. Researchers proved that as relative to free enzymes, enzyme immobilized on nanomaterials have improved thermostability characteristic, depicting stability up to 70 °C. Hydrolysis of agricultural waste was done in the presence of immobilized cellulase enzyme pursued by the employment of yeast for the production of bioethanol (21.96 g/L). After again and again, the use of immobilized enzyme for approximately five cycles, activity of enzyme remained 60%. Other several nanomaterials can be used in nanotechnology process in addition to magnetic nanoparticles, such as titanium oxide and silica, polymeric nanoparticles, and carbon materials like carbon nanotubes, graphene, fullerene, and others. All these materials have been successfully accounted for immobilization of various enzymes with respect to the processes of production of bioethanol (Cho et al. 2012; Pavlidis et al. 2012; Verma et al. 2013a). For example, to get enhanced catalytic activity for production of ethanol, carbon nanotubes entrapped with Rh particles can be used (Pan et al. 2007). In fact, the incorporation of materials of different interests is facilitated by free cores available on the carbon nanotubes. In some other research, Lupoi and Smith (Lupoi and Smith 2011) compared the efficiency of free cellulase enzyme with that of the cellulase enzyme immobilized on the silica nanoparticles in the hydrolysis of cellulose into glucose. They concluded that cellulase enzyme that is immobilized demonstrated high production of glucose relative to free enzyme, proving that in simultaneous saccharification and fermentation, immobilized enzymes can be employed. To employ for fermentation process of production of ethanol, microbial cells can also be immobilized on the nanoparticles. For example, a strategy was developed for immobilization of *Saccharomyces cerevisiae* cells onto the magnetic nanoparticles. Furthermore, continuous fermentation operation for the bioethanol production was performed, and reported immobilized *S. cerevisiae* cells depicted maximum ethanol production efficacy (Ivanova et al. 2011). Thus, the studies conducted either on the enzyme immobilization or whole microbial cells on the various nanomaterials proves that such approaches and technologies will be feasible for the secure and cost-effective synthesis of bioethanol from inexpensive lignocellulosic biomass.

## 11.5 Iron Oxide Nanomaterial Synthesis and Their Applications in Biofuel Production

Chemically or biologically nanoparticles could be synthesized. Many severe effects are linked with chemical synthesis procedures because of the existence of some toxicants which are absorbable on the surface. Biological method replaces physical and chemical methods that is an environment-friendly way of nanoparticles production using plants, microorganisms, enzymes, fungus, and plant extracts. Biological method being an environmental favorable replacement of physical and chemical methods is a way of nanoparticles production using plants, microorganisms, enzymes, fungus, and plant extracts. Development of these environmental favorable methods for production of nanoparticles is progressing into a significant domain of nanotechnology particularly silver nanocarriers, which have numerous implementations. Biosynthesis of nanoparticles by microorganisms is a green and eco-friendly technology. Green and environment favorable technology for the production of nanoparticles is biosynthesis. Variety of microorganisms both eukaryotes and prokaryotes are used for the production of metallic nanocarriers specifically palladium, gold, silver, zirconium, cadmium, iron platinum, and metal oxides such as zinc oxide, titanium oxide, etc. These microorganisms are actinomycetes, bacteria, algae, and fungi. According to the placement of nanoparticles, production of nanoparticles may be of two methods, i.e., intracellular or extracellular. Inorganic materials are formed by multicellular as well as unicellular organisms either extracellularly or intracellularly. Microorganisms such as bacteria and fungi are capable to tackle the production of metallic nanoparticles, and their ability is employed in the hunt for novel materials. Fungi have become the center of studies on biological production of metallic nanoparticles because of their resistance and metal bio-magnifications potential (Hasan 2015).

Currently iron oxide nanoparticles are getting much interest in atmospheric remediation because of their little size, magnetic attribute, and greater surface area. Among the most ubiquitous elements on earth is iron. Iron and oxygen atoms combine to form iron oxides. However, there are 16 identified iron oxides and oxyhydroxides. Feasible isolation of adsorbents from the process and its reutilization for further implementation become possible due to magnetic character of iron oxide nanoparticles. Iron oxide nanocarriers lead to a decline in the economic burden because of reusability. Feasibility of resource and facility in production presents nano-sized ferric oxides as cost-effective adsorbents for toxic metal absorption and adsorption. Elemental iron is therefore eco-favorable. Numerous encouraging applications involve utilization of iron oxide (Rasheed and Meera 2016). Several procedures have been investigated up till now for the production of iron oxide-based nanomaterials. Commonly these procedures can be categorized as chemical, biological, and physical methods. Literature exhibited that iron oxide nanoparticles are generally synthesized by chemical methods. Co-precipitation, hydrothermal synthesis, reverse micelles and micro-emulsion technology, thermal decomposition, sonochemical reactions, sol-gel production, hydrolysis and thermolysis of precursors, flow injection synthesis, electrospray production, and colloidal chemistry method are usually applied chemical methods. Chemical methods have some disadvantages, namely, the broad particle size scattering, the less dispensation in solvents, and the consistency of the size of particle of these nanomaterials that was somewhat of low quality (Nidhin et al. 2008). These procedures cause accumulation of nanoparticles. Therefore the interest in the employment of green resources for the production of nanomaterials is increasing. The following are the distinctive benefits of the biological synthesis procedures that make them superior over traditional employed chemical and physical procedures (Herlekar et al. 2014):

- Environment-friendly and clean approach because there is no use of toxicants.
- Production process is cost-effective as the active biological constituents such as enzyme itself perform as a capping and reducing agent.
- During pilot-scale synthesis, small nanocarriers can be formed.
- External investigational factors such as high pressure and high energy are not desired, thus leading to principal reduction in energy consumption.

In the area of magnetic nanomaterials in the last few years, explorations are increased on different types of iron oxide (usually involves the $Fe_3O_4$ magnetite, $FeIIFeIII_2O_4$, ferrimagnetic, superparamagnetic when the size is smaller than 15 nanometer), a-Fe2O3 (hematite, fragile, ferromagnetic, or antiferromagnetic), c-$Fe_2O_3$ (ferromagnetic, maghemite), FeO (wüstite, antiferromagnetic), e-$Fe_2O_3$ and b-$Fe_2O_3$ (wüstite, antiferromagnetic), b-$Fe_2O_3$, and e-$Fe_2O_0$ (Cornell and Schwertmann 2003). However, tackling stability, shape, size, and dispersibility of nanoparticles in interested solvents is a technological challenge. Due to greater surface to volume ratio, magnetic iron oxide nanocarriers have greater surface energies. Therefore, they try to accumulate so as to reduce the surface energies. Furthermore, the bare iron oxide nanocarriers can feasibly oxidized in atmosphere (particularly magnetite), owing to their enhanced chemical action, and usually lose dispersibility and magnetism. Therefore, it is very essential to provide appropriate surface layering and establish few effectual protection approaches to retain the stability of magnetic iron oxide nanomaterials. These approaches consist layering with or grafting of organic molecules such as little organic molecules or surfactants, biomolecules, and polymers or layering with an inorganic layer, like metal, metal oxide, silica, metal or nonmetal elementary substance, and metal sulfide. Certainly, it is notable that in several instances, the shelter shells can be employed for further functionalization too in addition to stabilizing the magnetic iron oxide nanoparticles (Wu et al. 2008).

Heterogeneous and homogeneous catalysts are used for the mass manufacture of biodiesel. The homogeneous ones have several disadvantages such as corrosiveness, catalyst recycle, influence on soap formation, and catalyst separation. The heterogeneous ones show a lot of demerits like less yields of biodiesel, costly in nature, and complex formation. Hence, it is essential to establish the cheap and potential catalyst to manufacture increased yield of biodiesel. Nowadays, metal nanoparticles perform a major role in the transesterification process as catalyst to manufacture increased productivity of biodiesel, having its large active surface area, enhanced resistance to saponification reaction, better rigidity, and increased catalytic activity. Conversely, the metal nanoparticles are unsteady in nature, and they have a propensity to make collections (Rengasamy et al. 2016). Xie and Ma (2009) described that the biodiesel were gained from soybean oil using immobilized lipase scattered on magnetic $Fe_3O_4$ nanoparticles. Anyhow, immobilization of enzymes needs extra cost and time. Regarding previous studies, the phytogenic metal nanoparticles are stable for long-time duration (Iravani 2011).

The most common procedures including co-precipitation, thermal decomposition, sonochemical production, hydrothermal production, microemulsion, and sonochemical synthetic route can all be directed to the manufacture of better quality of iron oxide nanoparticles. Moreover, these nanoparticles can also be manufactured by the other techniques like electrochemical formation, laser pyrolysis procedures, bacterial or microorganism formation (especially iron-reducing bacteria and the magnetotactic bacteria), etc. The most traditional method for obtaining $Fe_3O_4$ or c-$Fe_2O_3$ is by co-precipitation. In this procedure, we mix ferric and ferrous ions in a 1:2 molar ratio in very alkaline solutions at increased temperature or at room temperature. The shape and size of the iron oxide nanoparticles depend on the kind of salt used (like perchlorates, nitrates, sulfates, chlorides, etc.), the ferrous and ferric ion ratio, the ionic strength of the media, the pH value, the reaction temperature, and the other reaction factors (e.g., mixing rate, falling speed of alkaline solution). Currently it is investigated that the co-precipitation manufacture of $Fe_3O_4$ nanoparticles and their respective structure, apparent form, and magnetic characteristics at various temperature of reaction were studied (Wu et al. 2008). An organic solution-phase decomposition route has been largely used in iron oxide nanoparticles production, and decomposition of $Fe(cup)_3$ (cup = N-nitrosophenylhydroxylamine), $Fe(acac)_3$ (acac = acetylacetonate), or $Fe(CO)_5$ followed by oxidation can lead to excellent-quality monodispersed iron oxide nanoparticles, which mostly needs comparatively increased temperatures and a complex operation. Sun and Zeng (2002) described a common breakdown strategy for the manufacture of size-sensitive monodispersed magnetite nanoparticles established on increased temperature (265 °C) reaction of $Fe(acac)_3$ in phenyl ether in the availability of oleylamine, alcohol, and oleic acid. With the little magnetite nanoparticles as seeds, larger monodispersed magnetite nanoparticles of up to 20 nm in diameter can be manufactured and scattered into nonpolar solvent by seed-mediated growth procedure. The method does not need a size selection method and is quickly scaled up for synthesis on large scale. The as-synthesized $Fe_3O_4$ nanoparticle assemblies can be changed feasibly into c-$Fe_2O_3$ nanoparticles by annealing at increased temperature (250 °C) and oxygen for 2 h.

A thermodynamically sensible isotropic dispersion of two immiscible phases (oil and water) is microemulsion in the presence of surfactant; with the dissolved hydrophobic tails of the surfactant molecules in the oil phase, the surfactant molecules can produce a single layer at the coherence of oil and water, and in the water phase, the hydrophilic head groups are present. Self-fabricated assemblies of various kinds can be prepared, ranging, for instance, from (inverted) globular and columnar micelles to lamellar stage and bicontinuous microemulsions, which can co-occur with principally water or oil phases in the binary systems, e.g., oil/surfactant or water/surfactant (Solans et al. 2005). In that scenario, microemulsion and inverse micelles path can be applied for getting the size- and shape-checked iron oxide nanoparticles.

Iron oxide nanoparticles with authorized shape and size are significant technologically because of powerful association among these factors and magnetic characteristics. The thermal decomposition and microemulsion techniques generally take to complex method or demand comparatively increased temperatures. As a substitute, hydrothermal formation has several wet chemical strategies of crystallizing material in a closed vessel from the increased temperature aqueous solution (generally in the range from 130 to 250 °C) at increased vapor pressure (commonly in the range from 0.3 to 4 MPa). This procedure has also been used to grow dislocation-free single crystal carriers, and grains synthesized in this method could have a fitter crystallinity than that from other methods, so hydrothermal formation is susceptible to acquire the greatly crystalline iron oxide nanocarriers. Many investigators have described the production of iron oxide nanocarriers through hydrothermal technique (Hu et al. 2007).

According to whether or not use the particular surfactants, there are two main procedures. For instance, a one-step hydrothermal process to make very crystalline $Fe_3O_4$ nanopowders without using the surfactants has been reported by Wang et al. (2003). The nanoscale $Fe_3O_4$ powder (40 nm) acquired at 140 °C for 6 h possessed a saturation magnetization of 85.8 emu_g-1, a little less than that of the correspondent bulk $Fe_3O_4$(92 emu_g-1). It is recommended that the well-crystallized $Fe_3O_4$ grains made under suitable hydrothermal environment should be responsible for the enhanced saturation magnetization in nano-sized $Fe_3O_4$. Conversely, Zheng et al. (2006) described a hydrothermal route for synthesizing $Fe_3O_4$ nanoparticles with diameter of ca. 27 nm in the existence of a surfactant, sodium bis(2-ethylhexyl) sulfosuccinate (AOT). At room temperature, the magnetic characteristics of the nanoparticles showed a superparamagnetic performance. Furthermore, hydrothermal treatment is one of the well-to-do means of growing crystals for iron oxide nanoparticles.

As a ruthless substitute, the sonochemical procedure is broadly utilized to make novel materials with uncommon characteristics. The chemical effects of ultrasound emerge from acoustic cavitation, that is, the synthesis, development, and implosive bursting of bubbles in liquid. The implosive bursting of the bubble makes a confined hotspot via adiabatic compression or shock wave formation within the gas phase of the bursting bubble. The conditions formed in these hotspots have been experimentally found, with 1800 atm pressure, 5000 K transitory temperature, and cooling rates in excess of 1010 K/s (Suslick 1990). These utmost conditions were useful to make the new phase and have a shear effect for agglomeration, which is susceptible to make the very monodispersive nanoparticles. This procedure has been applied for the formation of several nanocomposites, and its versatility has been efficiently displayed in iron oxide nanoparticles synthesis (Bang and Suslick 2007). For example, magnetite nanoparticles can be effortlessly made by sonication of iron (II) acetate in water under an argon environment. Vijayakumar et al. (2000) described a sonochemical manufacturing course for synthesizing the pure nanometer-size $Fe_3O_4$ powder with particle size of ca. 10 nm. The synthesized $Fe_3O_4$ nanoparticles are superparamagnetic, and its magnetization at room temperature is very less (<1.25 emu g-1). The five aforementioned preparing ways have various merits and demerits for making iron oxide nanoparticles, respectively. In terms of size and morphology control of the iron oxide nanoparticles, thermal decomposition and hydrothermal preparing route seems the excellent procedure. For acquiring the water-soluble and biocompatible iron oxide nanoparticles, co-precipitation usually was engaged, but this procedure shows less control of the particle shape, wide dispersion of sizes, and gathering of particles. As a time ruthless substitute, sonochemical avenue can also be utilized to prepare iron oxide nanoparticles with rare magnetic characteristics. Additionally, it is notable that several green chemical preparation paths and biological construction paths are described for environment shelter causes.

## 11.6 Conclusion

The worldwide climatic issues like greenhouse effect caused by different chemicals such as the utilization of coal and petroleum have urged searching for substitute inexhaustible energy and biofuel means. The high requirement of substitute means that is also because of the quick consumption of occurring fuel withholds. Among the advance approaches, nanotechnology is acquiring much attention to deal with the problems of bioenergy and biofuel production by employing various implementations like alteration in raw materials and use of operative catalysts. Because of their phenomenal properties, iron oxide nanomaterials have become marvelous in biofuel industry in present years. Iron oxide nanoparticle technologies have great potentials to enhance production of biofuel.

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# Correction to: Application of Plant-Based Natural Product to Synthesize Nanomaterial

Mohammad Khajeh Mehrizi and Zahra Shahi

**Correction to:**
**Chapter 3 in: M. Srivastava et al. (eds.),**
***Nanomaterials in Biofuels Research,***
**Clean Energy Production Technologies,**
**https://doi.org/10.1007/978-981-13-9333-4_3**

The book was inadvertently published without including the name of the second author (Zahra Shahi) in Chapter 3. This has now been corrected.

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The updated online version of this chapter can be found at
https://doi.org/10.1007/978-981-13-9333-4_3

M. Srivastava et al. (eds.), *Nanomaterials in Biofuels Research*,
Clean Energy Production Technologies, https://doi.org/10.1007/978-981-13-9333-4_12